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CHARACTERIZATION OF ARTISANAL HONEYS FROM CASTILLA Y LEÓN (SPAIN)

PHD THESIS

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CARACTERIZACIÓN DE MIELES ARTESANALES DE CASTILLA Y LEÓN (ESPAÑA)

TESIS DOCTORAL

Ana Pascual Maté

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Memoria presentada por

Ana Pascual Maté

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UNIVERSIDAD DE BURGOS
DEPARTAMENTO DE BIOTECNOLOGÍA Y CIENCIA DE LOS ALIMENTOS

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Dña. Ana Pascual Maté ha realizado bajo su dirección el trabajo titulado "Characterization of artisanal honeys from Castilla y León (Spain)".

Considerando que dicho trabajo reúne los requisitos exigidos para ser presentado como Tesis Doctoral, expresan su conformidad con dicha presentación.

Y para que así conste, firman el presente certificado en Burgos a 2 de noviembre de dos mil quince.

Burgos, 2 de noviembre de 2015.

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CERTIFICA:

Que la memoria titulada "Characterization of artisanal honeys from Castilla y León (Spain)" presentada por Dña. Ana Pascual Maté para optar al grado de Doctor por la Universidad de Burgos, ha sido realizada bajo la dirección conjunta de los doctores D. Miguel Ángel Fernández Muiño y Dña. María Teresa Sancho Ortiz en el departamento de Biotecnología y Ciencia de los Alimentos de la Universidad de Burgos.

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Burgos, 2 de noviembre de 2015.

Fdo. Pilar Muñiz Rodríguez

« Ahora, voy a cantar a la miel, esa rosa del aire,
ese dulce regalo de los cielos... »

(Virgilio, Geórgicas, IV, 1)

ABSTRACT

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The purpose of the present PhD Thesis has been to characterize honeys of different botanical origins from Castilla y León (North-Central Spain) on the basis of their mellisopalinology, sensory and physicochemical parameters, including sugars' and phenolic compounds' profiles, different antioxidant-related parameters and antimicrobial analysis. A thorough review of the literature, as well as a critical review and set up of total flavonoids, trolox equivalent antioxidant capacity (TEAC) and antibacterial activity against *Staphylococcus aureus* were also done.

All honeys fulfilled the European regulations. The analysis of total flavonoids was done in neutral media on honeys' methanolic extracts, with a sample's colour correction. TEAC determination was simplified reading the absorbance at six minutes. Agar well diffusion procedure, followed by broth dilution assay, was proposed for honeys' antibacterial activity determination.

Dark honeys possessed higher TEAC, polyphenols, phenolic acids and ellagic acid mean values, and lower quercetin and kaempferol averages than light honeys. Chestnut and honeydew honeys were characterized by the highest electrical conductivity and pH, and the lowest fructose and glucose mean concentrations, as well as the lowest averages of quercetin and kaempferol. Honeydew samples had the highest mean values of optical rotation, proline, total di- and oligosaccharides, the main honeydew indicator sugars and ellagic acid. Chestnut samples possessed the highest mean concentration for the sum of all hydroxycinnamic-related compounds. Lavender and clover samples were characterized by the highest maltose, quercetin and kaempferol averages and the lowest electrical conductivity, pH, melezitose and ellagic acid mean values. Both honey types together with heather honeys possessed high monosaccharides averages. Clover samples had high moisture content, free and total acidity, and the lowest chlorogenic acid average, being the contrary to lavender honeys. Moreover clover honeys had the lowest pinocembrin, chrysin and galangin mean values, whereas lavender samples possessed the highest sucrose and the lowest isomaltose and raffinose averages. Finally, heather samples were characterized by the highest pinocembrin and chrysin mean values and the lowest specific rotation, disaccharides and trisaccharides mean contents.

Some significant correlations were found among the studied parameters. Bioactive compounds and other antioxidant-related parameters were more useful to classify honeys by their botanical origins than sugars and other physicochemical characteristics.

RESUMEN

El propósito de la presente Tesis Doctoral ha sido la caracterización de mieles de diferentes orígenes botánicos producidas en Castilla y León (España), en función de su análisis melisopalinológico, sensorial y de sus parámetros fisicoquímicos, destacando el perfil de azúcares y compuestos fenólicos, diferentes parámetros relacionados con la capacidad antioxidante y la evaluación de la actividad antibacteriana. También se llevó a cabo una revisión exhaustiva de la bibliografía, así como una revisión crítica y una puesta a punto del método de análisis de flavonoides totales, de la capacidad antioxidante equivalente de trolox (TEAC) y de la actividad antibacteriana contra *Staphylococcus aureus*.

Todas las mieles cumplieron con las regulaciones Europeas establecidas. El análisis de flavonoides totales se realizó en medio neutro en los extractos metanólicos de las mieles, corrigiendo el color de las muestras. La determinación TEAC se simplificó leyendo la absorbancia a seis minutos. Para la determinación de la actividad antibacteriana, se propuso el análisis de difusión en agar, seguido del ensayo de dilución en caldo.

Las mieles oscuras poseyeron mayores valores medios de TEAC, polifenoles, ácidos fenólicos y ácido elágico, y menores de quercetina y canferol que las mieles claras. Las mieles de castaño y mielada se caracterizaron por una conductividad y un pH muy elevados, y concentraciones bajas de fructosa, glucosa, quercetina y canferol. Las muestras de mielada tuvieron los valores medios más elevados de rotación específica, prolina, di- y oligosacáridos totales, de los principales azúcares indicadores de mielada y de ácido elágico. Las mieles de castaño poseyeron el promedio más elevado de compuestos hidroxicinámicos. Las mieles de lavanda y trébol se caracterizaron por los valores medios más elevados de maltosa, quercetina y canferol, y los más bajos de conductividad, pH, melecitosa y ácido elágico. Ambos tipos de miel, junto con las muestras de brezo, tuvieron la concentración media de monosacáridos más alta. Las mieles de trébol poseyeron una elevada humedad, acidez libre y acidez total, y el promedio de ácido clorogénico más bajo, siendo lo contrario en las de lavanda. Además, las mieles de trébol presentaron los valores medios de pinocembrina, crisina y galangina más bajos, y las de lavanda los más elevados de sacarosa y los más bajos de isomaltosa y rafinosa. Las mieles de brezo se caracterizaron por los mayores promedios de pinocembrina y crisina, y los menores de rotación específica, di- y oligosacáridos totales.

Se encontraron algunas correlaciones significativas entre los parámetros estudiados. Los resultados relacionados con la actividad antioxidante fueron más útiles que los otros análisis fisicoquímicos realizados a la hora de discriminar las mieles según su origen botánico.

PhD THESIS STRUCTURE

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This PhD Thesis has been divided into the following paragraphs: introduction, objectives, experimental work (including materials and methods, and results and discussion), future prospects, conclusions and annexes.

The introduction comprises three chapters. The first one is a brief general introduction in Spanish. The second chapter deals with physicochemical composition, nutritional and biological properties of honey. The third one tackles an extensive and thorough literature review about physicochemical analyses of honey.

The purposes of the thesis have been written both in English and in Spanish.

Chapters 4, 5, 6 and 7 refer to the paragraphs of the experimental part. These chapters have been structured with the format of scientific papers, because some of them were sent, accepted for publication and/or already published. The characterization of honeys from Castilla y León on the basis of their quality control parameters and sugars' profiles in particular, has been described in chapter 4. Chapter 5 has dealt with the setting up and optimization of different procedures related with antioxidant capacity, assessing the results of honeys from Castilla y León in chapter 6, together with the analysis of other bioactive compounds. Eventually, in chapter 7 the most common methods to determine antibacterial activity of honeys against *Staphylococcus aureus* have been researched and compared.

Finally, the conclusions of the research are summarized, both in English and in Spanish.

In respect of the attached annexes, annex 1 shows different tables commented in the experimental part, whereas annex 2 compiles the scientific articles derived from this PhD Thesis so far.

ESTRUCTURA DE LA TESIS DOCTORAL

Esta Tesis Doctoral se ha dividido en los siguientes apartados: introducción, objetivos, trabajo experimental (donde están incluidos materiales y métodos, y resultados y discusión), conclusiones, perspectivas futuras y anexos.

La introducción está compuesta por tres capítulos. El primer capítulo es una breve introducción en español. El segundo trata sobre la composición fisicoquímica, propiedades biológicas y nutricionales de la miel. El tercero aborda una extensa y detallada revisión bibliográfica de la metodología empleada para el análisis fisicoquímico de la miel.

Los objetivos de la tesis se han redactado en inglés y en español.

Los capítulos 4, 5, 6 y 7 hacen referencia a la parte experimental. Dichos capítulos se han estructurado como artículos, ya que algunos de ellos han sido enviados, aceptados y/o publicados. La caracterización de las mieles de Castilla y León por sus parámetros de control de calidad y en particular, de su perfil de azúcares, queda recogida en el capítulo 4. En el capítulo 5 se describe la optimización de distintas metodologías analíticas relacionadas con la capacidad antioxidante, evaluando en el capítulo 6 estos parámetros junto con otros compuestos bioactivos en las mieles castellano-leonesas. Para finalizar este apartado, en el capítulo 7 se investigan y comparan los métodos más utilizados para determinar la actividad antibacteriana de las mieles frente *Staphylococcous aureus*.

Por último, se exponen las conclusiones de la investigación realizada en inglés y en español.

En lo que respecta a los anexos adjuntos, el primero recoge distintas tablas comentadas en la parte experimental, mientras que el segundo anexo recopila los artículos científicos derivados de la presente tesis doctoral hasta el momento.

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INTRODUCTION

CHAPTER 1

INTRODUCCIÓN GENERAL

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1. Castilla y León y la apicultura

Castilla y León es una Comunidad Autónoma española formada por nueve provincias, que está situada en el centro-norte de la Península Ibérica (Figura 1). Es la Comunidad Autónoma más grande de España, y la tercera mayor región de la Unión Europea, con una extensión de 94.200 km^2 y una población de 2.495.765 de habitantes (INE, 2014).

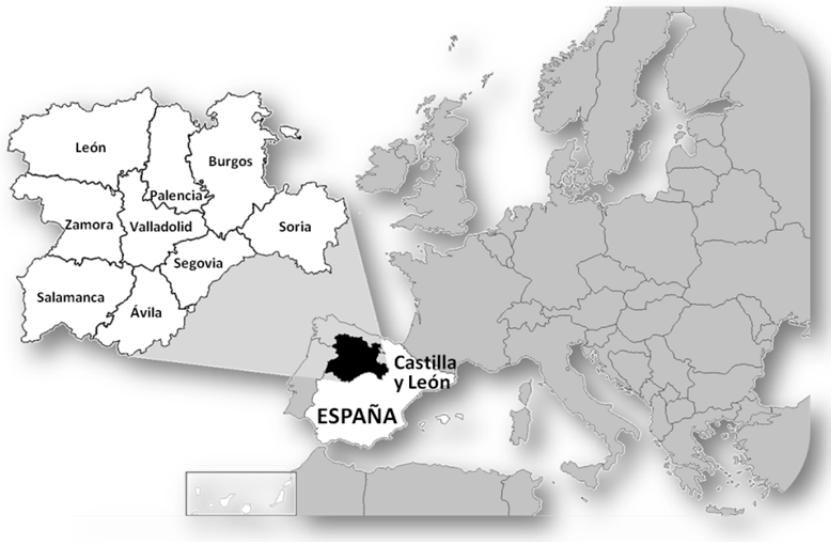

Figura 1. Mapa de Europa que muestra la localización de España y dentro de ésta, Castilla y León.

El clima de la región principalmente es mediterráneo continentalizado, caracterizado por inviernos fríos y largos, y veranos cortos y calurosos. La zona norte posee un clima oceánico, debido a la influencia del mar Cantábrico situado a unos 40 km de la región (García-Fernández, 1986).

En Castilla y León existe una gran diversidad forestal, destacando los bosques formados por encinas (*Quercus ilex*), robles (*Quercus pyrenaica* y *Quercus robur*), especies del género *Pinus*, hayas (*Fagus* sp.), fresnos (*Fraxinus* sp.), tilos (*Tilia* sp.) y castaños (*Castanea sativa*) (Guerra-Velasco, 2010).

Castilla y León posee el mayor número de explotaciones apícolas de España con 4.546 (un 16,5% del total), siendo la tercera en el número de colonias, con 399.961 (un 14,7% del total) y la cuarta en cuanto a producción de miel se refiere, con 3.983 toneladas (un 13,0% del total) (Subdirección General de Productos Ganaderos, 2015).

La flora melífera utilizada por las abejas para producir miel en Castilla y León proviene de ericáceas (*Erica* sp., *Calluna vulgaris*), leguminosas (tipo *Onobrychis* sp., *Trifolium* sp., *Genista* sp., entre otras), rosáceas (*Rubus* sp. y otras), labiadas (*Lavandula* sp., *Thymus* sp., entre otras) y castaños (*Castanea sativa*). También destacan plantas melíferas cultivadas

como el girasol (*Helianthus annuus*). Las abejas melíferas tanto en Castilla y León como en el resto de España, producen miel de mielada principalmente de árboles del género *Quercus* sp., fundamentalmente de encinas (*Quercus ilex*) y robles (*Quercus robur*, *Quercus pyrenaica*).

Las principales mieles monoflorales de Castilla y León son la miel de brezo (*Erica* sp., *Calluna vulgaris*), castaño (*Castanea sativa*), girasol (*Helianthus annuus*), lavanda (*Lavandula* sp.) y tomillo (*Thymus* sp.), así como miel de mielada (*Quercus* sp.).

2. Definición de la miel

La miel se encuentra definida en la Directiva 2001/110/CE (DOCE, 2002) y en el RD 1049/2003 (BOE, 2003) como la sustancia natural dulce producida por la abeja *Apis mellifera* a partir del néctar de plantas o de secreciones de partes vivas de plantas o de excreciones de insectos chupadores presentes en las partes vivas de plantas, que las abejas recolectan, transforman combinándolas con sustancias específicas propias, depositan, deshidratan, almacenan y dejan en colmenas para que madure.

Según su origen, la miel puede ser floral (miel de flores que a su vez puede ser monofloral o milflores) o de mielada (Figura 2). La mezcla de miel de flores y mielada se denomina miel de bosque.

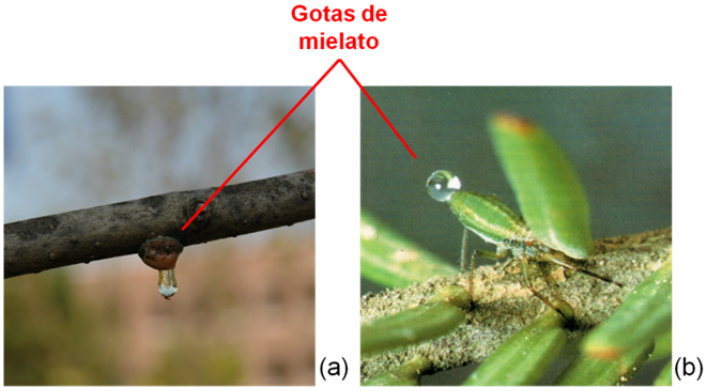

Figura 2. Los mielatos de las plantas son: (a) secreciones de partes vivas de las plantas o (b) excreciones de insectos.

Esta definición sólo es válida para la miel producida por las abejas *Apis mellifera* (especie existente en Europa), pero existen otras especies de abejas que producen miel con características totalmente distintas, por lo que sus requerimientos de calidad serán diferentes. Un ejemplo de ello es la miel producida en las regiones tropicales y subtropicales por las abejas sin aguijón o Meliponini (que incluye más de 500 especies), conocida en inglés como "pot honey", debido a que estas abejas, en lugar de almacenar la miel en panales de cera con celdillas hexagonales como la *Apis mellifera*, lo hacen en "potes o botijas" de cerumen y resina (Figura 3). Actualmente, no existe norma de calidad para esta miel, y los parámetros no cumplen con la legislación para *Apis mellifera*, ya que, mientras que en la miel de *Apis mellifera* la fermentación es un defecto grave que puede ser debido al deterioro de la miel, en

la miel de abejas sin aguijón, la fermentación se produce en el interior de la colmena. Esta miel, más húmeda y más ácida que la miel de *Apis mellifera*, es más valorada por su origen entomológico que por su origen botánico (muy difícil de evaluar debido a la gran diversidad de vegetación existente en estas regiones tropicales y subtropicales) y geográfico (Vit *et al.*, 2013).

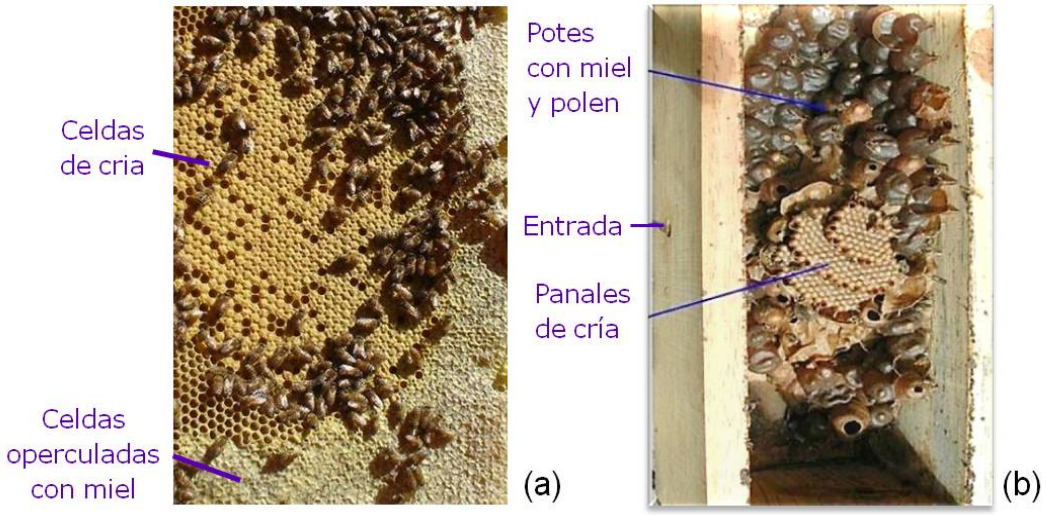

Figura 3. (a) Colmena de *Apis mellifera* y (b) colmena de Meliponini.

3. Producción de la miel

La colmena está constituida por la abeja reina, los zánganos y las abejas obreras (Figura 4). El único cometido de la reina es preservar la continuidad de la colmena, y el único cometido de los zánganos es fecundar a la reina. La reina casi no tiene cerebro con relación a la obrera, y esto ocurre en beneficio de sus órganos reproductivos; las obreras, en cambio, tienen sus órganos sexuales atrofiados, pero son más inteligentes, pudiendo cumplir diversas funciones, según su edad y tipo: abejas obreras limpiadoras, nodrizas (producen la jalea real), guardianas, y ventiladoras o evaporadoras, entre otras (Sainz-Laín y Gómez-Ferreras, 2000).

Figura 4. Miembros de una colmena.

Las fases de la producción de miel por parte de las abejas son las siguientes (Sainz-Laín y Gómez-Ferreras, 2000; Sabatini, 2007):

1) Succión de la solución azucarada que contiene más de un 90% de carbohidratos en sólidos totales (néctar o mielato) por parte de las abejas obreras pecoreadoras.

2) Almacenamiento en el buche o bolsa de la miel, que se expande pudiendo albergar hasta 70 mg de néctar o mielatos.

3) Trofalaxis: Proceso en el cual la abeja pecoreadora transfiere la solución azucarada a una abeja almacenadora, que a su vez se lo transfiere a otras abejas almacenadoras (Figura 5). El proceso dura unos 15-20 minutos, y en él intervienen aproximadamente unas cuatro abejas.

Figura 5. Trofalaxis.

Aquí da comienzo la conversión de la solución azucarada en miel, siendo la fase activa de su maduración, donde se produce la primera fase de deshidratación de la solución azucarada (facilitando así la posterior conservación de la miel), y la adición de secreciones glandulares enzimáticas que determinan una serie de transformaciones químicas sobre todo en los azúcares (como por ejemplo la adición de invertasa, que va a llevar a cabo la rotura de azúcares complejos a simples, rompiendo la sacarosa en fructosa y glucosa).

4) Almacenamiento: La abeja almacenadora deposita la miel en las celdillas del panal, siendo posteriormente el turno de la abeja ventiladora, que va a concentrar más el producto gracias a la evaporación indirecta originada por intensas corrientes de aire que provoca con su movimiento de alas. Esta es la fase pasiva de la maduración de la miel, donde tiene lugar la segunda fase de evaporación.

5) Operculación: Las abejas selladoras o cereras se encargan de cerrar u opercular las celdillas herméticamente con cera con el fin de evitar que la miel reabsorba el agua del medio y fermente.

4. Procesado de la miel

Las fases del procesado de la miel por parte del apicultor son las siguientes (Vangelisti, 2007):

1) Recogida: En los panales, se recogen los cuadros de las alzas melarias (que es donde se sitúa la miel) que se encuentren totalmente operculados, garantizando así la maduración de la miel.

2) Extracción: En primer lugar se realiza una desoperculación de los cuadros melarios (Figura 6), seguida de una centrifugación. Si la miel es de brezo *Calluna vulgaris* (también denominada miel de biércol o brecina), ésta se va a extraer de los cuadros con mucha dificultad debido a su tixotropía (propiedad que tienen algunos fluidos de pasar de estado gel a líquido por medio de agitación), por lo que como etapa previa a la centrifugación hay que realizar varios pases de un rodillo con púas sobre el cuadro melario (para provocar una agitación de la miel dentro de las celdillas y que ésta pase del estado gel a líquido), o calentar la miel a una temperatura máxima de 35ºC (aunque este procedimiento es menos eficaz).

Figura 6. Desoperculación.

3) Eliminación de las impurezas: Se realiza una filtración seguida de una decantación. Con la decantación se eliminan partículas extrañas a la miel que hayan pasado en la filtración (las más grandes tienden a depositarse en el fondo del contenedor, mientras que las más pequeñas emergen a la superficie), y además se eliminan las burbujas de aire que se encuentran englobadas en la miel y que se producen durante la fase de centrifugación.

4) Envasado.

Como tratamientos tecnológicos opcionales en este proceso de elaboración, destacan:

- Deshumidificación: Se realiza antes de la extracción en mieles con un contenido de agua excesivo, que no han terminado de madurar en los panales, previniendo con ello su fermentación.

- Cristalización inducida, preparación de miel de consistencia particular (líquida, cremosa, cristalizada), técnica de cristalización guiada o sembrado de la miel: Se realiza antes del envasado. Con ello se consigue dar homogeneidad y uniformidad al producto, garantizando que cuando la miel cristalice, los cristales formados sean más regulares y de dimensiones más finas. Es un tratamiento fundamental para la obtención de una miel de calidad.
- Calentamiento (30-40ºC): Facilita determinadas operaciones, pero sólo se debe aplicar si es estrictamente necesario.
- Pasteurización (72ºC, 6 minutos): Al disolver los cristales de glucosa, mantiene la miel en estado fluido durante más tiempo, además de eliminar las levaduras presentes que producen la fermentación.

5. Evaluación de la calidad y autenticidad de la miel

Los consumidores seleccionan la miel principalmente por sus propiedades sensoriales, siendo el sabor y el gusto los atributos más significativos (Castro-Vázquez *et al.*, 2008). Estas características organolépticas dependen sobre todo del tipo de flora donde las abejas recolectan el néctar y los mielatos, junto con otros factores como las condiciones climáticas y el origen geográfico. Esta variabilidad contribuye a la existencia de diferentes tipos de mieles (monoflorales o milflores), con una gran diversidad de características sensoriales (Días *et al.*, 2008).

Las mieles más demandadas son las monoflorales (que provienen predominantemente de una única fuente botánica), por lo que alcanzan precios más elevados en el mercado. Esto hace que a menudo se produzcan fraudes debido a un etiquetado incorrecto o a adulteraciones, por lo que, con el fin de prevenir dichos fraudes, es fundamental la aplicación de metodologías analíticas para la verificación del origen tanto botánico como geográfico (aunque este último es más difícil de evaluar), y para garantizar la calidad de la miel (Arvanitoyannis *et al.*, 2005; Aliferis *et al.*, 2010).

El método tradicional y de referencia hoy en día para la evaluación del origen botánico de la miel es el análisis polínico (melisopalinología), pero tiene el inconveniente de que algunas plantas producen una mayor o menor cantidad de polen en relación con la producción de néctar (Louveaux *et al.*, 1978) y, además, las flores femeninas no poseen polen. Por ello, esta técnica por sí sola no es suficiente para la autentificación de la miel, debiendo ser completada con la determinación de los parámetros fisicoquímicos y de las propiedades organolépticas del producto.

Tanto el análisis melisopalinológico como el sensorial son análisis muy complejos y laboriosos. Además, el análisis melisopalinológico requiere de un experto palinólogo para una

correcta interpretación de los resultados (Anklam, 1998), mientras que el análisis sensorial es un análisis que requiere de un grupo de personas entrenadas en las características organolépticas del producto (panel), pudiendo ser influenciadas por diferentes factores (Ampuero *et al.*, 2004).

Por estas razones, hoy en día la investigación se centra en la búsqueda de métodos alternativos que permitan la identificación de marcadores químicos específicos de las mieles monoflorales o en la combinación de métodos estadísticos junto con el análisis del perfil de una serie de parámetros ("fingerprint") que caractericen los diferentes tipos de mieles monoflorales (Ampuero *et al.*, 2004).

Por otro lado, la combinación de estos tres tipos de análisis es fundamental para evaluar la calidad de la miel: el análisis melisopalinológico no sólo nos proporciona el origen de la miel, sino que nos puede dar información muy importante acerca de la limpieza e impurezas que pueda poseer, la existencia de fermentación, y adulteraciones producidas por la adición de sustancias ajenas a la miel (Louveaux *et al.*, 1978); los análisis fisicoquímicos nos permiten evaluar si los parámetros cumplen con la legislación vigente, importantes para evitar fraudes y adulteraciones, y la ausencia de residuos de antibióticos y plaguicidas; mientras que mediante un análisis sensorial podemos detectar tanto defectos visuales (presencia de impurezas, espumas) y táctiles (consistencia desagradable) que afecten a la calidad de la miel, como defectos olfato-gustativos que nos den una idea de posibles adulteraciones o degradaciones del producto.

5.1. Análisis melisopalinológico

La melisopalinología es la ciencia que estudia la identificación de los granos de polen y los elementos de mielada presentes en la miel mediante un examen microscópico de su sedimento, ya que todas las mieles naturales tienen en su composición los elementos procedentes del néctar o de la mielada que la originaron (Louveaux *et al.*, 1978; Von der Ohe *et al.*, 2004).

La miel cuyo origen es el néctar, contiene los granos de polen (P) de las plantas visitadas por las abejas (Figura 7). Generalmente, para poder definir una miel como monofloral, el contenido de polen de la especie vegetal dominante debe ser al menos del 45% (polen predominante). Se establecen excepciones para especies en las que el polen puede estar infra o suprarrepresentado. El polen se definirá como secundario cuando su contenido se encuentre entre 16-45%.

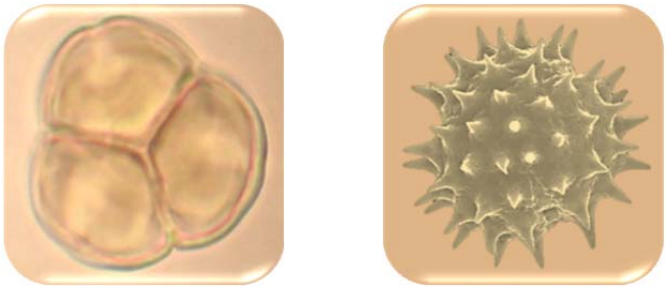

Figura 7. Pólenes vistos al microscopio: (a) polen de brezo (*Erica* sp.) y (b) polen de girasol (*Helianthus annuus*).

En el caso de las mieles cuyo origen es la mielada (Figura 8), en el sedimento existen elementos indicadores de mielada (HDE) como algas microscópicas, esporas, hifas o restos de micelios fúngicos, y polen tanto de las plantas que lo segregan como de otras especies anemófilas. En general, se requiere una relación HDE/P mayor de 3 para establecer que una miel es de mielada (Louveaux *et al.*, 1978; Von der Ohe *et al.*, 2004).

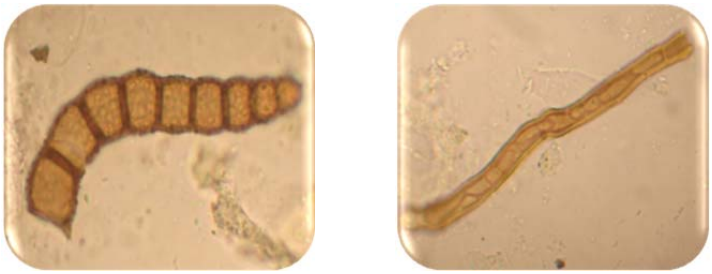

Figura 8. Elementos de mielada vistos al microscopio.

5.2. Análisis físico-químicos

La composición química de la miel depende en gran medida de su origen botánico y geográfico.

Los métodos oficiales de los análisis fisicoquímicos más importantes que se realizan en la miel son recogidos por la Legislación Española (BOE, 1986) y la "Association of Official Analytical Chemists" (AOAC, 2012), y han sido validados y normalizados por la Comisión Internacional de la Miel (Bogdanov, 2009). Algunos de estos parámetros, como el contenido en hidroximetilfurfural, agua, y residuos de antibióticos y pesticidas, sólo proporcionan información sobre la calidad de la miel, pero existen métodos basados en el análisis de componentes específicos que si pueden dar información sobre su origen. La combinación de métodos fisicoquímicos puede ser importante para autentificar una miel, especialmente si se aplican las nuevas técnicas de evaluación estadística.

Los parámetros más importantes a analizar en la miel son:

- Análisis fisicoquímicos legislados (CAC, 2001; DOCE, 2002; BOE, 2003):
  - Contenido en hidroximetilfurfural (HMF): Método espectrofotométrico o cromatográfico (HPLC). Máximo legal general: 40 mg/kg. Importancia: Indicador de frescura y detección de fraudes.
  - Índice de diastasas: Análisis colorimétrico. Mínimo legal general: 8 unidades Schade. Importancia: Indicador de frescura y detección de fraudes.
  - Contenido en agua: Refractometría o desecación a vacío. Máximo legal general: 20%. Importancia: Indicador de madurez, influencia en la conservación y en las propiedades físicas.
  - Conductividad eléctrica: Conductivimetría. Por regla general, las mieles con menos de 0,8 mS/cm van a ser mieles de flores (existen excepciones como la miel de brezo), y las mieles con más de 0,8 mS/cm, mieles de mielada y/o castaño. Importancia: Indicador del origen botánico y de posibles fraudes.
  - Sólidos insolubles: Gravimetría. Máximo legal general: 0,1 g/100 g. Importancia: Indicador de limpieza.
  - Azúcares (glucosa, fructosa, sacarosa). Métodos enzimáticos y cromatográficos.

    Los límites legales son:

    - Fructosa + glucosa: Miel de néctar mínimo 60 g/100 g; Miel de mielada o mezcla de mielada/néctar mínimo 45 g/100 g.
    - Sacarosa: En general, máximo 5 g/100 g.

    Importancia: Indicadores de madurez y de posibles fraudes.
  - Acidez libre: Potenciometría. Máximo legal 50 meq/kg. Importancia: Indicador de deterioro por fermentación.
- Parámetros no legislados que pueden servir para la autentificación o el control de calidad de las mieles:
  - Color.
  - Actividad de agua.
  - Rotación específica.
  - pH, acidez lactónica e índice de formol (potenciometría).
  - Perfil de azúcares (cromatografía).
  - Ácidos orgánicos (test enzimáticos, cromatografía, electroforesis capilar).
  - Aminoácidos (espectrofotometría, cromatografía).
  - Vitaminas (cromatografía).

- Sales minerales (espectrofotometría de absorción atómica, cromatografía, electroforesis capilar, plasma con acoplamiento inductivo).
- Compuestos volátiles (cromatografía).
- Actividad antibacteriana.
- Compuestos fenólicos y flavonoides (colorimetría, cromatografía, electroforesis).
- Actividad antioxidante mediante distintos métodos como ORAC (Capacidad de absorción de radicales de oxígeno), DPPH (Depleción del 2,2-difenil-1-picrilhydrazil), FRAP (Poder antioxidante reductor del hierro), TEAC o ABTS (Capacidad antioxidante equivalente de trolox o depleción del 2,2'-Azinobis-3-etil-benzotiazolina-6-ácido sulfónico), y actividad secuestrante de distintos radicales (hidroxilo, peroxilo, peroxinitrito, superóxido), entre otros.
- Nariz electrónica y lengua electrónica (potenciometría, voltametría).
- Residuos (BOE, 1998; DOUE, 2014): Métodos cromatográficos.

5.3. Análisis sensorial

El análisis sensorial en el caso de la miel se emplea fundamentalmente en tres aspectos: en primer lugar, la presentación del producto, donde, como se ha comentado anteriormente, es importante tanto el control de la calidad (limpieza, estado físico, ausencia de defectos), como el control del origen botánico y geográfico de dicha miel. En segundo lugar, la promoción del producto mediante la realización de degustaciones guiadas en ferias y mercados; y en tercer lugar, la valorización del producto, llevando a cabo diversos concursos (internacionales, nacionales, regionales, locales), y la obtención de distintas marcas de calidad, tales como las "Denominaciones de Origen Protegidas" (D.O.P.) y las "Indicaciones Geográficas Protegidas" (I.G.P.). El análisis sensorial se puede realizar con consumidores no adiestrados (pruebas de preferencia o aceptación mediante análisis hedónico-afectivos para establecer si el producto gusta o no), o sobre un grupo de catadores adiestrados o panel, en cuyo caso el análisis es útil para comparar muestras o para describir las propiedades organolépticas del producto (Giomo, 2007; Piana, 2007).

Para la técnica de evaluación sensorial, existen normas UNE y UNE-ISO de referencia, que marcan la metodología a seguir para la realización de las distintas pruebas sensoriales, para la selección y entrenamiento del panel y para el personal de los laboratorios de análisis sensorial.

Existen diversos factores que influyen en el análisis sensorial: factores genéticos (umbral de percepción, saturación), fisiológicos temporales (fatiga mental, física, saciedad o ayuno, el consumo de ciertos alimentos, el uso de perfumes y el embarazo, entre otros), psicológicos (como por ejemplo el orden de presentación, influencia del compañero, autoestima o falta de motivación), patológicos (enfermedades del aparato respiratorio como gripe, catarro o

enfermedades del aparato bucal), además de factores tales como el adiestramiento, la capacidad de expresión o la satisfacción personal (Giomo, 2007).

5.3.1. Análisis sensorial descriptivo

Es el análisis sensorial más importante y que aporta más información. El éxito de este tipo de caracterización organoléptica va a depender de la elección y entrenamiento de los jueces constituyentes del panel de cata (González y De Lorenzo, 2002a). Las fases de la percepción sensorial en el análisis descriptivo de la miel son (Persano-Oddo *et al.*, 1996; Piana *et al.*, 2004; Piana, 2007):

- Fase visual: Color y estado físico (es función del origen botánico y del envejecimiento del producto, influyendo en el color tanto el estado físico como el tipo de cristalización), limpieza, homogeneidad, deterioro, defectos (tanto en la elaboración como en la conservación).
- Fase olfativa por vía directa o nasal: Intensidad, persistencia, descripción del olor, ausencia de defectos.
- Fase olfato-gustativa: Sabor o gusto (con los sabores básicos dulce, ácido, salado y amargo), intensidad, persistencia, descripción del aroma por vía olfativa indirecta o retronasal, retrogusto y ausencia de defectos.
- Tanto en la fase olfativa directa (olor) como en la indirecta (aroma), se utilizan unos descriptores olfato-gustativos y un vocabulario específico desarrollado para la miel (Bentabol-Manzanares, 2002; Galán-Soldevilla *et al.*, 2005), basados en la "Rueda del olor y del aroma para la miel" (Figura 9), creada por Bruneau *et al.* (2000) y modificada por la Comisión Internacional de la Miel (Piana *et al.*, 2004).
- Otras percepciones bucales: Sensación táctil (textura de la miel como fluidez, viscosidad, granulación, consistencia y característica de los cristales formados), sensación química (como astringencia, picor o frescor) y sensación térmica.

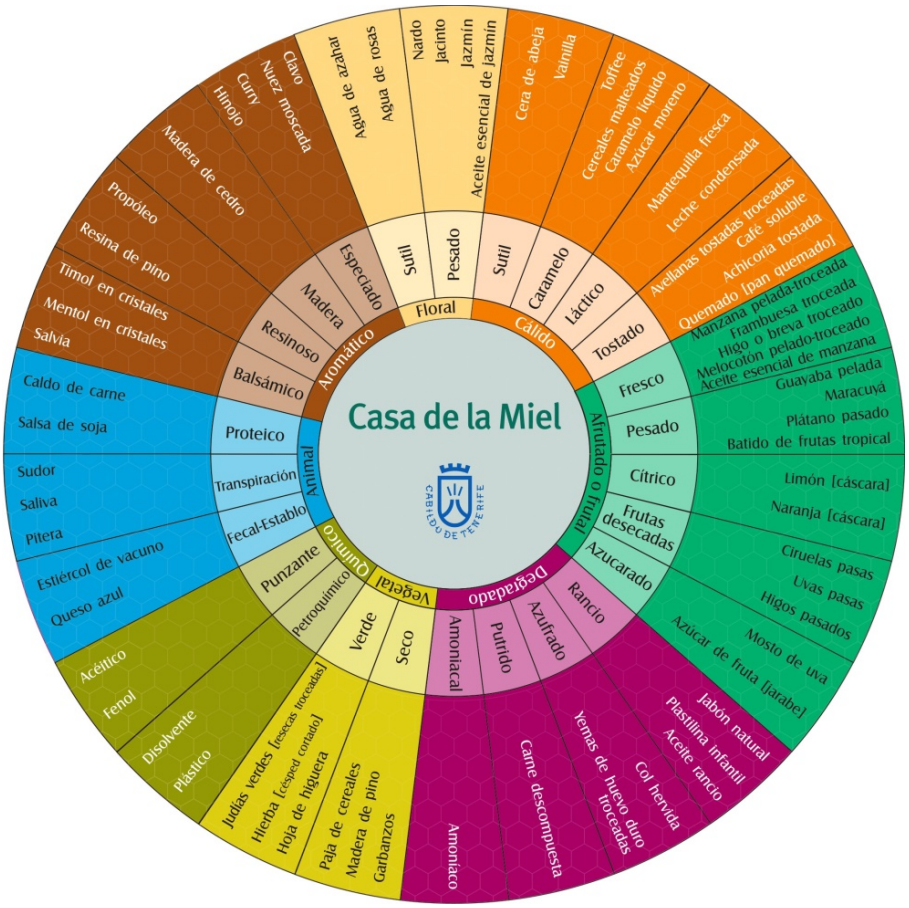

Figura 9. Rueda del olor y del aroma para la miel adaptada por la Casa de la Miel de Tenerife (Bentabol-Manzanares, 2002).

5.3.2. Defectos de la miel

Los defectos de la miel perceptibles a través de los órganos de los sentidos, son los siguientes (Sainz-Laín y Gómez-Ferreras, 2000; Colombo, 2007):

- Defectos en la elaboración
  - Impurezas: Debido a una mala filtración.
  - Espumas: Debido a una decantación insuficiente. No modifican la calidad de la miel, pero hacen que disminuya su valoración por parte del consumidor.
  - Tratamiento térmico excesivo o prolongado: Es un defecto grave, ya que aumentan las reacciones bioquímicas que acompañan al envejecimiento del producto (tonalidades más oscuras y alteración de los olores y sabores).
- Defectos en la conservación
  - Defectos de cristalización:
    - Gruesa no homogénea o lenta: Son mieles con baja tendencia a cristalizar (bajo contenido en glucosa, alto contenido en agua, y temperatura de conservación distinta de 14ºC). Se forman cristales de grandes dimensiones y angulosos, desagradables al paladar.

- Incompleta: Miel anterior que además ha sufrido un calentamiento excesivo o prolongado.
- Homogénea compacta o en reposo: Mieles con bajo contenido en agua en las que se forma una estructura cristalina muy ordenada y compacta. Son mieles duras y difíciles de manejar.
- Escarchado y jaspeado: Se produce en mieles como las anteriores. Consiste en la aparición de forma superficial (escarchado) o en el interior del tarro (jaspeado) de manchas blanquecinas, debido a la presencia de burbujas de aire en la miel que ascienden y entran en contacto con los cristales de glucosa, deshidratándolos. Defecto visual leve.
- Separación de fases: En mieles que poseen una estructura cristalina frágil (mieles cremosas), y con una humedad excesiva, la estructura cristalina cede, quedando en la parte superior una capa de miel líquida y en la inferior miel cristalizada. Es el defecto más grave de la cristalización. Indica que es una miel vieja con tendencia a fermentar.
- Fermentación: Es un defecto grave e irreversible que excluye al producto del consumo directo. Se produce debido a la presencia de levaduras osmófilas, en mieles con una humedad superior al 18% y/o conservadas a temperaturas mayores de 30ºC. Se desarrollan ácidos, gases y compuestos aromáticos nuevos que otorgan a las mieles fermentadas un olor alcohólico. Para prevenir la fermentación, se recomienda partir de una miel madura, un consumo rápido, una conservación en frio y/o la inactivación de las levaduras causantes de dicha fermentación (tratamiento térmico de pasteurización).

- Envejecimiento: Proceso irreversible que se produce debido a una mala conservación (temperaturas elevadas, luz), a tratamientos térmicos excesivos o al paso de tiempo. Se caracteriza por un oscurecimiento de la miel, la desaparición de los aromas típicos del producto y aparición de nuevos (acaramelados), desvanecimiento de la estructura cristalina y fermentación.

6. Las mieles españolas

En España existen actualmente distintas marcas de calidad para alimentos, entre las que destacan las D.O.P. y las I.G.P. Entre otras, están reconocidas con una calidad diferenciada mediante D.O.P. las mieles españolas de Campoo-Los Valles, Granada, La Alcarria, Liébana, Tenerife y Villuercas-Ibores, y con una I.G.P. las mieles de Galicia. Cada marca de calidad posee su legislación, que indica los tipos de mieles que se pueden producir, las características que tienen que cumplir y la zona geográfica de producción.

A continuación se presentan las principales mieles monoflorales españolas, sus principales territorios de obtención y sus características organolépticas más importantes (Persano-Oddo *et al.*, 1996; González y De Lorenzo, 2002b; Gómez-Pajuelo, 2004; Persano-Oddo y Piro, 2004; Colombo *et al.*, 2007):

- Acacia (*Robinia pseudoacacia*): Cantábrico. Color incoloro-amarillo muy claro, olor floral débil, aroma avainillado, delicado, sabor muy dulce.
- Azahar (*Citrus* sp.): Costa mediterránea. Color incoloro-amarillo claro, olor y aroma floral de media intensidad.
- Brezo (Ericaceae): Ampliamente distribuido por toda la península e Islas Canarias. Color ámbar oscuro con reflejos anaranjados cuando la miel es líquida, color marrón cuando está cristalizada. Olor y aroma de intensidad media-alta floral, acaramelado, a almendras amargas. Sabor a veces ligeramente amargo.
- Castaño (*Castanea sativa*): Norte de la península e Islas Canarias. Color ámbar medio-oscuro con tonalidades rojizas/verdosas. Olor y aroma intenso, pungente, animal, a madera seca, a jabón. Sabor más o menos amargo, a veces con notas saladas.
- Espliego y Lavanda (*Lavandula* sp.): Franja central de la península e Islas Baleares. Color anaranjado con tonalidades amarillentas las mieles de espliego, y amarillo claro las mieles de lavanda. Olor y aroma intenso a plantas aromáticas, a paja húmeda, a flores de camomila y a lavanda.
- Eucalipto (*Eucalyptus* sp.): Costa cantábrica y sur de la península. Color ámbar más o menos claro. Olor y aroma intenso, animal, acaramelado, ahumado, a regaliz, a champiñones secos. Sabor ligeramente ácido y salado.
- Girasol (*Helianthus annuus*): Franja central de la península. Color ámbar claro con reflejos amarillos cuando la miel está líquida, color amarillo-dorado en mieles cristalizadas. Olor y aroma de media intensidad, vegetal, a paja húmeda, tomate verde, oleoso.
- Mielada (Principalmente *Quercus* sp.): Franja central de la península e Islas Canarias. Color ámbar oscuro-muy oscuro. Olor y aroma intenso malteado, tostado, a madera, frutas desecadas. Sabor con ligeras notas saladas.
- Romero (*Rosmarinus officinalis*): Franja central de la península e Islas Baleares. Color amarillo más o menos claro en miel líquida y blanco-amarillento muy claro en miel cristalizada. Olor y aroma poco intenso, ligeramente aromático, floral.
- Tomillo (*Thymus* sp.): Franja central de la península. En miel líquida, color ámbar claro con tonalidades rojizas, y en miel cristalizada, color marrón claro. Olor intenso, fenólico, animal valeriánico. El aroma es algo más floral que el olor. Ligeramente picante.

En Tenerife, además de las mieles de brezo, castaño y mielada, debido a la exclusiva flora natural y cultivada de la isla, con gran número de endemismos y una dilatada temporada de floraciones que hace que prácticamente todo el año puedan estar trabajando las abejas, se recolectan gran variedad de mieles distintas y muchas de ellas únicas en el mundo (Bentabol-Manzanares, 2013). Las más importantes, junto con las de mielada, son las mieles de brezo (*Erica arborea*), castaño (*Castanea sativa),* aguacate (*Persea americana)*, barrilla (*Mesembryanthemum crystallinum*), hinojo (*Foeniculum vulgare*), malpica (*Carlina xeranthemoides*), pitera (*Agave americana*), poleo (*Bystropogon origanifolius*), relinchón (*Hirschfeldia incana*), retama del Teide (*Spartocytisus supranubius*), tajinaste rojo (*Echium wildpretii*) y tedera (*Aspalthium bituminosum*).

En cuanto a las mieles milflores, éstas contienen infinitos matices que las hacen únicas. Ninguna va a ser igual a otra.

¿Qué miel monofloral es mejor? ¿Son mejores las monoflorales que las milflores?
¡¡¡Para gustos están los colores!!!

REFERENCIAS BIBLIOGRÁFICAS

ALIFERIS, K A; TARANTILIS, P A; HARIZANIS, P C; ALISSANDRAKIS, E (2010) Botanical discrimination and classification of honey samples applying gas chromatography/mass spectrometry fingerprinting of headspace volatile compounds. *Food Chemistry* 121(3): 856-862. http://dx.doi.org/10.1016/j.foodchem.2009.12.098

AMPUERO, S; BOGDANOV, S; BOSSET, J O (2004) Classification of unifloral honeys with an MS-based electronic nose using different sampling modes: SHS, SPME and INDEX. *European Food Research and Technology* 218(2): 198-207. http://doi.org/10.1007/s00217-003-0834-9

ANKLAM, E (1998) A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry* 63(4): 549-562. http://doi.org/10.1016/S0308-8146(98)00057-0

ARVANITOYANNIS, I S; CHALHOUB, C; GOTSIOU, P; LYDAKIS-SIMANTIRIS, N; KEFALAS, P (2005) Novel quality control methods in conjunction with chemometrics (multivariate analysis) for detecting honey authenticity. *Critical Reviews in Food Science and Nutrition* 45(3): 193-203. http://doi.org/10.1080/10408690590956369

AOAC-ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (2012) Official Methods of Analysis of AOAC International. *Latimer J W (Ed).* Gaithersburg, Maryland, USA.

BENTABOL-MANZANARES, A (2002) Aportaciones metodológicas al análisis sensorial descriptivo de las mieles. Descripción de olores y aromas. *Alimentaria* 339: 49-52.

BENTABOL-MANZANARES, A (2013) *Las Mieles de Tenerife*; Ed. Apitén, Tenerife, España.

BOE-BOLETIN OFICIAL DEL ESTADO (1986) Orden de 12 de junio de 1986 por la que se aprueban los métodos oficiales de análisis para la miel (B.O.E nº 145 de 18 de junio de 1986). https://www.boe.es/boe/dias/1986/06/18/pdfs/A22195-22202.pdf

BOE-BOLETIN OFICIAL DEL ESTADO (1998) Real Decreto 1749/1998 de 31 de julio, por el que se establecen las medidas de control aplicables a determinadas sustancias y sus residuos en los animales vivos y sus productos (BOE nº 188 de 7 de agosto de 1998). http://www.boe.es/boe/dias/1998/08/07/pdfs/A26910-26927.pdf

BOE-BOLETIN OFICIAL DEL ESTADO (2003) Real Decreto 1049/2003 de 1 de agosto, por el que se aprueba la Norma de calidad relativa a la miel (B.O.E. nº 186 de 5 de agosto de 2003). https://www.boe.es/boe/dias/2003/08/05/pdfs/A30181-30183.pdf

BOGDANOV, S (2009) Harmonised methods of the European Honey Commission. http://www.ihc-platform.net/ihcmethods2009.pdf (Visitado 12/04/2015)

BRUNEAU, E; BARBIER, E; GALLEZ, L M; GUYOT-DECLERCK, C (2000) La roue des arômes des miels, *Abeilles & Cie.*77: 16-23.

CAC-CODEX ALIMENTARIUS COMMISION (2001) Revised Codex standard for honey. *Codex Stan* 12-1981, Rev. 1 (1987), Rev. 2 (2001). FAO. Roma. http://ftp.fao.org/codex/standard/en/CXS_012e.pdf (Visitado 08/08/2015)

CASTRO-VÁZQUEZ, L; DÍAZ-MAROTO, M C; GONZÁLEZ-VIÑAS, M A; DE LA FUENTE, E; PÉREZ-COELLO, M S (2008) Influence of storage conditions on chemical composition and sensory properties of citrus honey. *Journal of Agricultural and Food Chemistry* 56: 1999-2006. http://doi.org/10.1021/jf072227k

COLOMBO, R. (2007) I defetti del miele. En: Sabatini, A G; Bortolotti, L; Marcazzan, G L (2007) *Conoscere il miele*. Ed. Avenue media, Bolonia, Italia. pp. 257-267.

COLOMBO, R; MARCAZZAN, G L; SABATINI, A G; ACCORTI, M; PERSANO-ODDO, L; PIANA, M L; PIAZZA, M G; PULCINI, P (2007) Schede dei mieli uniflorali. En: Sabatini, A G; Bortolotti, L; Marcazzan, G L (2007) *Conoscere il miele*. Ed. Avenue media, Bolonia, Italia. pp. 271-320.

DÍAS, L A; PERES, A M; VILAS-BOAS, M; ROCHA, M A; ESTEVINHO, L; MACHADO, A A S C (2008) An electronic tongue for honey classification. *Microchimica Acta* 163(1-2): 97-102. http://doi.org/10.1007/s00604-007-0923-8

DOCE-DIARIO OFICIAL DE LAS COMUNIDADES EUROPEAS (2002) Directiva 2001/110/EC del Consejo de 20 de diciembre de 2001 relativa a la miel, Bruselas, Bélgica. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:010:0047:0052:ES:PDF

DOUE-DIARIO OFICIAL DE LA UNIÓN EUROPEA (2014) Reglamento (UE) 652/2014 del Parlamento Europeo y del Consejo, de 15 de mayo. Establece disposiciones para la gestión de los gastos relativos a la cadena alimentaria, la salud animal y el bienestar de los animales, y relativos a la fitosanidad y a los materiales de reproducción vegetal. https://www.boe.es/doue/2014/189/L00001-00032.pdf

GALÁN-SOLDEVILLA, H; RUIZ-PÉREZ-CACHO, M P; SERRANO-JIMÉNEZ, S; JODRAL-VILLAREJO, M; BENTABOL-MANZANARES, A (2005) Development of a preliminary sensory lexicon for floral honey. *Food quality and Preference* 16(1): 71-77. http://doi.org/10.1016/j.foodqual.2004.02.001

GARCÍA-FERNÁNDEZ, JESÚS (1986) *El clima en Castilla y León*. Ed. Ámbito, Valladolid, España.

GIOMO, A (2007) L'analisi sensoriale. En: Sabatini, A G; Bortolotti, L; Marcazzan, G L (2007) *Conoscere il miele*. Ed. Avenue media, Bolonia, Italia. pp.175-221.

GÓMEZ-PAJUELO, A (2004) *Mieles de España y Portugal: Conocimiento y Cata*. Ed. Montagud Editores S.A., Barcelona, España.

GONZÁLEZ, M M; DE LORENZO, C (2002a) El análisis sensorial. En: De Lorenzo C. (2002) *La miel de Madrid*. Ed. Instituto Madrileño de Investigación Agraria y Alimentaria de la Comunidad de Madrid, Consejería de Economía e Innovación Tecnológica, Madrid, España. pp.137-159. http://www.madrid.org/bvirtual/BVCM005574.pdf (Visitado 12/04/2014)

GONZÁLEZ, M M; DE LORENZO, C (2002b) Las mieles de Madrid. En: De Lorenzo C. (2002) *La miel de Madrid.* Ed. Instituto Madrileño de Investigación Agraria y Alimentaria de la Comunidad de Madrid, Consejería de Economía e Innovación Tecnológica, Madrid, España. pp. 161-173. http://www.madrid.org/bvirtual/BVCM005574.pdf (Visitado 12/04/2014)

GUERRA-VELASCO, J C (2010) *Las Guías del Duero-Flora y fauna de Castilla y León*. Ed. Edical, Valladolid, España.

INE-INSTITUTO NACIONAL DE ESTADÍSTICA (2014) http://www.ine.es/jaxi/menu.do?type=pcaxis&path=/t20/p321/serie&file=pcaxis (Visitado 12/04/2015)

LOUVEAUX, J; MAURIZIO, A; VORWOHL, G (1978) Methods of Melissopalynology. *Bee World* 59: 139-157. http://doi.org/10.1080/0005772X.1978.11097714

PERSANO-ODDO, L; PIAZZA, M G; SABATINI, A G; ACCORTI, M (1996) Characterization of unifloral honeys. *Apidologie* 26(6): 453-465. http://doi.org/10.1051/apido:19950602

PERSANO-ODDO, L; PIRO, R (2004) Main European unifloral honeys: descriptive sheets. *Apidologie* 35: S38-S81. http://doi.org/10.1051/apido:2004049

PIANA, L (2007) L'analisi sensoriale del miele. En: Sabatini, A G; Bortolotti, L; Marcazzan, G L (2007) *Conoscere il miele*. Ed. Avenue media, Bolonia, Italia. pp. 223-256.

PIANA, L; PERSANO-ODDO, L; BENTABOL, A; BRUNEAU, E; BOGDANOV, S; GUYOT, C (2004) Sensory analysis applied to honey: state of the art. *Apidologie* 35: S26-S37. http://dx.doi.org/10.1051/apido:2004048

SABATINI, A G (2007) Il miele: origine, composizione e proprietà. En: Sabatini, A G; Bortolotti, L; Marcazzan, G L (2007) *Conoscere il miele*. Ed. Avenue media, Bolonia, Italia. pp.3-37.

SAINZ-LAÍN, C; GÓMEZ-FERRERAS, C (2000) *Mieles españolas: características e identificación mediante el análisis del pole*n. Ed. Mundi-Prensa, Madrid, España.

SUBDIRECCIÓN GENERAL DE PRODUCTOS GANADEROS (2015) El sector de la miel en cifras. Principales indicadores económicos en 2014. http://www.magrama.gob.es/es/ganaderia/temas/produccion-y-mercados-ganaderos/indicadoreseconomicossectordelamiel2014_tcm7-381460.pdf (Visitado 12/09/2015)

VANGELISTI, M (2007) Miele di qualità: le tecniche di produzione e di lavorazione. En: Sabatini, A G; Bortolotti, L; Marcazzan, G L (2007) *Conoscere il miele*. Ed. Avenue media, Bolonia, Italia. pp.69-123.

VIT, P; PEDRO, A R M; ROUBIC, D (2013) *Pot honey: a legacy of stingless bees*. Ed. Springer, Nueva York, Estados Unidos.

VON DER OHE, W; PERSANO-ODDO, L; PIANA, M L; MORLOT, M; MARTIN, P (2004) Harmonized methods of melissopalynology. *Apidologie* 35: S18-S25. http://dx.doi.org/10.1051/apido:2004050

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CHAPTER 2

PHYSICOCHEMICAL COMPOSITION, NUTRITIONAL AND BIOLOGICAL PROPERTIES OF HONEY

PHYSICOCHEMICAL COMPOSITION, NUTRITIONAL AND BIOLOGICAL PROPERTIES OF HONEY

ABSTRACT

Honey has been used as a food with potential functional properties for thousands of years. Their physicochemical and biological properties vary according to the botanical source, geographical origin and climatic conditions, but additionally, harvest and extraction techniques, as well as the processing and storage conditions, can influence the honey characteristics. Firstly, it is described the chemical composition: water content (and water activity), sugar content and minor compounds, including substances with nutritional value, biological activity or toxic potential. The physical properties, such as colour, density, hygroscopicity, viscosity and crystallization have been also discussed. The physical characteristics of honey are particularly suitable for some culinary arts and can be decisive for the consumers' choice, so these points were mentioned. Then, the nutritional value and potentially functional properties have been widely discussed, focusing on antioxidant, antimicrobial, anti-parasite, antihypertensive and anti-inflammatory activity, as well as the probiotic and prebiotic properties of honey, and citing the most important constituents responsible for its biological potential. Finally, it is described the use of the honey as biomonitor for collecting information about the environment, identifying environmental contamination and characterizing the level of soil, water, plant and air pollution.

1. Introduction

Honey is an interesting nutritional sweetener composed mainly of carbohydrates (60-85%) and water (12-23%). It also contains low proportions of other compounds, such as organic acids, minerals, vitamins, enzymes, proteins, amino acids, Maillard reaction products, volatile compounds, and several bioactive substances (phenols and flavonoids, among others), as well as pollen grains (White, 1979a; Cano *et al.*, 2001; Gomes *et al.*, 2010; Almeida-Muradian *et al.*, 2013). Honey is usually consumed in natura, used in food systems and in human and veterinary medicine (Joseph *et al.*, 2007; Nigussi *et al.*, 2012). Honey's biological and nutritional properties are decisive for the consumers' choice, as well as for the commercialization management of this food.

2. Chemical composition

2.1. Water content and Water activity (aw)

Water content of honey is related to different factors such as the botanical and geographical origin of nectar, edaphic and climatic conditions, season of harvesting, intensity of nectar

flux, degree of maturation, manipulation by beekeepers during the harvest period, and extraction, processing and storage conditions (Estupiñán *et al.*, 1998; Sáinz-Laín and Gómez-Ferreras, 2000; González, 2002; Ojeda de Rodríguez, 2004; Sabatini, 2007; Pontara *et al.*, 2012).

Honeys from different botanical origins can have different moisture contents. Thus, heather, clover and strawberry tree honeys have higher natural water content (Piana *et al.*, 1989; Persano-Oddo *et al.*, 1995).

Moisture is a quality parameter related to honey shelf life. In general, honeys' percentage of water is appropriate when the beehive cells are totally capped with beeswax (Ortiz-Valbuena *et al.*, 1996). Normally, honey moisture ranges between 13% and 25% (Simal *et al.*, 1983), being the optimal about 17% (Doner, 1977; White, 1978; Sabatini, 2007). Honeys with very low moisture contents are difficult to handle and process (Estupiñán *et al.*, 1998). Conversely, honeys whose moisture is higher than 18% are prone to ferment, because sugars osmotic pressure is not powerful enough to avoid the osmophilic (sugar-tolerant) yeasts proliferation (Bogdanov and Martin, 2002). Depending on the initial number of yeast in honey, the water content from which honey ferments is different, so that the higher the honey moisture is, the lesser the amount of yeast is needed (White, 1975; Piana *et al.*, 1989; Estupiñán *et al.*, 1998).

Besides, some properties of honey (colour, crystallization, viscosity, flavour and density) are also affected by water content. As honey is a very hygroscopic product, it is important to avoid environmental moisture uptake during honey processing and packing (White, 1975).

For a given food, the water activity (aw) is the amount of water available to microorganisms. Sugar ties up part of the water and makes it unavailable for microorganisms' growth, thus being aw the criterion that determines bacterial spoilage, instead of the water content. Water activity is defined as the relation of the water vapour pressure of the food (p) to the vapour pressure of pure water (p0) at the same temperature. The water activity of pure water is 1, and each addition of water-fixing substances causes that $p<p0$. For this reason, the water activity is always lower than 1 (Gleiter *et al.*, 2006).

In honey, water activity ranges from 0.49 to 0.65, even though for some honeys it can reach a value of 0.75 (Cavia *et al.*, 2004; Costa *et al.*, 2013). The water activity needed for microorganisms' development is about 0.90 for bacteria, 0.80 for yeast and 0.70 for mould. Aw values below 0.60 are proper enough to inhibit the growth of osmophilic yeasts that cause honey fermentation (Sanz *et al.*, 1995; Gleiter *et al.*, 2006; Bogdanov, 2011b). Nevertheless, the aw influence on microorganisms growth depends on such factors as pH, temperature, oxygen and carbon dioxide concentration, as well as the presence of inhibitory substances (Cavia, 2002).

Water activity depends on the sugar composition (mainly glucose content and glucose/fructose ratio), honey crystallization and environmental conditions (Gleiter *et al.*,

2006). Water activity of honey is used to predict moisture exchange with the environment, since honey gains or losses moisture when is exposed to different ambient relative humidity values (Chirife *et al.*, 2006; Roudaut and Debeaufort, 2011). Moreover, for a given honey, the water activity of the crystallised state is higher than for the liquid state (Martin, 1958; Gleiter *et al.*, 2006). This is due to the fact that during the crystallization process, there is a release of the water linked to glucose. Gleiter *et al.* (2006) showed that in liquid state, blossom honeys had lower water activities than honeydew honeys having the same water content. However, they did not find significant difference between the water activities of different types of honeys in crystallised state.

Some researchers found significant correlations between honeys' moisture and honeys' water activity (Cavia *et al.*, 2004; Chirife *et al.*, 2006; Abramovic *et al.*, 2008; Pérez *et al.*, 2009). Actually, water activity should be considered a better honey quality control criterion than moisture, because it indicates the free water content that eventually is used by microorganisms to cause fermentation (Bogdanov, 2011b).

2.2. Sugar content

Honey is a supersaturated sugar solution, where carbohydrates are the main constituents accounting for about 95% dry matter (Bogdanov *et al.*, 2008). The most important physicochemical and nutritional properties of honey, such as sweetness, viscosity, granulation, hygroscopicity, specific rotation and energy value depend on sugars' composition (Estupiñán *et al.*, 1998; Cavia *et al.*, 2002; Sabatini, 2007). Moreover, the osmotic pressure produced by high sugars concentration is an important honeys' antimicrobial factor (Jeddar *et al.*, 1985). As food, honey has been used for centuries as a sweetener and human energy source.

Monosaccharides hexoses fructose (32-44%) and glucose (23-38%) are the main honey sugars. Very small amounts of other monosaccharides, such as galactose, were also identified in honeys (Val *et al.*, 1998). In almost all honey types, fructose is the main sugar, but there are exceptions such as rape (*Brassica napus*), dandelion (*Taraxacum officinale*) and blue curls (*Trichostema lanceolatumi*) honeys, where glucose is present in higher amounts (White, 1979a). They are produced by honeybees during the ripening process, by the transformation of nectar sucrose through the enzyme invertase from the bee's salivary glands. Furthermore, invertase has transglucosilation activity, producing more complex sugars from monosaccharides (White and Maher, 1953). Therefore, in honey the main disaccharides are α-glucosyl derivatives of monosaccharides, being likely trisaccharides and tetrasaccharides α-glucosyl derivatives of the main disaccharides and trisaccharides, respectively (Ruiz-Matute *et al.*, 2010). Other di- and trisaccharides in honey could be formed by microbial activity and enzymatic reactions in the intestinal tract of the plant-sucking insects (Hemiptera, mostly aphids) that excrete honeydew (Kolayli *et al.*, 2012).

More than 45 di-, tri- and other oligo- and polysaccharides have been detected in honey in small amounts (5-15%), like maltose, sucrose, turanose, trehalose, gentiobiose, isomaltose, lactose, kojibiose, raffinose, erlose, melezitose, maltotriose, panose, isomaltotriose and maltotetraose, among others (Val *et al.*, 1998; Lazaridou *et al.*, 2004; Ouchemoukh *et al.*, 2010; Ruiz-Matute *et al.*, 2010). Maltose (7%) and sucrose (1%) are the most important honey disaccharides (Shin and Ustunol, 2005). The presence of high amounts of sucrose in honey could be due to the fact that not all the sucrose from nectar or honeydew is hydrolysed by invertase enzyme (Sabatini, 2007). High sucrose values in honeys are related to its botanical origin, honey immaturity, high nectar flux or artificial feeding of bees (Ortiz-Valbuena *et al.*, 1996; Estupiñán *et al.*, 1998). For example, lavender (*Lavandula* sp.) and borage (*Borago officinalis*) honeys are allowed to contain higher sucrose amounts (OJEC, 2002). Melezitose, the main trisaccharide, is synthesized by the plant sucking insect that produces honeydew, through the enzymatic addition from their intestine and salivary glands. Other sugars such as erlose come from enzymatic reactions by the secretions of the bees' hypopharyngeal glands (Sabatini, 2007). Several sugars, such as galactose, lactose and raffinose were described as toxic to honey bees because of their lack of proper enzymes for its digestion (Herbert, 1992). The amount and type of carbohydrates vary among samples from different vegetal sources, being useful for the classification of unifloral honeys. For example, the concentration range of some carbohydrates were used to distinguish between blossom and honeydew honeys, because the latter contain lower levels of monosaccharides and higher values of trissacharides (mainly melezitose, erlose, raffinose and maltotriose), as well as other higher oligosaccharides (Bogdanov *et al.*, 2004; Diez *et al.*, 2004; Sanz *et al.*, 2004; Ouchemoukh *et al.*, 2010; Kolayli *et al.*, 2012). Heather honeys were characterized by the presence of erlose and nigerose, lavender honeys by the presence of sucrose and maltose, and forest honeys by the presence of trehalose and melezitose. Further, avocado honeys were characterized by the presence of perseitol, a sugar alcohol (Dvash *et al.*, 2002; Cotte *et al.*, 2004; Nozal *et al.*, 2005; Kaškonienė and Venskutonis, 2010).

Apart from the sugars' quantities, ratios between some of these compounds were proposed as suitable indicators to ascertain honey authenticity (Nozal *et al.*, 2005). For example, blossom honeys show a fructose-glucose ratio about 1.0, while in honeydew honeys the ratio ranges between 1.5 and 2.0 (Gleiter *et al.*, 2006). On the other hand, maltose/isomaltose ratio was particularly high in sunflower honeys, being conversely low in linden and honeydew honeys (Horváth and Molnár-Perl, 1997).

Honey ripeness and storage conditions modify honey sugars' composition (Sáinz-Laín and Gómez-Ferreras, 2000). During storage, the content of monosaccharides decreases, and the content of oligosaccharides increases (White *et al.*, 1961; Piana *et al.*, 1989; Sanz *et al.*, 2002) because of enzymatic activity and acid reversion (White, 1979a). Sugars' degradation, the acid-catalysed dehydration of hexoses or Maillard reactions, where sugars react with

aminoacids, darken honey colour (Sanz *et al.*, 2002; De Oliveira Resende Ribeiro *et al.*, 2012).

Honey quality criteria regulations establish minimum limits for the sum fructose and glucose as well as maximum limits for sucrose (Mercosur, 1999; Brasil, 2000; OJEC, 2002; Argentina, 2008; El Salvador, 2008), because the content of these sugars is related to the ripeness of honey and can reveal a possible adulteration (Lazaridou *et al.*, 2004; Belay *et al.*, 2013). However, as mentioned earlier, it is important to consider that these parameters also can vary according to the botanical origin.

2.3. Nitrogen compounds

Normally, the nitrogen content of honey is low, being proteins, free amino acids and enzymes the most important nitrogenous compounds. About 40 to 80 percent of the total honey's nitrogen comes from the protein fraction, and most of the remainder resides in the free amino acids (White, 1979a; Doner, 2003).

2.3.1. Proteins

Protein of honey comes from both bees (salivary glands), and plants (nectar, honeydew and mainly pollen). About 20 different nonenzymatic proteins have been identified in honey, many of which are common to all honeys, where albumins, globulins and nucleoproteins are included (White, 1979a; Sáinz-Laín and Gómez-Ferreras, 2000; Doner, 2003).

The total honey protein can vary from 0.1-0.5 %, although some honeys such as ling heather (*Calluna vulgaris*) content higher protein amounts (1-2%) (Sáinz-Laín and Gómez-Ferreras, 2000; Chua *et al.*, 2013). Ling heather honeys show a thixotropic behaviour that confers a gelatinous consistency, hindering extraction and processing. From technological standpoint, the presence of proteins can be undesirable. The higher the protein level is, the lower the surface tension of honey is, which produces a tendency to foam and form scum and, consequently, results in incorporation of air bubbles, as happens in buckwheat (*Fagopyrum esculentum*) honey (Doner, 2003).

Adulterated, overheated or long-time stored honeys show a reduction or absence of protein content (Almeida-Muradian *et al.*, 2013).

2.3.2. Aminoacids

Free aminoacids are honey compounds responsible for some honey properties such as antioxidant activities. Most of amino acids in honey are in the bound form and free amino acid content may be as low as one fifth of the total (González-Paramás *et al.*, 2006). The processing conditions that accelerate the occurrence of reactions, such as Maillard, lead to a loss of these compounds (Spano *et al.*, 2008).

Around 26 amino acids have been detected in honey, such as proline, glutamic acid, alanine, phenylalanine, tyrosine, leucine, isoleucine, lysine, methionine, histidine, arginine, aspartic acid, tryptophan, serine, valine, methionine, trypsin and threonine, among others (Sáinz-Laín and Gómez-Ferreras, 2000; Hermosín *et al.*, 2003).

The origin of honey's amino acids is attributable to both animal (bee secretions) and vegetal (nectar, honeydew and mainly pollen) origins. As the main source is pollen, the amino acid profile or some characteristics amino acids could be important for the botanical classification of honey, such as arginine in chestnut honey or tryptophan in acacia honey (Pirini *et al.*, 1992; Hermosín *et al.*, 2003). Nevertheless, free amino acids are also added by bees, which lead to a high variability of amino acid content for honeys from the same botanical source (Bogdanov and Martin, 2002).

Proline is the most abundant free amino acid in honey, ranging from 50% and 85% of the total (White, 1978; Hermosín *et al.*, 2003; Belitz *et al.*, 2009). It mainly comes from honeybee salivate secretions during the conversion of nectar or honeydew into honey (Bergner and Hahn, 1972). For this reason, this amino acid could not be a good indicator of the botanical origin of honey. In spite of this, Biino (1971) reported that high values of proline were typical for honeydew honeys. Proline could be correlated with the enzymatic content, since it could play an important role as regulator of nectar enzymatic transfer, mainly in the invertase secretions during the nectar transformation in honey (Ortiz-Valbuena *et al.*, 1996; Sabatini, 2007). The desirable proline content in honeys should be higher than 200 mg/kg (Bogdanov, 2011c). Some researchers analyse proline as quality criteria for honey ripeness estimation and as indicator of sugar adulteration, especially when the values of this aminoacid are significantly lower than 180 mg/kg, the minimum value that has been agreed for genuine honey (Bogdanov *et al.*, 1999).

2.3.3. Enzymes

Natural honey contains small amounts of enzymes, being diastase, invertase and glucose-oxidase the most important. Other enzymes that have been found in honey are acid phosphatase, catalase and β-glucosidase (White, 1979a). Enzymes such as invertase or glucose oxidase are mainly produced in the hypopharyngeal glands of the bees (animal origin). Honeybees add these enzymes in order to accomplish the nectar to honey ripening process (Doner, 2003). Some enzymes come from nectar, honeydew or pollen (vegetal origin), such as catalase and acid phosphatase. And finally, enzymes such as diastase have a double origin. Other possible origins could be honey microorganisms and in the case of honeydew honey, some enzymes could come from the plant sucking insects that produce honeydew (White, 1957; Cavia, 2002).

Enzymes are thermolabile, being used as indicator of aging and/or overheating, since their activities decrease in these conditions. In general, honeys that come from fast and abundant

nectar fluxes to be processed contain fewer enzymes because bees have less time to process the nectar. Moreover, nectars with high sugar content require less manipulation to become honeys than diluted ones, containing fewer enzymes (mainly diastase and invertase) (White, 1979a; Crane, 1985). The enzyme content also depends on temperature, honey botanical origin, nectar abundance flow and transformation degree, state and strength of the colony, seasonal activity of the bee hypopharyngeal glands, and specie, diet, age and physiological stage of the bee (Maurizio, 1962; White, 1979a; Persano Oddo *et al.*, 1999; González, 2002).

- Diastase

  Diastase (amylase) is the honey enzyme that best resists heat, so it is widely used as indicator of honey freshness, being its value regulated within several legislations. Diastase hydrolyses starch and dextrins, resulting in smaller carbohydrates. Its function in honey is not well known due to the fact that nectar does not possess starch, but probably takes part in the pollen digestion by bees (White, 1978; Crane, 1985; Doner, 2003). In addition to its animal origin (the hypopharyngeal glands secretions), it has a vegetal origin (nectar or honeydew) (Persano-Oddo *et al.*, 1990). For this reason, the activity of diastase also depends on the honey botanical origin (Juan-Borrás *et al.*, 2014), so that citrus and rosemary honeys, among others, are known to have low natural enzyme contents.

- Invertase

  Invertase (α-glucosidase) is an important honey enzyme, since it converts nectar and honeydew into honey, hydrolysing sucrose into fructose and glucose. Furthermore, the transglycosilase activity of invertase produces some oligosaccharides in the intermediate steps (White and Maher, 1953). Invertase activity keeps after honey extraction and during storage. Invertase makes honey a high-energy foodstuff that takes up a minimum area in the honeycomb (White, 1978; Crane, 1985; Sánchez *et al.*, 2001).

  Fructose has been described as a sugar that inhibits invertase, unlike glucose (Rinaudo *et al.*, 1973; Rodríguez-Delgado, 2010). Its activity decreases with heat and during storage (Serra-Bonvehí *et al.*, 2000; Sánchez *et al.*, 2001). Invertase has been proposed as better indicator of honey quality than diastase because it seems to be more sensitive to thermal process (White *et al.*, 1964; Persano-Oddo *et al.*, 1999). Some nectars require less manipulation by the bees in the hive to attain that thick consistency, so invertase levels are lower (Serra-Bonvehí *et al.*, 2000).

  Invertase values can be expressed in several units, such as invertase number (Hadorn number, IN) or as invertase per kg (Siegenthaler units, US). Values higher than 10 IN (73.5 US) have been proposed for fresh and not heated honeys, and higher than 4 IN (29.38 US) for honeys with low invertase activities (Bogdanov *et al.*, 1999; Bogdanov, 2011c).

- Glucose-oxidase

  Glucose-oxidase degrades glucose to gluconolactone, which yields in turn gluconic acid, increasing honey acidity, and releasing small quantities of hydrogen peroxide, compound responsible for honey's microbial resistance. This reaction is faster in immature o diluted honey during the water loss inside the hive, being slower in dry honeys (White *et al.*, 1963). The hydrogen peroxide production protects honey from bacterial decomposition, until achieving enough sugar concentration to avoid microbial growth due to its osmotic pressure (White, 1978; Sabatini, 2007; Rodríguez-Delgado, 2010). When honey is not diluted, the gluconic acid content decreases the pH, thus inhibiting the enzymatic activity. This enzyme is sensitive to light, visible radiation (mainly from 425 and 525 nm) and thermal processes, being inactivated at 60ºC (White, 1979a; Ortiz-Valbuena *et al.*, 1996; González, 2002).

- Others enzymes

  Other important enzymes present in honey in less quantity are catalase and acid phosphatase, whose origins are mainly pollen, and also nectar and honeydew (White, 1979a).

  Catalase converts the hydrogen peroxide produced by glucose-oxidase to water and oxygen (Huidobro *et al.*, 2005). Acid phosphatase produces inorganic phosphate from organic phosphates. It could be indicator of honey fermentation (Alonso-Torre *et al.*, 2006).

  Acid phosphatase activity depends on honey pH. The higher the pH is, the greater the acid phosphatase activity is, being the optimum pH range between 4.5 and 6.5. Acid phosphatase activity also decreases during storage (Alonso-Torre *et al.*, 2006).

  β-glucosidase is an enzyme added by bee secretions which hydrolyses glycosidic toxins ingested by honeybees and transforms β-glucans to oligosaccharides and glucose (Pontoh and Low, 2002; Labropoulos and Anestis, 2012).

  And finally, there are minority enzymes such as protease that hydrolyses proteins and polypeptides to yield peptides of lower molecular weight and esterase that breaks down esters (Labropoulos and Anestis, 2012).

2.4. Organic acids

Honey contains organic acids, in equilibrium with their corresponding lactones (White, 1979a; Gomes *et al.*, 2010). Honey acids represent less than 0.5% of total solids, but they are important for honey's taste, aroma, colour and honey's preservation, making it difficult for microorganisms to grow (Bogdanov, 2011c; Ananias *et al.*, 2013). They contribute to honey acidity and electrical conductivity. Some honeys' organic acids are likely to come directly from nectar or honeydew (citric, malic and oxalic), but the vast majority of them are produced

from nectar and honeydew sugars by the action of enzymes secreted by bees during ripeness and storage (formic acid and others) (White, 1979a; Ortiz-Valbuena *et al.*, 1996; Sancho *et al.*, 2013). Moreover, during storage, due to glucose oxidase action, the osmophilic yeasts produce alcohols and eventually organic acids from honey sugars, leading to the synthesis of such acids as acetic acid, whose levels are possible indicators of honey fermentation (Mato *et al.* 2003; Cavia *et al.*, 2007).

Gluconic acid is the main honey organic acid, representing the 70-90% of the total (Bogdanov, 2011c). It comes from glucose by the action of glucose oxidase. The metabolic activity of some Gluconobacter bacteria from bee intestine could produce gluconic acid (Ruiz-Argüeso and Rodríguez-Navarro, 1973). In honey, gluconic acid is in equilibrium with gluconolactone (Cavia *et al.*, 2007; Sancho *et al.*, 2013). Apart from gluconic acid, more than 30 different non-aromatic organic acids were found in honey such as acetic, butyric, citric, formic, lactic, maleic, malic, oxalic, fumaric, pyroglutamic, succinic, pyruvic and tartaric acids, among others (Mato *et al.*, 2003). Some organic acids are intermediates in the Krebs cycle of biological oxidation or other similar enzymatic pathways (Echigo and Takenaka 1974; White, 1979a). Honey organic acids are characteristic of the botanical origin of this food (Cherchi *et al.*, 1994; Anklam, 1998; Kaškonienė and Venskutonis, 2010). For example, Del Nozal *et al.* (1998) showed that *Erica* sp. honeys could be distinguished by their high content in quinic acid, *Quercus* sp. honeydew honeys by their low concentrations in pyruvic acid and high quantities of both malic and succinic acids, whereas high citric acid concentrations were described as a possible marker of *Thymus* sp. honeys. Suárez-Luque *et al.* (2006) found high levels of formic acid in *Castanea sativa* honeys, unlike the low levels of this acid in *Eucalyptus* sp. honeys.

The term acidity is related to the source of nectar, the bee specie and the action of enzymes or bacteria. Free acidity is due to organic acids (which are in equilibrium with their lactones), internal esters and inorganic ions, such as phosphates, chlorides, sulphates and nitrates, which could produce their corresponding acids (White, 1979a; González, 2002; Belay *et al.*, 2013). Honey's free acidity determination is included in the honey quality control regulations (OJEC, 2002). Other important acidity type is the lactonic acidity. Lactones (mainly glucolactones), may be considered to be a reserve acidity when the honey becomes alkaline (Terrab *et al.*, 2002).

Honey pH is not directly related to the acidity, because some honey components have buffer capacity, among them salts and some minerals compounds (White, 1979a; Terrab *et al.*, 2002; Ojeda de Rodríguez *et al.*, 2004). Honey pH ranges from 3.4 to 6.4, so that it is usually low enough to inhibit microorganisms' development (Cavia *et al.*, 2002; Gomes *et al.*, 2010). In general, pH values in nectar honeys vary from 3.3 to 4.6. An exception is chestnut honey, where pH values varying from 5 to 6. pH of honeydew honeys was described as higher than

that of blossom honeys, because of their higher mineral contents, ranging from 4.5 to 6.5 (Bogdanov, 2011c; Eleazu *et al.*, 2013).

2.5. Minerals

Mineral content in honey is generally low, ranging between 0.02% and 0.3% in blossom honeys, while in honeydew honeys can reach 1% of the total (Crane, 1985; Felsner *et al.*, 2004b). It is influenced by soil and climatic conditions, as well as the chemical composition of nectar that varies according to the different botanical sources involved in honey formation. Variations can also be related to harvesting, beekeeping techniques (such as extraction methods) and the material collected by the bees during foraging on flowers (White, 1975; Piana *et al.*, 1989; Ortiz-Valbuena *et al.*, 1996; Sabatini, 2007).

Minerals are absorbed in their salts forms dissolved in water, moving from the roots to the plant sap and then being pumped to the nectar or honeydew and pollen (Estupiñán *et al.*, 1998; Sabatini, 2007). The most important minerals found in honeys are potassium, sodium, calcium and magnesium. Less abundant elements are iron, copper, manganese and chloro, and in minor quantities, trace elements such as boron, phosphorus, sulfur, silicon, barium and nickel, among others (Doner, 2003). Potassium is the main one, standing for 80% of the total, as a result of its quick secretion by nectaries (Ortiz-Valbuena *et al.*, 1996). The amount of minerals present in honey does not significantly contribute to the dietary recommendations.

Many researchers classified different unifloral honeys by their trace elements profile (Fernández-Torres *et al.*, 2005; Alda-Garcilope *et al.*, 2012; Chen *et al.*, 2014). In general, dark honeys contain more minerals than the light ones, being higher in honeydew honeys (Anklam, 1998; Sáinz-Laín and Gómez-Ferreras, 2000; Fernández-Torres *et al.*, 2005; Nozal-Nalda *et al.*, 2005). Chudzinska and Baralkiewicz (2010) distinguished honeydew honeys by the content of K, Al, Ni, Cd, and Zn, while Na, Ba, and Pb amounts were characteristics of rape honeys.

Both ash percentage and the electrical conductivity are related to the honeys mineral content (Felsner *et al.*, 2004a; Pires *et al.*, 2009; Santos *et al.*, 2014). In respect of botanical origins, generally nectar honeys have a lower ash content or electrical conductivity than honeydew honeys (Felsner *et al.*, 2004b).

Honey has been considered as a potential environment pollution indicator, as a result of a bio-accumulative process in the outskirts of urban and industrial areas, as well as in extraurban crossroads, where traces of some mineral compounds and/or heavy metals were found (Popa *et al.*, 2013).

2.6. Vitamins

Honey contains vitamins that come mainly from the pollen of the flowers visited by bees, as well as from nectar or honeydew (Sáinz-Laín and Gómez-Ferreras, 2000). The amounts of vitamins in honey are so small that this food cannot be considered as a good source of these nutrients. The content of water-soluble vitamins is higher than the quantity of fat-soluble vitamins, because honey hardly contains lipidic substances (León-Ruiz *et al.*, 2013b).The most important vitamin of honey is vitamin C, which has antioxidant effect. Vitamins of B group were also detected in different quantities (León-Ruiz *et al.*, 2013b). Some fat-soluble vitamins such as vitamin A, D, E and K have been found in small quantities (Sáinz-Laín and Gómez-Ferreras, 2000).

2.7. Polyphenols compounds

Potential therapeutic properties of honey have been attributed to bioactive compounds, which provides this food with antioxidant, antibacterial, and anti-inflammatory activities, among others (White, 1979a; Chen *et al.*, 2000; Gheldof and Engeseth 2002; Ferreira *et al.*, 2009; Pichichero *et al.*, 2009). Most of these substances are phenolic compounds. Other components with biological activity include enzymes (catalase, glucose oxidase), organic acids, Maillard reaction products (melanoidins), aminoacids, proteins, vitamins (such as ascorbic acid, alpha-tocopherol), carotenoid derivatives (as β-carotene, precursor of vitamin A), and other compounds such as hydrogen peroxide, methylglyoxal, royalisin and acetil-coline (White, 1979b; Gheldof *et al.*, 2002; Baltrusaityte *et al.*, 2007; Bertoncelj *et al.*, 2007; Kwakman *et al.*, 2010). Many of these components have been suggested as markers for botanical and/or geographical origins of honeys (Yao *et al.*, 2004).

Honey phenolic phytochemicals are important aromatic secondary metabolites derived from plants, whose nectars or honeydew is sipped by bees, as well as from pollen or propolis (Ferreres *et al.*, 1992). Their range in honeys is about 5 to 1300 mg/kg (Al-Mamary *et al.*, 2002; Gheldof and Engeseth, 2002).

Polyphenols are divided into several classes, according to the phenolic structural features (Grassi *et al.*, 2010). In honey they are mainly phenolic acids, phenolic acid derivatives and flavonoids (Tomás-Barberán *et al.*, 2001).

Phenolic acids comprise molecules with one phenolic ring (Grassi *et al.*, 2010). They are non-flavonoid polyphenolic compounds derivatives of benzoic acid (such as gallic, ellagic and protocatechuic acids) and cinnamic acid (such as caffeic, sinapic, ferulic and coumaric acids) (Amiot *et al.*, 1989).

Flavonoids are a large family of plant phenolic pigments. They contain several phenolic hydroxyl functions attached to ring structures (Rice-Evans *et al.*, 1997). Depending on the structural complexity of flavonoids, particularly on the oxidation state of the central ring, the

flavonoids are subdivided in flavonols (such as myricitin, galangin, quercetin, rutin and kaempferol), flavones (such as chrysin, tectochrysin, luteolin and apigenin), flavanols (such as catechin), flavanones (such as hesperetin, naringenin, pinocembrin and pinobanksin), isoflavones, anthocyanins and chalcones (Rice-Evans *et al.*, 1997; Wollgast and Anklam, 2002; Forester and Waterhouse, 2009; Chandrasekara and Shahidi, 2010; Grassi *et al.*, 2010).

The main flower-derived flavonoids in honey are aglycones (Soler *et al.*, 1995). The conversion of natural glycosides present in nectar to the corresponding aglycones detected in honey is due to the hydrolysis by bee saliva enzymes (Ferreres *et al.*, 1992; Tomás-Barberán *et al.*, 1993b). At first, it was thought that only aglycones were present in honey, but in 2008, Truchado *et al.* detected flavonoid glycosides in honey for first time. The reason was the existence of flavonoid rhamnosides and rutinosides in the nectar of some plants that could not be hydrolysed by any bee enzyme (Truchado *et al.*, 2009a).

In general, more than 90% honey flavonoids come from propolis (such as pinobanskin, pinocembrin and chrysin) (Ferreres *et al.*, 1992; Martos *et al.*, 1997). Propolis-derived flavonoids are relatively lipophilic, and they are found in honey in variable quantities, depending on the degree of propolis contamination in the hive and beeswax, with no relation with its botanical origin (Ferreres *et al.*, 1994d; Martos *et al.*, 2000a). However, they were proposed as useful for the identification of its geographical origin (Ferreres *et al.*, 1991; Ferreres *et al.*, 1992; Gil *et al.*, 1995).

In temperate climate areas, flavonoids are originated from poplar bud exudates, diffusing to beeswax and honey in the hives (Tomás-Barberán *et al.* 1993b; Gil *et al.*, 1995; Martos *et al.*, 2000a). In areas where *Populus* species are not native plants, as in the case of the Equatorial regions, tropics and very arid areas, bees seek different plan exudates. The honeys produced in these areas show flavonoid profiles characterized by the absence of propolis-derived flavonoids (Tomás-Barberán *et al.*, 1993b). Finally, in countries where poplars have been introduced for gardening or agro-industrial purposes, propolis-derived flavonoids are present in different relative amounts, being the pollen-nectar-derived flavonoids the major constituents (Tomás-Barberán *et al.*, 1993b; Martos *et al.*, 1997). There are flavonoids which come from both origins (for example, kaempferol in rosemary honey could be originated from propolis and nectar, but mainly from nectar) (Gil *et al.*, 1995).

Nectar-pollen-derived flavonoids could be useful for honeys' botanical characterization, being the contribution of nectar more important than the contribution of pollen (Ferreres *et al.*, 1992; Ferreres *et al.*, 1993).

Polyphenol content of dark coloured honeys (such as heather), is usually higher than that of light ones (such as citrus). Dark honeys have been reported to contain more phenolic acid derivatives but less flavonoids than light ones (Amiot *et al.*, 1989).

The polyphenols profile and/or the identification of some individual components or a group of compounds are important tools for the characterization of both botanical and geographical origin of honeys. Some substances were described as chemical markers, among them quercetin for sunflower (Tomás-Barberán *et al.*, 2001), kaempferol for rosemary (Gil *et al.*, 1995), hesperitin for citrus (Ferreres *et al.*, 1993), naringenin and luteolin for lavender (Ferreres *et al.*, 1994b; Andrade *et al.*, 1997b), myricetin, tricetin, luteolin, gallic acid and abscisic acid for eucalyptus (Martos *et al.*, 2000a, b; Yao *et al.*, 2003, 2004), kaempferol-rhamnosides and rhamnosyl-glucosides for acacia (Truchado *et al.*, 2008), ellagic, benzoic, phenylacetic, mandelic and β-phenyllactic acids for heather (Ferreres *et al.*, 1994a; Soler *et al.*, 1995; Ferreres *et al.*, 1996a, b; Andrade *et al.*, 1997a; Dimitrova *et al.*, 2007), homogenistic acid for Strawberry tree (Cabras *et al.*, 1999), caffeic, p-coumaric and ferulic acids for chestnut (Tomás-Barberán *et al.*, 2001; Dimitrova *et al.*, 2007), protocatechuic acid for honeydew (Steeg and Montag, 1988; Anklam, 1998; Tomás-Barberán *et al.*, 2001; Haroun *et al.*, 2012;), phenyl-propanoic acid for rape, 4-hydroxybenzoic acid and no phenylacetic acid for buckwheat (Steeg and Montag, 1988) and rosmarinic acid for thyme (Andrade *et al.*, 1997b).

2.8. Volatile and semivolatile compounds

Honeys from different botanical sources have distinctive organoleptic characteristics that influence the acceptance and selection of the product by consumers. The honey aroma and taste are related to the volatile compounds (Piana *et al.*, 2004; Montenegro *et al.*, 2008), together with sugars, acids, aminoacids, tannins and phenolics (White, 1979a).

More than 600 low molecular weight compounds have been identified in honeys at very small concentrations as complex mixtures of different chemical structures such as monoterpenes, terpenes, terpenoids, norisopreniods, phenolic compounds, benzene derivatives, alcohols, ketones, aldehydes, esters, fatty acids, acids, hydrocarbons and cyclic compounds (Alissandrakis *et al.*, 2003; Castro-Vázquez *et al.*, 2003; Soria *et al.*, 2005; Castro-Vázquez *et al.*, 2006; Jerković *et al.*, 2009). Their impact on honey aroma depends on their concentrations in relation with their odour thresholds (Castro-Vázquez *et al.*, 2007).

Honeys aromatic substances derive from the botanical source, the physiology of the honeybee, and the climatic conditions (Serra-Bonvehí and Ventura-Coll, 2003; Soria *et al.*, 2003; Bianchi *et al.*, 2005). Some alcohols and branched aldehydes are likely to be produced by microbial metabolism (Castro-Vázquez *et al.*, 2009), while furan and pyran derivatives are artefacts of the Maillard reaction, generated during honey thermal processing and storage conditions (Guyot-Declerck *et al.*, 2002; Jerković *et al.*, 2006). Finally, other aroma compounds are honey off-flavour pollutants, originated in the surrounding environment (Tananaki *et al.*, 2005).

Honey aromatic profile, as well as the identification of volatile chemical markers, proved to be effective to assess the honey botanical and geographical origins and to detect possible adulterations (D'Arcy *et al.*, 1997; Cuevas-Glory *et al.*, 2007; Escriche *et al.*, 2009). Methyl anthranilate has been proposed as marker of citrus honey (Ferreres *et al.*, 1994c), isophorone of strawberry tree honey (De la Fuente *et al.*, 2007; Tuberoso *et al.*, 2010), phenylacetic acid of ling heather honey (Guyot *et al.*, 1999), and some acetophenone derivatives such as 3-aminoacetophenone of chestnut honey (Alissandrakis *et al.*, 2011).

2.9. Pigments

They are responsible for the honey colours, being the most important of which polyphenols, carotenoids, xanthopylls and anthocyanins, which can be grouped in water soluble and lipid soluble pigments. Other compounds that can contribute to honey colour are sugars, minerals and aminoacids (Ortiz-Valbuena *et al.*, 1996; Sáinz-Laín and Gómez-Ferreras, 2000; Sabatini, 2007).

2.10. Lipids

Small quantities of lipid compounds (about 0.04%) have been found in honey, among them glycerides, sterols, phospholipids and different acids such as palmitic, oleic, lauric, miristic, stearic and linoleic (White, 1979a; Sáinz-Laín and Gómez-Ferreras, 2000). They come mainly from rests of wax and also from plants (Sabatini, 2007).

2.11. Hydroxymethylfurfural

Hydroxymethylfurfural (HMF) is a furanic compound produced by sugar degradation, from dehydration of hexoses in acidic medium and to a lesser extent, as an intermediate in the Maillard reactions (Wunderlin *et al.*, 1998; González, 2002; Sanz *et al.*, 2002; Spano *et al.*, 2006; Cavia *et al.*, 2008; Turhan *et al.*, 2008; De Oliveira Resende Ribeiro *et al.*, 2012; Islam *et al.*, 2014).

HMF is a parameter of honey freshness, since it is absent or present in trace amounts in fresh honeys. HMF concentration increases during honey processing by heat treatment, and also by adulteration with commercial sugars and throughout storage (Fallico *et al.*, 2004; Fallico *et al.*, 2006; Sodré *et al.*, 2011; Belay *et al.*, 2013; Kesic *et al.*, 2014). HMF content is also affected by the use of metallic containers, pH, bee species and botanical source (Estupiñán *et al.*, 1998; Wunderlin *et al.*, 1998; Gokmen and Morales, 2014). High acidity, moisture content, sugars (mainly fructose), aminoacids (such as alanine) and minerals (such as magnesium, manganese, iron and zinc) speed up HMF production (Ortiz-Valbuena and Silva-Losada, 1991; Anam and Dart, 1995; Estupiñán *et al.*, 1998). Kesic *et al.* (2014) observed a negative correlation between the fructose/glucose ratio and HMF contents.

High values of HMF are naturally present in honeys from warm climate areas, such as tropical and subtropical countries (Sodré *et al.*, 2011).

2.12. Toxic compounds

Some honeys possess toxic compounds. They are synthetized by some plants or insects in low quantities. The main toxic components are polyhydroxylated cyclic hydrocarbons (diterpenoids) or pyrrolizidine alkaloid (Vit and Barrera, 2002; Sabatini, 2007; Bogdanov, 2011c; Islam *et al.*, 2014).

Environment pollutants, pesticides and antibiotics are other potentially toxic substances that honey can contain.

3. Physical properties

Honey has been often used as a food ingredient, because its physical characteristics are particularly suitable for some culinary arts.

3.1. Colour

Colour is a physical property immediately perceived by consumers. In honeys varies from colourless and light yellow to dark amber or nearly black, sometimes with green or reddish reflexes (White, 1975; Accorti *et al.*, 1986; Bogdanov, 2011b; Eteraf-Oskouei and Najafi, 2013) and it is related to the botanical origin, climate and soil conditions. Some authors have reported that pollen, sugars related products, carotenoids, xanthophylls, anthocyanins, minerals, aminoacids and phenolic compounds, mainly flavonoids, influence the colour (Ortiz-Valbuena *et al.*, 1996; Sabatini, 2007; Almeida-Muradian *et al.*, 2014).

Dark honeys present higher minerals, dextrin and polyphenol contents, and higher acidity than light honeys (White, 1975; Ortiz-Valbuena *et al.*, 1996; González, 2002). The colour of dark honeys is strongly correlated with concentrations of Cd, Fe and Pb, while the colour of pale honeys with concentrations of Al and Mg (González-Miret *et al.*, 2005). In addition, this parameter is affected by storage, heat, enzymatic reactions and crystallization (Sodré *et al.*, 2011; Eteraf-Oskouei and Najafi, 2013). Granulated honey tends to set a lighter colour than when liquid, depending on the crystal size. The finest crystals always give a paler appearance (White, 1979b; Crane, 1985).

In some countries, the honey colour influences the price of honey, varying according to the consumers' preferences. Generally, the lightest ones achieve higher prices in the market, but there are some countries such as Germany, Switzerland, Greece and Turkey where dark honeydew honeys are preferably chosen (Bogdanov, 2011b; Tuberoso *et al.*, 2014).

The browning or darkening of the honey depends on the initial colour of the honey, chemical composition, storage and heating (Crane, 1985; Gonzales *et al.*, 1999). Components that

could affect darkening are sugars, nitrogen content, free aminoacids and moisture (Gonzales *et al.*, 1999). Factors that produce honey darkening are the reaction between amino acids and sugars in acid medium (Maillard reactions) that leads to the melanoidin formation, the caramelization process of sugars (instability of fructose in acid solution), high colloids content by macromolecules hydrolysis, and ascorbic acid, polyphenols and lipid oxidation reactions (Huidobro and Simal, 1984; Ortiz-Valbuena *et al.*, 1996; Gonzales *et al.*, 1999; Sáinz-Laín and Gomez-Ferreras, 2000; González, 2002; Almeida-Muradian *et al.*, 2014). Some beekeepers' practices can also intensify honey colour, such as the use of old combs, the light exposure during honey storage or the use of inappropriate materials that could lead to reactions between tannic acids derivatives and oxidized polyphenols with iron salts (Huidobro and Simal, 1984; Crane, 1985; Gonzales *et al.*, 1999; Gonzalez, 2002; Sabatini, 2007).

3.2. Electrical conductivity

Electrical conductivity (EC) refers to the ability of a material to conduct an electric current. It is directly related to the botanical origin, as well as to the mineral content and inorganic ions, and somehow to organic acids, proteins and other components such as sugars, polyols and pollen grains, that can act as electrolytes (White, 1979b). EC is correlated to honey ash content and alkalinity of ash (Sancho *et al.*, 1991b; Sancho *et al.*, 1992). According to the current European regulation (OJEC, 2002), electrical conductivity of blossom must be lower than 0.8 mS/cm of EC, while electrical conductivity of honeydew honey and chestnut honey must be higher than 0.8 mS/cm. Exceptions are honeys from *Arbutus*, *Banksia*, *Erica*, *Leptospermum*, *Melaleuca*, *Eucalyptus*, *Tilia* and blends.

3.3. Density

Another honey property strongly influenced by the high sugar content is density. Density is expressed as specific gravity (White, 1979b), and the values are related to the water content, temperature and solids concentration (Oroian, 2013). Density decreases linearly while temperature or water content increases, and increases linearly with an increase of the solids content (Sabatini, 2007; Oroian, 2013). Medium values of relative density in honeys at 20ºC vary from 1.40 to 1.44 g/l, depending on honey botanical origin (Crane, 1985; Sáinz-Laín and Gómez-Ferreras, 2000). Because of density variation of honeys stored in tanks, sometimes different honey stratification layers are observed, in which the upper layer has lower density and higher moisture, thus being more prone to ferment (White, 1975; Krell, 1996; Bogdanov, 2011b).

3.4. Viscosity and rheological properties

Viscosity is an important property for handling, processing (affecting a number of technological operations), storage and sensory quality, so that it determines the acceptance of honey by consumers.

Honey viscosity depends on the botanical origin, moisture, temperature, fructose/glucose ratio, honey granulation and chemical composition. The more the temperature and water contents are, and the less the polysaccharide content is, the lower the honey viscosity is (Fattori, 2004). It has been possible to predict honey viscosity values at specific temperatures with an Arrhenius-type model (Bhandari *et al.*, 1999; Mossel *et al.*, 2000).

Also, some honey compounds such as dextrins, proteins and other colloidal substances tend to increase honey viscosity (Rybak-Chmielewska, 2004; Ren *et al.*, 2010; Sodré *et al.*, 2011). Honey granulation results in a dramatic increase of viscosity of a factor of 10 (Bogdanov, 2011b). Viscosity and surface tension are responsible for the foaming characteristics of honey (Olaitan *et al.*, 2007; Manzoor *et al.*, 2013). Viscosity also has a relationship with the fluidity of particles. The more viscous the fluid is, the lower its fluidity is (Kolayli *et al.*, 2014), which may hinder its homogenization in preparations, limiting its use in the food industry. For this reason, the use of honey powder (dry honey), has become an attractive option for the industry, particularly for bread-baking industries (Tong *et al.*, 2010).

According to their viscosity, the vast majority of honeys can be described as Newtonian, but certain types exhibit non-Newtonian behaviour, due to the presence of high molecular compounds. *Piptadenia moniliformis* honey has a pseudoplastic fluid behaviour (Stelmakiené *et al.*, 2012), several *Eucalyptus* honeys and *Nigerian Opuntia* engelmanni honey present dilatancy behaviour and Weissenberg effect mainly due to the presence of high-molecular weight dextrans (Sone, 1972; Fattori, 2004; Juszczak and Fortuna, 2006; Yanniotis *et al.*, 2006), and honeys from ling heather (*Calluna vulgaris*), *Leptospermum* sp. (such as New Zealand Manuka), buckwheat (*Fagopyrum esculentum*), white clover (*Trifolium repens*), and Indian Karvi (*Carvia callosa*) exhibit shear-thinning and thixotropic behaviour due to their high protein content (Mossel *et al.*, 2000; Witczak *et al.*, 2011; Stelmakiené *et al.*, 2012). Thixotropy confers to honeys a gel-like structure that makes more difficult their extraction from the combs and their handling. This gelatinous consistency can be turned to liquid, by agitation or by mechanical energy application (Sabatini, 2007; Bogdanov, 2011b; Witczak *et al.*, 2011).

3.5. Optical rotation

Honey has the property of rotating the plane of polarised light due to its carbohydrate composition. Each sugar has a specific angle of polarized light rotation (specific rotation). Some sugars rotate the polarized light angle to the left, presenting a negative optical rotation value (laevorotatory sugars such as fructose [α]D20=-92.4º), while others rotate it to the right, with positive optical activity (dextrorotatory, such as glucose [α]D20=+52.7º) (García-Álvarez *et al.*, 2002; Sanz *et al.*, 2002; Dinkov, 2003). The overall value for the optical rotation depends on the concentration of the different honey sugars (Bogdanov *et al.*, 1999). Nectar honeys, that usually present higher fructose content, are laevorotatory. On the other

hand, some honeydew and other honeys can be dextrorotatory due to its lower fructose content and its higher oligosaccharide mass fraction, mainly melezitose ([α]D20=+88.2°) and erlose ([α]D20=+121.8°) (White, 1979b; Dinkov, 2003; Primorac *et al.*, 2011). Some adulterated honeys, also present low fructose values, being normally dextrorotatory (García-Álvarez *et al.*, 2002). Although some researchers determined optical rotation to botanical characterization of honeys (Persano-Oddo *et al.*, 1995; Bogdanov *et al.*, 2004; Bertoncelj *et al.*, 2011), its measurement was not described as suitable for that purpose (Dimins *et al.*, 2008).

3.6. Refractive index

The refractive index is an optical property that varies between 1.504 and 1.4815, increasing when solid content is high (or water content is low), depending on the temperature (White, 1979b; Sáinz-Laín and Gómez-Ferreras, 2000).

3.7. Hygroscopicity

Honey is a strongly hygroscopic product due to its high content of sugars (mainly fructose), absorbing or holding moisture from the environment depending upon the temperature and the relative humidity (White, 1979b; Sabatini, 2007; Bogdanov, 2011b; Eteraf-Oskouei and Najafi, 2013). This property must be taken into account when packing, storing and for industrial uses. If honey absorbs moisture, it becomes diluted, being more prone to ferment. The hygroscopicity is a desirable property in the management of some products in which honey is incorporated, mainly those subjected to heating, because it helps keep the softness or non-drying in baked goods (White, 1979b; Crane, 1985).

3.8. Osmotic pressure

The chemical composition of honey, mainly sugars, makes honey as a high osmotic pressure food. Together with the acid pH, the organic acid content, the hydrogen peroxide produced by glucose oxidase action and other non-peroxide factors, the high honey osmotic pressure avoids the microbial growth, increasing the stability and shelf life of the product (Manyi-Loh *et al.*, 2011b; Alvarez-Suarez *et al.*, 2014).

3.9. Crystallization

Honey crystallization or granulation is a natural and spontaneous complex physical process. The glucose, less soluble than fructose, separates from water and precipitates out of the supersaturated solution, becoming glucose monohydrate crystals by water losses (Gleiter *et al.*, 2006). Honeys rich in fructose such as acacia and sage, may remain liquid for long period, while honeys rich in glucose such as rape or dandelion, often granulate immediately after harvesting or sometimes within the cells of the comb (Dyce, 1931; Maurizio, 1962).

This process can be undesirable by beekeepers, since some consumer think that if honey is crystallized, is because it has been somehow adulterated (Kabbani *et al.*, 2011; Costa *et al.*, 2013). In contrast, creamy crystallized honey is desirably for some purposes such as the commercialization of spread honey. Crystallization only affects the honey colour and texture, preserving the flavour and quality characteristics of the liquid honey. In principle, crystallized honey is not a spoiled product, but if a non-homogeneous crystallization occurs, the sugar concentration of the upper part decreases, increasing the moisture content of the liquid phase (Dyce, 1979; Crane, 1985; Sancho *et al.*, 1991a; Kolayli *et al.*, 2012).

Honey crystallization depends on such factors as temperature, viscosity, water content, sugars (mainly glucose and melezitose content), dextrin content, the glucose supersaturation coefficient and the presence of particles that could act as crystallization nuclei (proteins and other colloids, pollen grains, dust and other suspended particles, yeast, wax, propolis or air bubbles, among others) (Crane, 1985; Serra-Bonvehí, 1986; Sancho *et al.*, 1991a; Estupiñán *et al.*, 1998; Bhandhari *et al.*, 1999).

- Temperatures of 5-7ºC facilitate the creation of crystallization nuclei (Serra-Bonvehí, 1986) and for honey crystal growing process the optimum temperature range lies between 10 and 18ºC, being 14ºC the most suitable temperature. At low temperatures, crystallization is slowed down because despite decreasing sugar solubility (thereby, favouring granulation), there is an increase of honey viscosity that reduces the glucose diffusion, making it more difficult for crystals to move (Dyce, 1931; Serra-Bonvehí, 1986; Jéanne, 1991; Lupano, 1997). Temperatures higher than 25ºC allow the dissolution of the glucose crystals (Serra-Bonvehí, 1989; Jéanne, 1991; Sancho *et al.*, 1991a; Bogdanov, 2011a). Sudden temperature changes lead to the formation of glucose crystals that produce a mobility of air bubbles, acting as granulation catalysts. The same happens with agitation, because of the formation of air bubbles (Serra-Bonvehí, 1986, 1989).
- Regarding water content, honeys between 15 and 18% of moisture crystallize optimally (Bogdanov, 2011a). Too high water content decreases crystallization speed, because sugar saturation also decreases. However, glucose can easily precipitate, remaining the fructose on the liquid surface. This sugar bounds water from the environment, which increases the risk of fermentation (Jéanne, 1991).
- The higher the glucose (more than 28-30%) and melezitose (more than 10%) contents are, the faster the crystallization is (White, 1979b; Sabatini, 2007; Bogdanov, 2011a). Fructose and maltose act as granulation inhibitors because they increase the glucose solubility (Tabouret, 1979; Crane, 1985; Sancho *et al.*, 1991a).
- Samples with fructose/glucose (F/G) ratio higher than 1.3 (such as acacia and sage, among others) and glucose/water (G/W) ratio lower than 1.7 crystallize slowly while in samples with ratios fructose/glucose lower than 1.0 (such as dandelion, rape and sunflower, among others) and glucose/water higher than 2.0, crystallization is faster

(White *et al.*, 1962; Cavia *et al.*, 2002; Amir *et al.*, 2010; Buba *et al.*, 2013). These ratios together with other relations such as (G-W)/F, the supersaturation index (based on the ternary system water-fructose-glucose), the Codounis index ((100/G)-(W/G)-1) and the Tabouret index ((G/H)/(1-aw)n, being n=1-1.5 if water content is higher than 17% and n=2 if is lower) have been proposed to predict the honey granulation (Tabouret, 1979; Serra-Bonvehí, 1989; Sancho *et al.*, 1991a). F/G and G/W are not the most suitable ratios to predict honey crystallization, because the former does not take into account water content, and the latter does not consider the inhibitory action of fructose. Moreover, G/W ratio has not been proved as satisfactory for honeys with low moisture contents (Tabouret, 1979). Conversely, (G-W)/F and Tabourent index have demonstrated to be better because fructose and water activity are included, respectively (Serra-Bonvehí, 1989; Sancho *et al.*, 1991a).

- Decantation, filtration of centrifugation processes (where honey impurities, air bubbles and other particles are removed), storage at freezing temperatures and the removal of yeast and glucose nuclei by pasteurization, decrease the crystallization speed (Townsend, 1979; Bhandari *et al.*, 1999).

The number of crystallization nuclei in honey determines the crystals size. At higher number of crystallization nuclei, higher will be the crystals number and less will be the size, being the crystallization faster. A fast crystallization leads to a fine granulation that produces a more or less compact structure, while a slow granulation produces non compact structures of fewer and thick crystals (White, 1978; Serra-Bonvehí, 1986; Ortiz-Valbuena *et al.*, 1996; Bhandari *et al.*, 1999). Some honey defects due to a bad crystallization are the formation of frosting, the rough granulation and the separation in two phases (Bogdanov, 2011a).

When crystallized product is preferred, it is possible to make an induced granulation process of honey in order to avoid the building of frost or coarse crystallization (Bogdanov, 2011a). The objective of this process is to form an adequate creamy consistency with small crystals (Chen *et al.*, 2009). The procedure is a mechanical cutting of the crystals up by agitating the honey, followed by the inoculation of 5-10% of the honey with a starter honey that presents the desired consistency (Bogdanov, 2011a). On the other hand, when honey needs to remain or become liquid, it is possible to slow the rate of crystallization or undo the crystals in products already crystallized by heating. However, heating under improper conditions can result in inactivation of enzymes, loss of flavour and aroma, darkening the honey colour and also favour the formation of hydroxymethylfurfural (HMF), with a consequent reduction in the honey quality. Although heating is not recommended, honey liquefaction process at low temperatures is often studied and the use of ultrasounds waves to accelerate the liquefaction process is an alternative to the heat treatment only (Assil *et al.*, 1991). But one problem of this liquefaction is that honey recrystallization is usually not uniform (Rybak-Chmielewska, 2004).

4. Nutritional value and potentially functional properties of honey

In general, the vast majority of health benefits attributed to honeys have been related to both antioxidant and antimicrobial activities of this food (Boukraâ, 2014; Bogdanov, 2015). Furthermore, honey has shown other potential functional properties that are worth commenting, the most important of which are antihypertensive capacity, anti-inflammatory activity, as well as prebiotic and probiotic effects.

4.1. Honey nutritional value

In human nutrition, honey is an excellent source of energy. 100 g of honey supplies about 1283 kJ of energy (306 kcal). 20 g honey is the usual quantity per serving or tablespoon that provides about 256.6 kJ (61.2 kcal), which represents more or less 3% of the energy necessary per day (Bogdanov *et al.*, 2008). The main constituents of honey are the simple carbohydrates, fructose and glucose that are used for human body energy requirements after being rapidly absorbed into the blood without previous digestion (Ajibola *et al.*, 2012).

Blasa *et al.* (2006) claimed that honey is a good food for people of all ages. It helps improve elderly people's health (Alvarez-Suarez *et al.*, 2009), and sportspeople performance, increasing the rejuvenation of muscles with no further needs of other more expensive sporting activities enhancers. Thereby, honey has been described as a well-tolerated food and an effective carbohydrate source for athletes (Earnest *et al.*, 2000; Kreider *et al.*, 2002; Ajibola *et al.*, 2012).

The proteins, vitamins and minerals' contents of honey are very low, so that their contribution to human needs regarding these nutrients is marginal (Bogdanov *et al.*, 2008). However, if compared with sugar, honey has proved to be healthier as a sweetening agent. According to some researchers, several honey enzymes can enhance the digestion of sugars and starch (Ajibola *et al.*, 2012). In contrast, other researchers claim that saliva already has those enzymes, so that their contribution to digestion is insignificant. Nevertheless, the hydrogen peroxide produced by honey's glucose oxidase could have an antimicrobial effect within the mouth (Bogdanov, 2015). Unlike sugar, on the people's digestive system honey intake gives rise to a laxative effect and improves calcium absorption that could help to reduce the risk of bone mass loss (Ariefdjohan *et al.*, 2008; Ajibola *et al.*, 2012). Honey contains small amounts of beneficial nutrients. Flavonoids and other honey's phenolic compounds provide this food with functional properties such as antioxidant capacity. The amount of flavonoids depends on the honey type (Gheldof and Engeseth, 2002), and appears to be higher in honeys harvested during dry seasons with high temperatures (Kenjeric *et al.*, 2007). Honey also contains healthy substances like choline and acetylcholine. Choline is crucial for cardiovascular and brain function, as well as for cellular membrane composition and repair. Acetylcholine functions as a neurotransmitter (Bogdanov, 2015).

With regard to glycemic index, honey has also been described as a healthier food choice compared to sucrose (Al-Khalidi *et al.*, 1980; Jawad *et al.*, 1981). The main honey monosaccharide, fructose, has a glycemic index of 19 and sucrose of 68 (Bogdanov, 2015). Arcot and Brand-Miller (2005) observed a negative correlation between honey glycemic index and fructose concentration, so that honeys rich in fructose could be beneficial in respect of some diseases (Jenkins *et al.*, 2002).

Honey intake can also lead to some problems, the most important of which are the potential allergenicity of some bees or plant proteins that could be present in honey (Simon *et al.*, 2009), as well as the possible presence of *Clostridium botulinum* spores in honey. In respect of the last issue, within the stomach of toddlers younger than one year, the spores of that microorganism can germinate, grow and excrete the toxin (Brown, 2000; Manyi-Loh *et al.*, 2011a). Therefore, honey must not be eaten by toddlers under the age of one year. However, there is no risk regarding honey ingestion by humans older than 12 months (Bogdanov, 2015).

4.2. Antioxidant activity of honeys

Unlike other sweeteners, honey has shown antioxidant activity, which provides this food with nutritional and technological advantages. Honey has proved to prevent or delay food spoilage due to oxidative reactions, protecting meats against lipid oxidation (Antony *et al.*, 2000; McKibben and Engeseth, 2002), and vegetable products against enzymatic browning (Oszmianski and Lee, 1990; McLellan *et al.*, 1995; Chen *et al.*, 2000). Therefore, honey has a great potential to be used as a natural antioxidant for foods (Gheldof *et al.*, 2002; Nagai *et al.*, 2006). In vitro studies have revealed that honey intakes inhibit oxidation of human serum lipoproteins (Gheldof and Engeseth, 2002; Al-Waili, 2003; Schramm *et al.*, 2003). Buckwheat honey in particular was able to increase human's serum antioxidant activity (Gheldof *et al.*, 2003).

Phenolic compounds (flavonoids, phenolic acids), as well as melanoidins (Maillard reaction products), appear to be the most important constituents of honey responsible for its antioxidant activity (Bogdanov, 2015). These compounds, together with glucose-oxidase, catalase, carotenoids, organic acids, ascorbic acid, aminoacids and proteins have been described as honey antioxidants (Frankel *et al.*, 1998; Al-Mamary *et al.*, 2002; Fahey and Stephenson, 2002; Gheldof *et al.*, 2002; Aljadi and Kamaruddin, 2004; Beretta *et al.*, 2005; D'Arcy, 2005; Inoue *et al.*, 2005; Blasa *et al.*, 2006; Nagai *et al.*, 2006; Pérez *et al.*, 2007; Brudzynski and Miotto, 2011; Boukraâ, 2014). Dark honeys have more phenolic compounds and consequently, they are supposed to show a higher antioxidant activity (Gheldof *et al.*, 2002; Beretta *et al.*, 2005; Bertoncelj *et al.*, 2007; Vela *et al.*, 2007; Mărghitaş *et al.*, 2009; Piljac-Zegarac *et al.*, 2009; Pyrzynska and Biesaga, 2009; Escuredo *et al.*, 2013; D'Oliveira-Sant'Ana *et al.*, 2014).

Antioxidant substances have different mechanisms of action, among them decrease of the adverse consequences of reactive oxygen and nitrogen species, inhibit the enzymes responsible for producing superoxide anions, metal chelation, radical chain reactions breaking, and eventually, they can play a preventive role inhibiting the reactive oxidants from being formed (Ou *et al.*, 2005; Pyrzynska and Biesaga, 2009).

Antioxidant activity of honey depends on its botanical source, and moreover such factors as environmental and seasonal changes have a strong influence on this property (Frankel *et al.*, 1998; Al-Mamary *et al.*, 2002; Gheldof *et al.*, 2002; Baltrusaityte *et al.*, 2007; Küçük *et al.*, 2007; Vela *et al.*, 2007; Ulsoy *et al.*, 2010), as well as the packaging material, processing ways and storage conditions (Gheldof *et al.*, 2002; Wang *et al.*, 2004; Díaz-Moreno, 2009). Several researchers have studied the evolution of antioxidant activity of honeys with time, leading to contradictory results. After six months of storage, some papers describe that antioxidant activity of honey decreased with time and/or after heating (Nagai *et al.*, 2001; Wang *et al.*, 2004; Díaz-Moreno, 2009), whereas in other study it increased with temperature and storage time (Turkmen *et al.*, 2006). Honey's antioxidant capacity was significantly lower after being stored at room temperature throughout one year (Saric *et al.*, 2012). With regard to raw and processed honeys, antioxidant activity of both of them after storage was described as analogous (Wang *et al.*, 2004).

The advantages of honey as a protective agent against liver damage, radiation, inflammation, emotional tension, and other pathologies related to oxidative stress, have been recently reviewed (Boukraâ, 2014; Bogdanov, 2015). Up-to-date research has highlighted that, because of its antioxidant activity, honey could play an interesting role in the management of oxidative stress-associated chronic diseases (Erejuwa *et al.*, 2011; Erejuwa *et al.*, 2012a; Boukraâ, 2014).

4.3. Antimicrobial and anti-parasite activity of honeys

Since 1892 honey has been described as a food with antibacterial activity (Paulus *et al.*, 2012). Nowadays, due to the fact that there are many microbial strains resistant to antibiotics, the possible uses of honey as antibacterial agent are increasing (Cooper *et al.*, 2002 a, b), both to preserve other foods (Taormina *et al.*, 2001; Mundo *et al.*, 2004; Nagai *et al.*, 2006; Krusna *et al.*, 2007), and in medicine applications (Efem, 1988; Molan, 1999; Molan, 2001; The National Honey Board, 2002; Oelschlaegel *et al.*, 2012b). For clinical uses, honey is previously sterilized, generally by gamma irradiation (Postmes *et al.*, 1995), so that potentially present spores are destroyed (Paulus *et al.*, 2012).

There are many bacteria sensitive to honey (*Staphylococcus aureus, Escherichia coli, Bacillus subtilis*, *Pseudomonas aeruginosa*, *Paenibacillus larvae*, as well as some *Streptococcus* sp., *Salmonella* sp., *Shigella* sp., and *Proteus* sp., among others), most of them Gram-positive, and many of them pathogenic (Molan, 1992a,b; Bogdanov, 1997; Kwakman *et al.*, 2010). As an

antibacterial agent, honey has been reported to possess bacteriostatic and bactericidal effects, both optimum in fresh and unheated honeys. The former mainly depends on honey concentration, whereas bactericidal action depends both on honey concentration and time of action (Bogdanov, 2015).

In addition to its natural antibacterial activity, Maddocks and Jenkins (2013) demonstrated that honey showed anitivirulent effects against microorganisms, reducing their ability to obtain iron from their host, thus helping minimize progression of infections.

Apart from antibacterial activity, antimicrobial activity of honey against fungi, yeasts, some viruses and parasites has also been researched.

With regard to the activities of honeys against fungi and yeasts, there are studies that show that, according to their composition, several honeys possess antifungal capacity (Al-Waili *et al.*, 2013). More specifically, some honeys were reported to have activity against dermatophytes (Molan, 2001; Bogdanov, 2015). In the research carried out by Abdelmonem *et al.* (2012), vulvovaginal candidiasis of pregnant women was successfully controlled with a mixture of honey and yogurt. Furthermore, as food, honey showed immunomodulator effect against invasive aspergillosis suffered by mice (Nikaein *et al.*, 2014). Taking into account geographical and botanical origins of honeys, the activity of different Turkish samples was assessed against several *Candida* and *Trichosporon* strains of yeasts (Koc *et al.*, 2009). Other studies revealed that some Chilean *Quillaja saponaria* honeys had both antibacterial and antifungic activities (Montenegro *et al.*, 2009). At concentrations higher than 10% several unifloral honeys from Slovakia were active against *Penicillium* species (Kacaniová *et al.*, 2011). Some Iranian samples also demonstrated capacity against *Candida*, *Aspergillus* and *Trichophyton rubrum* (Katiraee *et al.*, 2014).

In respect of the possible antiviral activity of honey, different investigations reported that this food could be efficient against Rubella virus (Zeina *et al.*, 1996) and Herpes virus (Al-Waili, 2004; Hashemipour *et al.*, 2014).

Honey has also exhibited activity against several parasites, such as *Leishmania* (Zeina *et al.*, 1997) and the nematode *Caenorhabditis elegans* (Sajid and Azim, 2012), being the major nematicidal component a glycoconjugate with the molecular mass of 5511.

Honeys' antimicrobial activity depends on botanical sources, honeybees' metabolism, and environmental, seasonal and climatic conditions, which have a strong influence on physical and chemical properties of this food (Basualdo *et al.*, 2007). Antimicrobial components of honeys and their effectiveness have been described and summarized by several researchers (Molan, 1992 a, b; Molan, 1997; Taormina *et al.*, 2001; Sherlock *et al.*, 2010; Al Waili *et al.*, 2011; Paulus *et al.*, 2012; Boukraâ, 2014; Bogdanov, 2015).

In general, honeys' antibacterial compounds can be divided into those with peroxide action and those with non-peroxide action.

Hydrogen peroxide is the component responsible for honey's antibacterial peroxide activity and was the first antibacterial compound studied in this food (Adcock, 1962; White *et al.*, 1963; Molan, 1992b; Taormina *et al.*, 2001; Brudzynski, 2006). It is produced under aerobic conditions from glucose by the action of glucose oxidase (White and Subers, 1963), and its possible function is preventing unripe honey spoilage (Paulus *et al.*, 2012). Antibacterial activity of honeys' hydrogen peroxide accumulation was, at first, expressed as inhibine (White *et al.*, 1963; Dustmann, 1979). According to Paulus *et al.* (2012), a possible explanation for the different hydrogen peroxide accumulation in honeys could be due to differences in activity of glucose oxidase. The peroxide antibacterial activity of honey is sensitive to heat, light and storage (White and Subers, 1964 a, b; Dustmann, 1972; Bogdanov, 1997), so that Bogdanov (2015) has recommended honey's storage in cool and dark places, and honey's consumption when fresh.

Studies carried out by Allen *et al.* (1991) and Mundo *et al.* (2004) showed that after neutralizing hydrogen peroxide, some honeys still had antibacterial activity, demonstrating that there were other honey compounds with antimicrobial properties, as it had been previously suggested by Lavie and Grassé (1963). Non-peroxide antimicrobial activity of honey is less sensitive to heat and light (Bogdanov, 1984; Bogdanov and Blumer, 2001). Methylglyoxal and royalisin (bee defensin-1) have been reported as the main compounds responsible for the non-peroxide honey's antibacterial activity. Manuka honeys from *Leptospermum scoparium* bushes (native to New Zealand and Australia), are known to contain very high levels of methylglyoxal (Mavrick *et al.*, 2008), which is formed nonenzymatically from manuka nectar's dihydroxyacetone (Adams *et al.*, 2009) during honey storage (Paulus *et al.*, 2012). Methylglyoxal has also been found in honeys from different botanical sources, but its concentrations have been considerably lower as those of manuka honeys (Paulus *et al.*, 2012). Antibacterial activity of manuka honeys varies among different batches (Allen *et al.*, 1991; Oelschlaegel *et al.*, 2012a), so that each manuka honey batch must be analysed for antibacterial activity, which is usually expressed as "unique manuka factor" (UMF). UMF represents the concentration of a phenol solution that yields a similar zone of Staphylococcus aureus growth inhibition as the honey tested, by using a radial diffusion assay (Allen *et al.*, 1991). Manuka honeys were also reported to exhibit inhibitory effects against the influenza virus (Watanabe *et al.*, 2014) and methylglyoxal, in particular, showed effects against HIV1 virus (Behbahani, 2014).

Royalisin is a peptide secreted by the honeybee hypopharingeal gland that has demonstrated a strong activity against Gram-positive bacteria (Kwakman *et al.*, 2011; Paulus *et al.*, 2012). In honeys in which methylglyoxal had been neutralized, royalisin was reported to show additive antibacterial activity with other antibacterial components such as hydrogen peroxide, sugars

and/or low pH (Paulus *et al.*, 2012). Other proteinaceous substances with antibacterial activity were found in some honeys (Mundo *et al.*, 2004; Gallardo-Chacón *et al.*, 2008). Qualitative and quantitative analysis of royalisin and antibacterial proteinaceous compounds in different unifloral and multifloral honeys is interesting for future research about antibacterial activity of this food.

Low honey pH and acidic substances such as aromatic acids and royal jelly acids, have been reported to play an important role on antibacterial activity of honeys (Bogdanov, 1997; Kwakman *et al.*, 2010; Isidorov *et al.*, 2011; Paulus *et al.*, 2012). Furthermore, the low water activity of honeys and the osmolality due to the high sugar amount also contribute to antimicrobial activity of this food (White, 1979b; Münstedt *et al.*, 2011; Boukraâ, 2014; Bogdanov, 2015). Maillard reaction products (Brudzynski and Kim, 2011; Brudzynski and Miotto, 2011) have shown antibacterial activities, as well as lysozyme (León-Ruiz *et al.*, 2013a) and the combination of honey phenolic compounds (Molan, 1992a; Aljadi and Yusoff, 2003; Truchado *et al.*, 2009b; Bogdanov, 2015). Honey flavonoids have also been reported to have antifungal activity against *Candida albicans* (Candiracci *et al.*, 2011).

When cultured in vitro, some bacteria present in honey (Lee *et al.*, 2008 a, b), produce such antimicrobial agents as bacillomycin F, or antifungal peptides (Zhao *et al.*, 2013).

Antimicrobial activity of honey can also be indirect, because this food has been reported to show immune-activating, anti-inflammatory and prebiotic effects (Bogdanov, 2015).

4.4. Antihypertensive activity of honeys

Hypertension is one of the most important cardiovascular risk factors (Poulter, 2003), because continuous elevation of blood pressure produces changes in the myocardial structure and coronary vasculature, leading to left ventricular hypertrophy with such heart dysfunctions as arrhythmias and congestive heart failure, among others (Standridge, 2005). Cardiovascular diseases are the main cause of death globally (Erejuwa *et al.*, 2012a). Therefore, hypertension has become a relevant health problem, because blood pressure increases with aging and nowadays life expectancy is longer.

Recent research has shown that honey and honey-derivative intakes can help reduce hypertension risk. Hiwatashi *et al.* (2010) carried out a study with spontaneously hypertensive rats, feeding them with a honey-based beverage containing γ-aminobutyric acid(GABA)-fermented rice bran. They observed beneficial effects against hypertension after 7 weeks, attributing those effects to GABA.

Erejuwa *et al.* (2011, 2012b) researched the potential antihypertensive effects of honey intakes on spontaneously hypertensive rats. They observed that honey supplementation significantly reduced renal malondialdehyde levels, as well as systolic blood pressure via

amelioration of oxidative stress in the kidney of the rats. More specifically, Malaysian tualang honey were able to reduce systolic blood pressure in diabetic spontaneously hypertensive rats.

Inhibition of angiotensin-I-converting enzyme (ACE) is one of the most well-known antihypertensive mechanisms. ACE catalyses the conversion of angiotensin I to the strong vasoconstrictor angiotensin II. *Echium vulgare* honeys showed potent ACE inhibition by spectrophotometric analysis at 228 nm (Nagai *et al.*, 2012). Another study carried out with 20 Spanish honeys from the autonomous community of Castilla-La Mancha, whose ACE inhibitory capacity had been determined by HPLC with ultraviolet detection, revealed that rosemary (*Rosmarinus officinalis* L.), lavender (*Lavandula latifolia* Medik.), thyme (*Thymus vulgaris* L.) and chestnut (*Castanea sativa* Mill.) honeys evidenced great ACE inhibitory activity variations, generally ranging from about 13% to 71%, being chestnut honeys the ones which showed the highest antihypertensive capacity (León-Ruiz *et al.*, 2013a). These researchers (León-Ruiz *et al.*, 2013b), did not find relationships between ACE inhibitory capacity and antioxidant activities of the honeys studied, but they suggested that the biological activities of honey melanoidins should be studied in relation with their potential antihypertensive activity.

4.5. Anti-inflammatory activity of honeys

As well as hypertension, inflammation is considered one of the main causes of cardiovascular risk and other illnesses (Willerson and Ridker, 2004). Reactive oxygen species are important factors responsible for the injuries during inflammation (Singer and Clark, 1999), because they help release free oxygen radicals, which, in turn, induce proinflammatory cytokines (Feldmann and Steinman, 2005; Tracey *et al.*, 2008).

Honey has shown that can reduce inflammation in several experiments carried out with laboratory animals (Owoyele *et al.*, 2014; Bogdanov, 2015). In wounds with no bacterial infection, honey proved to exhibit anti-inflammatory activity (Postmes, 2001). Honey intake by rats with bowel disease was an efficient treatment for inflammatory colitis, possibly due to the prevention of free radicals production that, according to the researchers, could be also indirectly related to the antibacterial activity of this food (Bilsel *et al.*, 2002). Kassim *et al.* (2010a, b) showed that in rats both honey and its extracts could inhibit edema, producing inhibitory activities against inflammatory mediators. In rabbits, artificial inflammation was reduced by honey, probably because of a drop of both neutrophils infiltration and myeloperoxidase activity (Kassim *et al.*, 2012). Gelam honey from Malaysia attenuated carrageenan-induced rat paw inflammation (Hussein *et al.*, 2013).

In humans, honey ingestion demonstrated to be able to reduce such inflammatory mediators as thromboxane and prostaglandins (Al-Waili and Boni, 2003). In vitro synthesis of human neutrophil superoxide was decreased by New Zealand rewarewa, manuka and kanuka honeys (Leong *et al.*, 2012).

Anti-inflammatory activity of honeys has been attributed to flavonoids (Candiracci *et al.*, 2012), which could inhibit the delivery of proinflammatory cytokines, as well as the expressions of the inducible nitric oxide synthase and the production of reactive oxygen species. Woo *et al.* (2005) and Kao *et al.* (2010) demonstrated that such flavonoids as chrysin and quercetin had similar anti-inflammatory activities but with different mechanisms. However, according to Farooqui and Farooqui (2014), the molecular mechanisms of flavonoids' anti-inflammatory activity have not been clarified yet. Further research is needed to study the potentially interesting anti-inflammatory activity of honey.

4.6. Probiotic and prebiotic properties of honey

Fresh honey has been reported to have probiotic *Bifidus* and *Lactobacilus* bacteria (Olofsson and Vasquez, 2008) that are beneficial for human health.

Prebiotics are food ingredients that potentially stimulate the activity of the gut flora, altering its composition and providing energy to selected microbial species (Abdellah and Abderrahim, 2014). Possible prebiotics effects of honeys have been attributed to oligosaccharides, whose actions have been proved to be similar to that of fructooligosaccharides (Yun, 1996; Sanz *et al.*, 2005). Panose has been described as the most active oligosaccharide. The mechanism of action of honey oligosaccharides appears to be synergistic, leading to an increase of lactobacilli and bifidobacteria (Ustunol, 2000; Ustunol and Gandhi, 2001). Nevertheless, the study carried out by Popa and Ustunol (2011), questioned the probiotic effects of honey oligosaccharides, because other sweeteners, apart from several unifloral honeys containing different oligosaccharide amounts, supported the growth, activity and viability of lactic acid bacteria and bifidobacteria.

Conversely to sucrose, honey proved to increase in vitro and in vivo the population of *Lactobacillus acidophilus* and *Lactobacillus plantarum* of rats (Shamala *et al.*, 2000). An in vitro research using five bifidobacteria strains, showed that honey provided with a growth-promoting effect comparable to that of fructose and glucose oligosaccharides (Kajiwara *et al.*, 2002). In addition, the lactobacilli in the small intestine could well be affected advantageously by honey components, such as sugars (Haddadin *et al.* 2007).

Honey exhibited prebiotic action towards three Lactobacillus species isolated from human faeces (Tejpal and Goyal, 2009). Furthermore, the growth, activity and viability of lactobacilli could be enhanced by adding honey to dairy products (Altman, 2010).

In respect of botanical origins, sour-wood, alfalfa, sage and clover honey showed prebiotic capacity (Kajiwara *et al.*, 2002), the three first of them stimulating the growth of five human intestinal bifidobacteria, as well (Shin and Ustunol, 2005). Honeydew honeys were also described to contain prebiotic oligosaccharides (Sanz *et al.*, 2005). Lucan *et al.* (2009) observed that, comparing to acacia honeys, chestnut samples better enhanced the growth and

acidity of *Bifidobacterium lactis*, finding, as well, inhibitory potential effects of honey-sweetened fermented goat and cow milk against a strain of Listeria monocytogenes. Abdellah and Abderrahim (2014), claimed that several honey compounds might also inhibit the development of pathogens such as Helicobacter pylori or Staphylococcus aureus. The possible mechanism of action would be the attachment of oligosaccharides to the cell walls of the bacteria, preventing adhesion to human tissues.

However, it is still unclear if all honeys have prebiotic effects and, if so, whether some honeys have a stronger prebiotic activity (Bogdanov, 2015), so future research is needed.

5. Honey as environmental bioindicator

Honey, as a result of a bio-accumulative process, has been used as biomonitor for collecting information about the environment, identifying environmental contamination (Jones, 1987; Kacaniová *et al.*, 2009) and characterizing the level of soil, water, plant and air pollution (Fodor and Molnar, 1993).

5.1. Honey as indicator of radioactive contamination

Despite the fact that honey has been used as an indicator of radioactive contamination near nuclear power plants or in different places after radioactive accidents, Gilber and Lisk (1978) did not detect beta or gamma emission in any of the sample collected in the vicinity of a nuclear reprocessing plant. Tonelly *et al.* (1990) claimed that, among bee products, honey was the worst indicator of radioactive environmental contamination, being bee pollen the best one.

5.2. Honey as indicator of minerals and heavy metals contamination

Honey has been studied as a mineral content environmental indicator, having been considered as a potential pollution indicator of the concentration of heavy metal in different zones such as rural, urban and industrial areas, as well as extraurban crossroads (Enrich *et al.*, 2007; Popa *et al.*, 2013). Jones (1987) found low and variable concentrations of heavy metals in honey, attributing the variability to factors such as the floral source, season, time of year, and rainfall, among others. For this reason, he concluded that honey could not be used as a reliable and sensitive indicator. Fakhimzadeh and Lodenius (2000) agreed, also claiming that bees themselves were better bioindicators of industrial and urban heavy metal pollution. Conversely, other researchers reported higher concentrations of heavy metals in honeys from urban and industrial polluted areas, than in honeys from unpolluted rural zones (Fodor and Molnar, 1993; Rodríguez-García *et al.*, 2006; Rashed *et al.*, 2009; Lambert *et al.*, 2012). Furthermore, Leita *et al.* (1996) found a linear relationship between Cd and honey.

5.3. Honey as indicator of pesticides contamination

Rissato *et al.* (2007) and Malhat *et al.* (2015) performed a multiresidue analysis in honey for monitoring pesticides' residues in several regions. Balayiannis and Balayiannis (2008) investigated the pollution of different agricultural areas of Greece by insecticides, using honey from those areas as bioindicator. Panseri *et al.* (2014) found residues in samples harvested in industrial areas. On the contrary, these authors did not find residues in honeys coming from organic production zones. They concluded that honey's pesticide contamination was closely related to the contamination source, being able to reveal the precise environmental pollutant.

5.4. Honey as indicator of other environmental pollutants

Ponikvar *et al.* (2005) showed that the amounts of sulphate in honey depended on the emissions of SO_2. Fermo *et al.* (2013) found higher concentrations of some cations and anions in the Western Balkans than in Italian regions, attributing this fact to local industrial and agricultural activities.

REFERENCES

ABDELLAH, F; ABDERRAHIM L A (2014) Honey for gastrointestinal disorders. In *Boukraâ, L (Ed). Honey in Traditional and Modern Medicine.* CRC Press Taylor & Francis Group; Boca Raton (FL), USA. pp. 159-186. http://dx.doi.org/10.1201/b15608-9

ABDELMONEM, A M; RASHEED, S M; MOHAMED, A S (2012) Bee-honey and yogurt: a novel mixture for treating patients with vulvovaginal candidiasis during pregnancy. *Archives of Gynecology and Obstetrics* 286(1): 109-114. http://dx.doi.org/10.1007/s00404-012-2242-5

ABRAMOVIC, H; JAMNIK, M; BURKAN, L; KAC, M (2008) Water activity and water content in Slovenian honeys. *Food Control* 19(11): 1086-1090. http://dx.doi.org/10.1016/j.foodcont.2007.11.008

ACCORTI, M; PERSANO-ODDO, L; PIAZZA, M G; SABATINI, A G (1986) Schede di caratterizzazione delle principali qualità di miele italiano. *Apicoltura* 2: 5-35.

ADAMS C J; MANLEY-HARRIS M; MOLAN P C (2009) The origin of methylglyoxal in New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydrate Research* 344(8): 1050-1053. http://dx.doi.org/10.1016/j.carres.2009.03.020

ADCOCK, D (1962) The effect of catalase on the inhibine and peroxide values of various honeys. *Journal of Apicultural Research* 1(1): 38-40. http://dx.doi.org/10.1080/00218839.1962.11100047

AJIBOLA, A; CHAMUNORWA, J; ERLWANGER, K H (2012) Nutraceutical values of natural honey and its contribution to human health and wealth. *Nutrition & Metabolism* 9: 61-73. http://dx.doi.org/10.1186/1743-7075-9-61

AL WAILI, N S; SALOM, K; BUTLER, G; AL GHAMDI, A A (2011) Honey and microbial infections: a review supporting the use of honey for microbial control. *Journal of Medicinal Food* 14(10): 1079-1096. http://dx.doi.org/10.1089/jmf.2010.0161

ALDA-GARCILOPE, C; GALLEGO-PICÓ, A; BRAVO-YAGÜE, J C; GARCINUÑO-MARTÍNEZ, R M; FERNÁNDEZ-HERNANDO, P (2012) Characterization of Spanish honeys with protected designation of origin ‘‘Miel de Granada’’ according to their mineral content. *Food Chemistry* 135(3): 1785-1788. http://dx.doi.org/10.1016/j.foodchem.2012.06.057

ALISSANDRAKIS, E; DAFERERA, D; TARANTILIS, P A; POLISSIOU, M; HARIZANIS, P C (2003) Ultrasound-assisted extraction of volatile compounds from citrus flowers and citrus honey. *Food Chemistry* 82(4): 575-582. http://dx.doi.org/10.1016/S0308-8146(03)00013-X

ALISSANDRAKIS, E; TARANTILIS, P A; PAPPAS, C; HARIZANIS, P C; POLISSIOU, M (2011) Investigation of organic extractives from unifloral chestnut (*Castanea sativa* L.) and eucalyptus (*Eucalyptus globulus* Labill.) honeys and flowers to identification of botanical marker compounds. *LWT-Food Science and Technology* 44(4): 1042-1051. http://dx.doi.org/10.1016/j.lwt.2010.10.002

ALJADI, A M; YUSOFF K M (2003) Isolation and identification of phenolic acids in Malaysian honey with antibacterial properties. *Turkish Journal of Medical Sciences* 33(4): 229-236.

ALJADI, A M; KAMARUDDIN, M Y (2004) Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chemistry* 85(4): 513-518. http://dx.doi.org/10.1016/S0308-8146(02)00596-4

AL-KHALIDI, A; JAWAD, F H; TAWFIQ, N H (1980) Effects of bees honey, zahdi dates and its syrup on blood glucose and serum insulin of diabetics. *Nutrition reports international* 21(5): 631-643.

ALLEN, K L; MOLAN, P C; REID G M (1991) A survey of the antibacterial activity of some New Zealand honeys. *Journal of Pharmacy and Pharmacology* 43(12): 817-822. http://dx.doi.org/10.1111/j.2042-7158.1991.tb03186.x

AL-MAMARY, M; AL-MEERI, A; AL-HABORI, M (2002) Antioxidant activities and total phenolics of different types of honey. *Nutrition Research* 22(9): 1041-1047. http://dx.doi.org/10.1016/S0271-5317(02)00406-2

ALMEIDA-MURADIAN, L B; STRAMM, K M; HORITA, A; BARTH, O M; FREITAS, A S; ESTEVINHO, L M (2013) Comparative study of the physicochemical and palynological characteristics of honey from *Melipona subnitida* and *Apis mellifera*. *International Journal of Food Science and Technology* 48(8): 1698-1706. http://dx.doi.org/10.1111/ijfs.12140

ALMEIDA-MURADIAN, L B; STRAMM, K M; ESTEVINHO, L M (2014) Efficiency of the FT-IR ATR spectrometry for the prediction of the physicochemical characteristics of *Melipona subnitida* honey and study of the temperature’s effect on those properties. *International Journal of Food Science and Technology* 49(1): 188-195. http://dx.doi.org/10.1111/ijfs.12297

ALONSO-TORRE, S R; CAVIA, M M; FERNÁNDEZ-MUIÑO, M A; MORENO, G; HUIDOBRO, J F; SANCHO, M T (2006) Evolution of acid phosphatase activity of honeys from different climates. *Food Chemistry* 97(4): 750-755. http://dx.doi.org/10.1016/j.foodchem.2005.06.010

ALTMAN, N (2010) *The honey prescription: the amazing power of honey as medicine*. Healing Arts Press. Inner Traditions, Bear & Company; Rochester (VT), USA. 243 pp.

ALVAREZ-SUAREZ, J M; TULPANI, S; ROMANDINI, S; VIDAL, A; BATTINO, M (2009) Methodological aspects about determination of phenolic compounds and in vitro evaluation of antioxidant capacity in the honey: A review. *Current Analytical Chemistry* 5(4): 293-302. http://dx.doi.org/10.2174/157341109789077768

ALVAREZ-SUAREZ, J M; GASPARRINI, M; FORBES-HERNÁNDEZ, T Y; MAZZONI, L; GIAMPIERI, F (2014) The composition and biological activity of honey: A focus on Manuka honey. *Foods* 3: 420-432. http://dx.doi.org/doi:10.3390/foods3030420

AL-WAILI, N S (2003) Effects of daily consumption of honey solution on hematological indices and blood levels of minerals and enzymes in normal individuals. *Journal of Medicinal Food* 6(2): 135-140. http://dx.doi.org/10.1089/109662003322233549

AL-WAILI, N S (2004) Topical honey applications *vs.* acyclovir for the treatment of recurrent herpes simplex lesions. *Medical Science Monitor* 10(8): 94-98.

AL-WAILI, N S; BONI, N S (2003) Natural honey lowers plasma prostaglandin concentrations in normal individuals. *Journal of Medicinal Food* 6(2): 129-133. http://dx.doi.org/10.1089/109662003322233530

AL-WAILI, N; AL-GHAMDI, A; JAVED ANSARI, M; AL-ATTAL, Y; AL-MUBARAK, A; SALOM, K (2013) Differences in composition of honey samples and their impact on the antimicrobial activities against drug multiresistant bacteria and pathogenic fungi. *Archives of medical research* 44(4): 307-316. http://dx.doi.org/10.1016/j.arcmed.2013.04.009

AMIOT, M J; AUBERT, S; GONNET, M; TACCHINI, M (1989) Les composes phénoliques des miels: étude préliminaire sur l'identification et la quantification par familles. *Apidologie* 20(2): 115-125. http://dx.doi.org/10.1051/apido:19890202

AMIR, Y; YESLI, A; BENGANA, M; SADOUDI, R; AMROUCHE, T (2010) Physico-chemical and microbiological assessment of honey from Algeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 9(9): 1485-1494.

ANAM, O O; DART, R K (1995) Influence of metal ions on hydroxymethylfurfural formation in honey. *Analytical Proceedings Including Analytical Communications* 32: 515-517. http://dx.doi.org/10.1039/AI9953200515

ANANIAS, K R; DE-MELO, A A M; MOURA, C J (2013) Analysis of moisture content, acidity and contamination by yeast and molds in *Apis mellifera* L. honey from central Brazil. *Brazilian Journal of Microbiology* 44(3): 679-683. http://dx.doi.org/10.1590/S1517-83822013000300003

ANDRADE, P; FERRERES, F; AMARAL, M T (1997a) Analysis of honey phenolic acids by HPLC, its application to honey botanical characterization. *Journal of Liquid Chromatography & Related Technologies* 20(14): 2281-2288. http://dx.doi.org/10.1080/10826079708006563http://dx.doi.org/

ANDRADE, P; FERRERES, F; GIL, M I; TOMÁS-BARBERÁN, F A (1997b) Determination of phenolic compounds in honeys with different floral origin by capillary zone electrophoresis. *Food Chemistry* 60(1): 79-84. http://dx.doi.org/10.1016/S0308-8146(96)00313-5

ANKLAM, E (1998) A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry* 63(4): 549-562. http://dx.doi.org/10.1016/S0308-8146(98)00057-0

ANTONY, S M; RIECK, J R; DAWSON, P L (2000) Effect of dry honey on oxidation in turkey breast meat. *Poultry Science* 79(12): 1846-1850. http://dx.doi.org/ 10.1093/ps/79.12.1846

ARCOT, J; BRAND-MILLER, J (2005) A preliminary assessment of the glycemic index of honey. *Rural Industries Research and Development Corporation; Australian Government*. Publication Nº 05/027. 28 pp. https://rirdc.infoservices.com.au/downloads/05-027.pdf (Accessed 12/04/2015)

ARGENTINA. Código Alimentario Argentino of February 2008 in Capitulo 10: Alimentos azucarados. Ministerio da la Salud. Administración Nacional de Medicamentos, Alimentos y Tecnología Médica. 72 pp.

ARIEFDJOHAN, M W; MARTIN, B R; LACHCIK, P J; WEAVER, C M (2008) Acute and chronic effects of honey and its carbohydrate constituents on calcium absorption in rats. *Journal of Agricultural and Food Chemistry* 56: 2649-2654. http://dx.doi.org/10.1021/jf073357w

ASSIL, H I; STERLING, R; SPORNS, P (1991) Crystal control in processed liquid honey. *Journal of Food Science* 56(4): 1034-1041. http://dx.doi.org/ 10.1111/j.1365-2621.1991.tb14635

BALAYIANNIS, G; BALAYIANNIS, P (2008) Bee Honey as an Environmental Bioindicator of Pesticides' Occurrence in Six Agricultural Areas of Greece. *Archives of Environmental Contamination and Toxicology* 55(3): 462-470. http://dx.doi.org/10.1007/s00244-007-9126-x

BALTRUSAITYTĖ, V; VENSKUTONIS, P R; CEKSTERYTĖ, V (2007) Radical scavenging activity of different floral origin honey and beebread phenolic extracts. *Food Chemistry* 101(2): 502-514. http://dx.doi.org/ 10.1016/j.foodchem.2006.02.007

BASUALDO, C; SGROY, V; FINOLA, M S; MARIOLI, J M (2007) Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Veterinary Microbiology* 124(3-4): 375-381. http://dx.doi.org/ 10.1016/j.vetmic.2007.04.039

BEHBAHANI, M (2014) Anti-HIV-1 activity of eight monofloral Iranian honey types. *Plos One* 9(10): e108195. http://dx.doi.org/ 10.1371/journal.pone.0108195

BELAY, A; SOLOMON, W K; BULTOSSA, G; ADGABA, N; MELAKU, S (2013) Physicochemical properties of the Harenna forest honey, Bale, Ethiopia. *Food Chemistry* 141(4): 3386-3392. http://dx.doi.org/10.1016/j.foodchem.2013.06.035

BELITZ, H -D; GROSCH, W; SCHIEBERLE, P (2009) *Food Chemistry (4th revised and extended edition)*. Springer; Berlin, Germany. 1170 pp. http://dx.doi.org/10.1007/978-3-540-69934-7

BERETTA, G; GRANATA, P; FERRERO, M; ORIOLI, M; FACINO, R M (2005) Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytica Chimica Acta* 533(2): 185-191. http://dx.doi.org/ 10.1016/j.aca.2004.11.010

BERGNER, K G; HAHN, H (1972) Zum vorkommen und zur herkunft der freien aminosäuren in honig. *Apidologie* 3(1): 5-34. http://dx.doi.org/10.1051/apido:19720101

BERTONCELJ, J; DOBERŠEK, U; JAMNIK, M; GOLOB, T (2007) Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chemistry* 105(2): 822-828. http://dx.doi.org/10.1016/j.foodchem.2007.01.060

BERTONCELJ, J; GOLOB, T; KROPF, U; KOROSEC, M (2011) Characterisation of Slovenian honeys on the basis of sensory and physicochemical analysis with a chemometric approach. *International Journal of Food Science and Technology* 46: 1661-1671. http://dx.doi.org/10.1111/j.1365-2621.2011.02664.x

BHANDARI, B; D'ARCY, B; KELLY, C (1999) Rheology and crystallization kinetics of honey: present status. *International Journal of Food Properties* 2(3): 217-226. http://dx.doi.org/ 10.1080/10942919909524606

BIANCHI, F; CARERI, M; MUSCI, M (2005) Volatile norisoprenoids as markers of botanical origin of Sardinian strawberry-tree (*Arbutus unedo* L.) honey: characterization of aroma compounds by dynamic headspace extraction and gas chromatography-mass spectrometry. *Food Chemistry* 89(4): 527-532. http://dx.doi.org/10.1016/j.foodchem.2004.03.009

BIINO, L (1971) Ricerca di alcuni aminoacidi in due varieta di miele. *Rivista Italiana delle Essenze e Profumi* 53: 80-84.

BILSEL, Y; BUGRA, D; YAMANER, S; BULUT, T; CEVIKBAS, U; TURKOGLU, U (2002) Could honey have a place in colitis therapy? Effects of honey, prednisolone, and disulfiram on inflammation, nitric oxide, and free radical formation. *Digestive Surgery* 19(4): 306-311. http://dx.doi.org/ 10.1159/000064580

BLASA, M; CANDRACCI, M; ACCORSI, A; PIACENTINI, M P; ALBERTINI, M C; PIATTI, E (2006) Raw millefiori honey is packed full of antioxidants. *Food Chemistry* 97: 217-222. http://dx.doi.org/10.1016/j.foodchem.2005.03.039

BOGDANOV, S (1984) Characterisation of antibacterial substances in honey. *Lebensmittel-Wissenschaft und Technologie* 17: 74-76.

BOGDANOV, S (1997) Nature and origin of the antibacterial substances in honey. *Lebensmittel-Wissenschaft und Technologie* 30(7): 748-753. http://dx.doi.org/10.1006/fstl.1997.0259

BOGDANOV, S (2011a) Honey Technology. In *Bogdanov, S (Ed). The Honey Book*. pp. 15-18. http://www.bee-hexagon.net/honey/ (Accessed 12/04/2015).

BOGDANOV, S (2011b) Physical Properties. In *Bogdanov, S (Ed). The Honey Book*. pp. 19-27. http://www.bee-hexagon.net/honey/ (Accessed 12/04/2015).

BOGDANOV, S (2011c) Honey Composition. In *Bogdanov, S (Ed). The Honey Book*. pp. 27-36. http://www.bee-hexagon.net/honey/ (Accessed 12/04/2015).

BOGDANOV, S (2015) Honey as Nutrient and Functional Food. In *Bogdanov, S (Ed). The Honey Book*. http://www.bee-hexagon.net/files/file/fileE/HealthHoney/8HoneyNutrientFunctionalReview.pdf (Accessed 01/03/2015).

BOGDANOV, S; LÜLLMAN, C; MARTIN, P; VON DER OHE, W; RUSSMANN, H; VORWOHL, G; PERSANO-ODDO, L; SABATINI, A G; MARCAZZAN, G L; PIRO, R; FLAMINI, C; MORLOT, M; HERITIER, J; BORNECK, R; MARIOLEAS, P; TSIGOURI, A; KERKVLIET, J; ORTIZ, A; IVANOV, T; D'ARCY, B; MOSSEL, B; VIT, P (1999) Honey quality and international regulatory standards: review by the international honey commission. *Bee World* 80(2): 61-69. http://dx.doi.org/ 10.1080/0005772X.1999.11099428

BOGDANOV, S; BLUMER, P (2001) Natürliche antibiotische Eigenschaften des Honigs. *Schweizerische Bienen-Zeitung* 124(2): 18-21.

BOGDANOV, S; MARTIN, P (2002) Honey authenticity: a review. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene* 93: 232-254.

BOGDANOV, S; RUOFF, K; PERSANO-ODDO, L (2004) Physico-chemical methods for the characterization of unifloral honeys: a review. *Apidologie* 35(1): S4-S17. http://dx.doi.org/ 10.1051/apido:2004047

BOGDANOV, S; JURENDIC, T; SIEBER, R; GALLMANN, P (2008) Honey for nutrition and health: A review. *Journal of the American College of Nutrition* 27(6): 677-689.http://dx.doi.org/10.1080/07315724.2008.10719745

BOUKRAÂ, L (2014) Healing properties of honey. In *Boukraâ, L (Ed). Honey in Traditional and Modern Medicine.* CRC Press Taylor & Francis Group; Boca Raton (FL), USA. pp. 37-52. http://dx.doi.org/10.1201/b15608-5

BRASIL. Normative Instruction 11/MAPA/Brazil of 20 October 2000 that approved the Technical Regulations of Identity and Quality of Honey. Union Official Diary section 1, 23.10.2000. pp.16-17.

BROWN, K L (2000) Control of bacterial spores. *British Medical Bulletin* 56(1): 158-171. http://dx.doi.org/10.1258/0007142001902860

BRUDZYNSKI, K (2006) Effect of hydrogen peroxide on antibacterial activities of Canadian honeys. *Canadian Journal of Microbiology* 52(12): 1228-1237. http://dx.doi.org/ 10.1139/w06-086

BRUDZYNSKI, K; KIM, L (2011) Storage-induced chemical changes in active components of honey de-regulate its antibacterial activity. *Food Chemistry* 126(3): 1155-1163. http://dx.doi.org/ 10.1016/j.foodchem.2010.11.151

BRUDZYNSKI, K; MIOTTO, D (2011) The relationship between the content of Maillard reaction-like products and bioactivity of Canadian honeys. *Food Chemistry* 124(3): 869-874. http://dx.doi.org/ 10.1016/j.foodchem.2010.07.009

BUBA, F; GIDADO, A; SHUGABA, A (2013) Analysis of biochemical composition of honey samples from North-East Nigeria. *Biochemistry & Analytical Biochemistry* 2(3): 1-7. http://dx.doi.org/10.4172/2161-1009.1000139

CABRAS, P; ANGIONI, A; TUBEROSO, C; FLORIS, I; RENIERO, F; GUILLOU, C; GHELLI, S (1999) Homogentisic acid: A phenolic acid as marker of strawberry-tree (*Arbutus unedo*) honey. *Journal of Agriculture and Food Chemistry* 47(10): 4064-4070. http://dx.doi.org/ 10.1021/jf990141o

CANDIRACCI, M; CITTERIO, B; DIAMANTINI, G; BLASA, M; ACCORSI, A; PIATTI, E (2011) Honey flavonoids, natural antifungal agents against *Candida Albicans*. *International Journal of Food Properties* 14(4): 799-808. http://dx.doi.org/ 10.1080/10942910903453355

CANDIRACCI, M; PIATTI, E; DOMINGUEZ-BARRAGAN, M; GARCIA-ANTRAS, D; MORGADO, B; RUANO, D; GUTIERREZ, J F; PARRADO, J; CASTANO, A (2012) Anti-inflammatory activity of a honey flavonoid extract on lipopolysaccharide-activated N13 microglial cells. *Journal of agricultural and food chemistry* 60(50): 12304-12311. http://dx.doi.org/ 10.1021/jf302468h

CANO, C B; FELSNER, M L; MATOS, J R; BRUNS, R E; WHATANABE, H M; ALMEIDA-MURADIAN, L B (2001) Comparison of methods for determining moisture content of citrus and eucalyptus Brazilian honeys by refractometry. *Journal of Food Composition and Analysis* 14(1): 101-109. http://dx.doi.org/10.1006/jfca.2000.0951

CASTRO-VÁZQUEZ, L; PÉREZ-COELLO, M S; CABEZUDO, M D (2003) Analysis of volatile compounds of Rosemary honey. Comparison of different extraction techniques. *Chromatographia* 57(3): 227-233. http://dx.doi.org/ 10.1007/BF02491721

CASTRO-VÁZQUEZ, L; DÍAZ-MAROTO, M C; GUCHU, E; PÉREZ-COELLO, M S (2006) Analysis of volatile compounds of eucalyptus honey by solid phase extraction followed by gas chromatography coupled to mass spectrometry. *European Food Research and Technology* 224: 27-31. http://dx.doi.org/10.1007/s00217-006-0284-2

CASTRO-VÁZQUEZ, L; DÍAZ-MAROTO, M C; PÉREZ-COELLO, M S (2007) Aroma composition and new chemical markers of Spanish citrus honeys. *Food Chemistry* 103(2): 601-606. http://dx.doi.org/10.1016/j.foodchem.2006.08.031

CASTRO-VÁZQUEZ, L; DÍAZ-MAROTO, M C; GONZÁLEZ-VIÑAS, M A; PÉREZ-COELLO, M S (2009) Differentiation of monofloral citrus, rosemary, eucalyptus, lavender, thyme and heather honeys based on volatile composition and sensory descriptive analysis. *Food Chemistry* 112(4): 1022-1030. http://dx.doi.org/ 10.1016/j.foodchem.2008.06.036

CAVIA, M M (2002) Estudio del envejecimiento de mieles de Burgos y Galicia: influencia de la granulación inducida. PhD Thesis. University of Burgos (Spain). Advisors: Dr. María Teresa Sancho Ortiz, Dr. Miguel Ángel Fernández Muiño and Dr. José Francisco Huidobro Canales.

CAVIA, M M; FERNÁNDEZ-MUIÑO, M A; GÓMEZ-ALONSO, E; MONTES-PÉREZ, M J; HUIDOBRO, J F; SANCHO, M T (2002) Evolution of fructose and glucose in honey over one year: influence of induced granulation. *Food Chemistry* 78(2): 157-161. http://dx.doi.org/ 10.1016/S0308-8146(01)00393-4

CAVIA, M M; FERNÁNDEZ-MUIÑO, M A; HUIDOBRO, J F; SANCHO, M T (2004) Correlation between moisture and water activity of honeys harvested in different years. *Journal of Food Science* 69(5): C368-C370. http://dx.doi.org/ 10.1111/j.1365-2621.2004.tb10699.x

CAVIA, M M; FERNÁNDEZ-MUIÑO, M A; ALONSO-TORRE, S R; HUIDOBRO, J F; SANCHO, M T (2007) Evolution of acidity of honeys from continental climates: influence of induced granulation. *Food Chemistry* 100(4): 1728-1733. http://dx.doi.org/ 10.1016/j.foodchem.2005.10.019

CAVIA, M M; ÁLVAREZ, C; HUIDOBRO, J; FERNÁNDEZ-MUIÑO, M A; SANCHO, M T (2008) Evolution of hydroxymethylfurfural content of honeys from different climates: Influence of induced granulation. *International Journal of Food Sciences and Nutrition* 59(1): 88-94. http://dx.doi.org/ 10.1080/10253890701560176

CHANDRASEKARA, A; SHAHIDI, F (2010) Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. *Journal of Agricultural and Food Chemistry* 58(11): 6706-6714. http://dx.doi.org/ 10.1021/jf100868b

CHEN, H; FAN, C; CHANG, Q; PANG, G; HU, X; LU, M; WANG, W (2014) Chemometric Determination of the botanical origin for chinese honeys on the basis of mineral elements determined by ICP-MS. *Journal of Agricultural and Food Chemistry* 62(11): 2443-2448. http://dx.doi.org/10.1021/jf405045q

CHEN, L; MEHTA, A; BERENBAUM, M; ZANGERL, A R; ENGESETH, N J (2000) Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. *Journal of Agricultural and Food Chemistry* 48(10): 4997-5000. http://dx.doi.org/10.1021/jf000373j

CHEN, Y W; LIN, C H; WU, F Y; CHEN, H H (2009) Rheological properties of crystallized honey prepared by a new type of nuclei. *Journal of Food Process Engineering* 32(4): 512-527. http://dx.doi.org/10.1111/j.1745-4530.2007.00227.x

CHERCHI, A; SPANEDDA, L; TUBEROSO, C; CABRAS, P (1994) Solid-phase extraction and high-performance liquid chromatographic determination of organic acids in honey. *Journal of Chromatography A* 669(1-2): 59-64. http://dx.doi.org/ 10.1016/0021-9673(94)80336-6

CHIRIFE, J; ZAMORA, M C; MOTTO, A (2006) The correlation between water activity and % moisture in honey: fundamental aspects and application to Argentine honeys. *Journal of Food Engineering* 72(3): 287-292. http://dx.doi.org/10.1016/j.jfoodeng.2004.12.009

CHUA, L S; LEE, J Y; CHAN, G F (2013) Honey protein extraction and determination by mass spectrometry. *Analytical and Bioanalytical Chemistry* 405(10): 3063-3074. http://dx.doi.org/10.1007/s00216-012-6630-2

CHUDZINSKA, M; BARALKIEWICZ, D (2010) Estimation of honey authenticity by multielements characteristics using inductively coupled plasma-mass spectrometry (ICP-MS) combined with chemometrics. *Food and Chemical Toxicology* 48(1): 284-90. http://dx.doi.org/ 10.1016/j.fct.2009.10.011

COOPER, R A; HALAS, E; MOLAN, P C (2002a) The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns. *Journal of Burn Care Rehabilitation* 23(6): 366-370. http://dx.doi.org/10.1097/01.BCR.0000036453.98917.41

COOPER, R A; MOLAN, P C; HARDING K G (2002b) The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. *Journal of Applied Microbiology* 93(5): 857-863. http://dx.doi.org/10.1046/j.1365-2672.2002.01761.x

COSTA, P A; MORAES, I C F; BITTANTE, A M Q B; SOBRAL, P J A; GOMIDE, C A; CARRER, C C (2013) Physical properties of honeys produced in the Northeast of Brazil. *International Journal of Food Studies* 2(1): 118-125. http://dx.doi.org/ 10.7455/ijfs/2.1.2013.a9

COTTE, J F; CASABIANCA, H; CHARDON, S; LHERITIER, J; GRENIER-LOUSTALOT, M F (2004) Chromatographic analysis of sugars applied to the characterization of monofloral honey. *Analytical and Bioanalytical Chemistry* 380(4): 698-705. http://dx.doi.org/ 10.1007/s00216-004-2764-1

CRANE, E (1985) *El libro de la miel*. Fondo de Cultura Económica; México D.F., México. 289 pp.

CUEVAS-GLORY, L F; PINO, J A; SANTIAGO, L S; SAURI-DUCH, E (2007) A review of volatile analytical methods for determining the botanical origin of honey. *Food Chemistry* 103(1): 1032-1043. http://dx.doi.org/10.1016/j.foodchem.2006.07.068

D'ARCY, B R (2005) Antioxidants in Australian floral honeys-identification of health-enhancing nutrient components. *Rural Industries Research and Development Corporation; Australian Government.* Publication Nº 05/040. 84 pp. https://rirdc.infoservices.com.au/downloads/05-040

D'ARCY, B R; RINTOUL, G B; ROWLAND, C Y; BLACKMAN, A J (1997) Composition of Australian honey extractives. 1. Norisoprenoids, monoterpenes, and other natural volatiles from blue gum (*Eucalyptus leucoxylon*) and yellow box (*Eucalyptus melliodora*) honeys. *Journal of Agricultural and Food Chemistry* 45(5): 1834-1843. http://dx.doi.org/ 10.1021/jf960625+

DE LA FUENTE, E; MARTÍNEZ-CASTRO, I; SANZ, J (2007) Characterization of Spanish unifloral honeys by solid phase microextraction and gas chromatography-mass spectrometry. *Journal of Separation Science* 28(9-10): 1093-1100. http://dx.doi.org/ 10.1002/jssc.200500018

DE OLIVEIRA RESENDE RIBEIRO, R; DA SILVA CARNEIRO, C; TEIXEIRA MÁRSICO, E; LIMA CUNHA, F; CONTE JUNIOR, C A; BORGES MANO, S (2012) Influence of the time/temperature binomial on the hydroxymethylfurfural content of floral honeys subjected to heat treatment. *Ciência e Agrotecnologia* 36(2): 204 -209. http://dx.doi.org/10.1590/S1413-70542012000200009

DEL NOZAL, M J; BERNAL, J L; MARINERO, P; DIEGO, J C; FRECHILLA, J I; HIGES, M; LLORENTE, J (1998) High performance liquid chromatographic determination of organic acids in honeys from different botanical origin. *Journal of Liquid Chromatography & Related Technologies* 21(20): 3197-3214. http://dx.doi.org/10.1080/10826079808001268

DÍAZ-MORENO, A C (2009) Influencia de las condiciones de almacenamiento sobre la calidad físico-química y biológica de la miel. PhD Thesis. University of Zaragoza (Spain). Advisors: Dr. Consuelo Pérez Arquillué and Dr. Teresa Juan Esteban.

DIEZ, M J; ANDRÉS, C; TERRAB, A (2004) Physicochemical parameters and pollen analysis of Moroccan honeydew honeys. *International Journal of Food Sciences and Technology* 39(2): 167-176. http://dx.doi.org/ 10.1046/j.0950-5423.2003.00769.x

DIMINS, F; KUKA, P; CAKSTE, I (2008) Content of carbohydrates and specific rotation angle of honey. In *3rd Baltic Conference on Food Science and Technology FOODBALT-2008. Latvia University of Agriculture, Faculty of Food Technology*. Conference Proceedings available at: http://agris.fao.org/agris-search/search.do?recordID=LV2008000468

DIMITROVA, B; GEVRENOVA, R; ANKLAM, E (2007) Analysis of phenolic acids in honeys of different floral origin by solid-phase extraction and high-performance liquid chromatography. *Phytochemical Analysis* 18(1): 24-32. http://dx.doi.org/10.1002/pca.948

DINKOV, D (2003) A scientific note on the specific rotation of three honey types from Bulgaria. *Apidologie* 34(1): 319-320. http://dx.doi.org/10.1051/apido:2003017

D'OLIVEIRA SANT'ANA, L D; FERREIRA, A B B; LORENZON, M C A; BERBARA, R L L; CASTRO, R N (2014) Correlation of total phenolic and flavonoid contents of Brazilian honeys with colour and antioxidant capacity. *International Journal of Food Properties* 17(1): 65-76. http://dx.doi.org/ 10.1080/10942912.2011.614368

DONER, L W (1977) The sugars of honey: A review. *Journal of the Science of Food and Agriculture* 28(5): 443-456. http://dx.doi.org/ 10.1002/jsfa.2740280508

DONER, L W (2003) Honey. In *Caballero, B; Finglas, P M; Trugo, L C (Eds). Encyclopedia of Food Sciences and Nutrition. 2nd edition*. Academic Press; London, UK. pp. 3125-3130. http://dx.doi.org/10.1016/B0-12-227055-X/00600-3

DUSTMANN, J H (1972) Über den Einfluss des Lichtes auf den Peroxid-Wert (Inhibin) des Honigs. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* 148(5): 263-268. http://dx.doi.org/ 10.1007/BF01458717

DUSTMANN, J H (1979) Antibacterial effect of honey. *Apiacta* 14: 7-11.

DVASH, L; AFIK, O; SHAFIR, S; SCHAFFER, A; YESELSON, Y; DAG, A; LANDAU, S (2002) Determination by near-infrared spectroscopy of perseitol used as a marker for the botanical origin of avocado (*Persea americana* Mill.) honey. Journal of Agriculture and Food Chemistry 50(19): 5283-5287. http://dx.doi.org/ 10.1021/jf020329z

DYCE, E J (1931) Crystallization of honey. *Journal of Economic Entomology* 24(4): 597-602. http://dx.doi.org/ 10.1080/0005772X.2010.11417371

DYCE, E J (1979) Producing finely granulated or creamed honey. In *Crane, E (Ed). Honey: A comprehensive survey (2nd Edition)*. Heinemann; London, UK. pp. 293-306.

EARNEST, C; KREIDER, R; LUNDBERG, J; RASMJSSEN, C; COWAN, P; GREENWOOD, M; ALMADA, A (2000) Effects of pre-exercise carbohydrate feedings on glucose and insulin responses during and after resistance exercise. *Journal of Strength and Conditioning Research* 14: 259-372.

ECHIGO, T; TAKENAKA, T (1974) Production of organic acids in honey by honeybees. Nippon Nogei Kagaku Kaishi 48(4): 225-230. http://dx.doi.org/ 0.1271/nogeikagaku1924.48.225

EFEM, S E E (1988) Clinical observations on the wound-healing properties of honey. *British journal of Surgery* 75(7): 679-681. http://dx.doi.org/ 10.1002/bjs.1800750718

EL SALVADOR. NSO 67.19.01:08 of 2008 relating to Miel de Abejas: Especificaciones (segunda actualizacion). Consejo Nacional de Ciencia y Tecnologia, 12 pp.

ELEAZU, C O; IROAGANACHI, M A; OKORONKWO, J O (2013) Determination of the physico-chemical composition, microbial quality and free radical scavenging activities of some commercially sold honey samples in Aba, Nigeria: 'The Effect of Varying Colors'. *Journal of Nutrition & Food Science* 3(2): 7 pp. http://dx.doi.org/10.4172/2155-9600.1000189

ENRICH, C; BOEYKENS, S; CARACCIOLO, N; CUSTO, G; VÁZQUEZ, C (2007) Honey characterization by total reflection x-ray fluorescence: evaluation of environmental quality and risk for the human health. *X-Ray Spectrometry* 36(4): 215-220. http://dx.doi.org/10.1002/xrs.944

EREJUWA, O O; SULAIMAN, S A; AB WAHAB, M S; SIRAJUDEEN, K N S; SALLEH, M S M; GURTU, S (2011) differential responses to blood pressure and oxidative stress in Streptozotocin-induced diabetic Wistar-Kyoto rats and spontaneously hypertensive rats: effects of antioxidant (honey) treatment. *International Journal of Molecular Sciences* 12(3): 1888-1907. http://dx.doi.org/ 10.3390/ijms12031888

EREJUWA, O O; SULAIMAN, S A; AB WAHAB, M S (2012a) Honey: a novel antioxidant. *Molecules* 17(4): 4400-4423. http://dx.doi.org/ 10.3390/molecules17044400

EREJUWA, O O; SULAIMAN, S A; AB WAHAB, M S; SIRAJUDEEN, K N S; SALLEH, M S M; GURTU, S (2012b) Honey supplementation in spontaneously hypertensive rats elicits antihypertensive effect via amelioration of renal oxidative stress. *Oxidative Medicine and Cellular Longevity*. Article ID 374037: 1-14. http://dx.doi.org/10.1155/2012/374037

ESCRICHE, I; VISQUERT, M; JUAN-BORRÁS, M; FITO, P (2009) Influence of simulated industrial thermal treatments on the volatile fractions of different varieties of honey. *Food Chemistry* 112(2): 329-338. http://dx.doi.org/10.1016/j.foodchem.2008.05.068

ESCUREDO, O; MIGUEZ, M; FERNÁNDEZ-GONZÁLEZ, M; CARMEN SEIJO, M (2013) Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. *Food Chemistry* 138(2-3): 851-856. http://dx.doi.org/ 10.1016/j.foodchem.2012.11.015

ESTUPIÑÁN, S; SANJUAN, E; MILLÁN, R; GONZÁLEZ-CORTÉS, M A (1998) Parámetros de calidad de la miel I. Microbiología, caracteres fisicoquímicos y de envejecimiento. *Alimentaria* 296: 89-94.

ETERAF-OSKOUEI, T; NAJAFI, M (2013) Traditional and modern uses of natural honey in human diseases: a review. *Iranian Journal of Basic Medical Sciences* 16(6): 731-742.

FAHEY, J W; STEPHENSON, K K (2002) Pinostrobin from honey and Thai ginger (*Boesenbergia pandurata*): A potent flavonoid inducer of mammalian phase 2 chemoprotective and antioxidant enzymes. *Journal of agricultural and food chemistry* 50(25): 7472-7476. http://dx.doi.org/ 10.1021/jf025692k

FAKHIMZADEH, K; LODENIUS, M (2000) Honey, pollen and bees as indicator of metal pollution. *Acta Universitatis Carolinae Environmentalica* 12: 13-20.

FALLICO, B; ZAPPALÁ, M; ARENA, E; VERZERA, A (2004) Effects of conditioning on HMF content in unifloral honeys. *Food Chemistry* 85(2): 305-313. http://dx.doi.org/ 10.1016/j.foodchem.2003.07.010

FALLICO, B; ARENA, E; VERZERA, A; ZAPPALA, M (2006) The European Food Legislation and its impact on honey sector. *Accreditation and Quality Assurance* 11(1-2): 49-54. http://dx.doi.org/ 10.1007/s00769-006-0128-6

FAROOQUI, T; FAROOQUI, A A (2014) Honey for cardiovascular diseases. In *Boukraâ, L (Ed). Honey in Traditional and Modern Medicine.* CRC Press Taylor & Francis Group. Boca Raton (FL), USA. pp. 187-216. http://dx.doi.org/10.1201/b15608-10

FATTORI, S B (2004) *La miel: propiedades, composición y análisis físico-químico*. Comisión Apimondia "Tecnología y Productos"; Buenos Aires, Argentina. 243 pp.

FELDMAN, M; STEINMAN, L (2005) Design of effective immunotherapy for human autoimmunity. *Nature* 435: 612-619. http://dx.doi.org/10.1038/nature03727

FELSNER, M L; CANO, C B; BRUNS, R E; WATANABE, H M; ALMEIDA-MURADIAN, L B; MATOS, J R (2004a) Characterization of monofloral honeys by ash contents through a hierarchical design. *Journal of Food Composition and Analysis* 17(6): 737-747. http://dx.doi.org/10.1016/j.jfca.2003.11.001

FELSNER, M L; CANO, C B; MATOS, J R; ALMEIDA-MURADIAN, L B; BRUNS, R E (2004b) Optimization of thermogravimetric analysis of ash content in honey. *Journal of the Brazilian Chemical Society* 15(6): 797-802. http://dx.doi.org/10.1590/S0103-50532004000600002

FERMO, P; BERETTA, G; FACINO, R M; GELMINI, G; PIAZZALUNGA, A (2013) Ionic profile of honey as a potential indicator of botanical origin and global environmental pollution. *Environmental Pollution* 178: 173-181. http://dx.doi.org/10.1016/j.envpol.2013.03.029

FERNÁNDEZ-TORRES, R; PÉREZ-BERNAL, J; BELLO-LÓPEZ, M A; CALLEJÓN-MOCHÓN, M; JIMÉNEZ-SÁNCHEZ, J C; GUIRAÚM-PÉREZ, A (2005) Mineral content and botanical origin of Spanish honeys. *Talanta* 65(3): 686-691. http://dx.doi.org/10.1016/j.talanta.2004.07.030.

FERREIRA, I C F R; AIRES, E; BARREIRA, J C M; ESTEVINHO, L M (2009) Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry* 114(4): 1438-1443. http://dx.doi.org/10.1016/j.foodchem.2008.11.028

FERRERES, F; TOMÁS-BARBERÁN, F A; GIL, M I; TOMÁS-LORENTE, F (1991) An HPLC technique for flavonoid analysis in honey. *Journal of the Science of Food and Agriculture* 56(1): 49-56. http://dx.doi.org/10.1002/jsfa.2740560106

FERRERES, F; ORTIZ, A; SILVA, C; GARCÍA-VIGUERA, C; TOMÁS-BARBERÁN, F A; TOMÁS-LORENTE, F (1992) Flavonoids of "La Alcarria" honey: A study of their botanical origin. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* 194(2): 139-143. http://dx.doi.org/10.1007/BF01190185

FERRERES, F; GARCÍA-VIGUERA, C; TOMÁS-LORENTE, F; TOMÁS-BARBERÁN, F (1993) Hesperetin: A marker of the floral origin of citrus honey. *Journal of the Science of Food Agriculture* 61(1): 121-123. http://dx.doi.org/10.1002/jsfa.2740610119

FERRERES, F; ANDRADE, P; TOMÁS-BARBERÁN, F A (1994a) Flavonoids from Portuguese heather honey. *Zeitschrift für Lebensmittel-Untersuchung und Forshung* 199(1): 32-37. http://dx.doi.org/10.1007/BF01192949

FERRERES, F; BLÁZQUEZ, M A; GIL, M I; TOMÁS-BARBERÁN, F A (1994b) Separation of honey flavonoids by micellar electrokinetic capillary chromatography. *Journal of Chromatography* 669(1-2): 268-274. http://dx.doi.org/10.1016/0021-9673(94)80359-5

FERRERES, F; GINER, J M; TOMÁS-BARBERÁN, F A (1994c) A comparative study of hesperetin and methyl anthranilate as markers of the floral origin of citrus honey. *Journal of the Science of Food Agriculture* 65(3): 371-372. http://dx.doi.org/10.1002/jsfa.2740650316

FERRERES, F; TOMÁS-BARBERÁN, F A; SOLER, C; GARCÍA-VIGUERA, C; ORTIZ, A; TOMÁS-LORENTE, F (1994d) A simple extractive technique for honey flavonoid HPLC analysis. *Apidologie* 25(1): 21-30. http://dx.doi.org/10.1051/apido:19940103

FERRERES, F; ANDRADE, P; GIL, M I; TOMÁS-BARBERÁN, F A (1996a) Floral nectar phenolics as biochemical markers for the botanical origin of heather honey. *Zeitschrift für Lebensmittel-Untersuchung und Forshung* 202(1): 40-44. http://dx.doi.org/10.1007/BF01229682

FERRERES, F; ANDRADE, P; TOMÁS-BARBERÁN, F A (1996b) Natural occurrence of abscisic acid in heather honey and floral nectar. *Journal of Agricultural and Food Chemistry* 44(8): 2053-2056. http://dx.doi.org/10.1021/jf9507553

FODOR, P; MOLNAR, E (1993) Honey as an environmental indicator: effect of sample preparation on trace element determination by ICP-AES. *Mikrochimica Acta* 112(1-4): 113-118. http://dx.doi.org/10.1007/BF01243327

FORESTER, S C; WATERHOUSE, A L (2009) Metabolites are key to understanding health effects of wine polyphenolics. *Journal of Nutrition* 139(9): 1824S-1831S. http://dx.doi.org/10.3945/jn.109.107664

FRANKEL, S; ROBINSON, G E; BERENBAUM, M R (1998) Antioxidant capacity and correlated characteristics of 14 unifloral honeys. *Journal of Apicultural Research* 37(1): 27-31. http://dx.doi.org/10.1080/00218839.1998.11100951

GALLARDO-CHACON, J J; CASELLIES, M; IZQUIERDO-PULIDO, M; RIUS, N (2008) Inhibitory activity of monofloral and multifloral honeys against bacterial pathogens. *Journal of Apicultural Research* 47(2): 131-136. http://dx.doi.org/10.1080/00218839.2008.11101439

GARCÍA-ÁLVAREZ, M; CERESUELA, S; HUIDOBRO, J F; HERMIDA, M; RODRÍGUEZ-OTERO, J L (2002) Determination ofpolarimetric parameters of honey by near-infrared transflectance spectroscopy. *Journal of Agricultural and Food Chemistry* 50(3): 419-425. http://dx.doi.org/10.1021/jf0105438

GHELDOF, N; ENGESETH, N J (2002) Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *Journal of Agricultural and Food Chemistry* 50(10): 3050-3055. http://dx.doi.org/10.1021/jf0114637

GHELDOF, N; WANG, X H; ENGESETH, N J (2002) Identification and quantification of antioxidant components of honeys from various floral sources. *Journal of Agricultural and Food Chemistry* 50(21): 5870-5877. http://dx.doi.org/10.1021/jf0256135

GHELDOF, N; WANG, X H; ENGESETH, N J (2003) Buckwheat honey increases serum antioxidant capacity in humans. *Journal of Agricultural and Food Chemistry* 51(5): 1500-1505. http://dx.doi.org/10.1021/jf025897t

GIL, M I; FERRERES, F; ORTIZ, A; SUBRA, E; TOMÁS-BARBERÁN, F A (1995) Plant phenolic metabolites and floral origin of rosemary honey. *Journal of Agricultural and Food Science* 43(11): 2833-2838. http://dx.doi.org/10.1021/jf00059a012

GILBER, M D; LISK, D J (1978) Honey as an environmental indicator of radionuclide contamination. *Bulletin of Environmental Contamination & Toxicology* 19(1): 32-34.http://dx.doi.org/10.1007/BF01685763

GLEITER, R A; HORN, H; ISENGARD, H D (2006) Influence of type and state of crystallisation on the water activity of honey. *Food Chemistry* 96(3): 441-445. http://dx.doi.org/10.1016/j.foodchem.2005.03.051

GOKMEN, V; MORALES, F J (2014) Processing contaminants. In *Motarjemi, Y; Moy, G; Tood, E (Eds). Encyclopedia of Food Safety, Volume 2*. Academic Press Inc; San Diego (CA), USA. pp. 404-408. http://dx.doi.org/10.1016/B978-0-12-378612-8.000024-X

GOMES, S; DIAS, L G; MOREIRA, L L; RODRIGUES, P; ESTEVINHO, L (2010) Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. *Food and Chemical Toxicology* 48(2): 544-548. http://dx.doi.org/10.1016/j.fct.2009.11.029

GONZALES, A P; BURIN, L; BUERA, M P (1999) Color changes during storage of honeys in relation to their composition and initial color. *Food Research International* 32(3): 185-191. http://dx.doi.org/10.1016/S0963-9969(99)00075-7

GONZÁLEZ, M M (2002) El origen, la calidad y la frescura de la miel: la interpretación de un análisis. In *De Lorenzo, C (Ed). La miel de Madrid*. Consejería de Economía e Innovación Tecnológica. Comunidad de Madrid. Instituto Madrileño de investigación Agraria y alimentaria. pp. 27-45. Available at: http://www.madrid.org/bvirtual/BVCM005574.pdf (Accessed 12/02/2015)

GONZÁLEZ-MIRET, M L; TERRAB, A; HERNANZ, D; FERNÁNDEZ-RECAMALES, M A; HEREDIA, F J (2005) Multivariate correlation between color and mineral composition of honeys and by their botanical origin. *Journal of Agricultural and Food Chemistry* 53(7): 2574-2580. http://dx.doi.org/10.1021/jf048207p

GONZÁLEZ-PARAMÁS, A M; GÓMEZ-BAREZ, J A; CORDÓN-MARCOS, C; GARCÍA-VILLANOVA, R J; SÁNCHEZ-SÁNCHEZ, J (2006) HPLC-fluorimetric method for analysis of amino acids in products of the hive (honey and bee-pollen). *Food Chemistry* 95(1): 148-156. http://dx.doi.org/10.1016/j.foodchem.2005.02.008

GRASSI, D; DESIDERI, G; FERRI, C (2010) Flavonoids: antioxidants against atherosclerosis. *Nutrients* 2(8): 889-902. http://dx.doi.org/10.3390/nu2080889

GUYOT, C; SCHEIRMAN, V; COLLIN, S (1999) Floral origin markers of heather honeys: *Calluna vulgaris* and *Erica arborea*. *Food Chemistry* 64(1): 3-11. http://dx.doi.org/10.1016/S0308-8146(98)00122-8

GUYOT-DECLERCK, C; RENSON, S; BOUSETA, A; COLLIN, S (2002) Floral quality and discrimination of *Lavandula stoechas*, *Lavandula angustifolia*, and *Lavandula angustifolia* x *latifolia* honeys. *Food Chemistry* 79(4): 453-459. http://dx.doi.org/10.1016/S0308-8146(02)00216-9

HADDADIN, M S Y; NAZER, I; ABU RADDAD SARA' JAMAL, I; ROBINSON, R K (2007) Effect of honey on the growth and metabolism of two bacterial species of intestinal origin. *Pakistan Journal of Nutrition* 6(6): 693-697. http://dx.doi.org/10.3923/pjn.2007.693.697

HAROUN, M I, POYRAZOGLU, E S; KONAR, N; ARTIK, N (2012) Phenolic acids and flavonoids profiles of some Turkish honeydew and floral honeys. *Journal of Food Technology* 10(2): 39-45. http://dx.doi.org/10.3923/jftech.2012.39.45

HASHEMIPOUR, M A; TAVAKOLINEGHAD, Z; ARABZADEH, S A M; IRANMANESH, Z; NASSAB, S A H G (2014) Antiviral activities of honey, royal jelly, and acyclovir against HSV-1. *Wounds-A Compendium of Clinical Research and Practice* 26(2): 47-54.

HERBERT, E W JR. (1992) Honey Bee Nutrition. In *Graham, J M (Ed). The Hive and the Honey Bee.* Dadant and Sons; Hamilton (IL), USA. pp. 197-233.

HERMOSÍN, I; CHICÓN, R M; CABEZUDO, M D (2003) Free amino acid composition and botanical origin of honey. *Food Chemistry* 83(2): 263-268. http://dx.doi.org/10.1016/S0308-8146(03)00089-X

HIWATASHI, K; NARISAWA, A; HOKARI, M; TOEDA, K (2010) Antihypertensive effect of honey-based beverage containing fermented rice bran in spontaneously hypertensive rats. *Nippon Shokuhin Kagaku Kogaku Kaishi* 57(1): 40-43. http://dx.doi.org/10.3136/nskkk.57.40

HORVÁTH, K; MOLNÁR-PERL, I (1997) Simultaneous quantitation of mono-, di- and trisaccharides by GC-MS of their TMS ether oxime derivatives: II. In honey. *Chromatographia* 45(1): 328-335. http://dx.doi.org/10.1007/BF02505579

HUIDOBRO, J F; SIMAL, J (1984) Determinación del color y de la turbidez en las mieles. *Anales de Bromatología* XXXVI-2: 225-245.

HUIDOBRO, J F; SÁNCHEZ, M P; MUNIATEGUI, S; SANCHO, M T (2005) Precise method for the measurement of catalase activity in honey. *Journal of AOAC International* 88(3): 800-804.

HUSSEIN, S Z; YUSOFF, K M; MAKPOL, S; YUSOF, Y A M (2013) Gelam honey attenuates carrageenan-induced rat paw inflammation via NF-kappa B pathway. *Plos One* 8(8): e72365. http://dx.doi.org/10.1371/journal.pone.0072365

INOUE, K; MURAYARNA, S; SESHIMO, F; TAKEBA, K; YOSHIMURA, Y; NAKAZAWA, H (2005) Identification of phenolic compound in manuka honey as specific superoxide anion radical scavenger using electron spin resonance (ESR) and liquid chromatography with coulometric array detection. *Journal of the Science of Food and Agriculture* 85(5): 872-878. http://dx.doi.org/10.1002/jsfa.1952

ISIDOROV, V A; CZYZEWSKA, U; JANKOWSKA, E; BAKIER, S (2011) Determination of royal jelly acids in honey. *Food Chemistry* 124(1): 387-391. http://dx.doi.org/10.1016/j.foodchem.2010.06.044

ISLAM, M N; KHALIL, M I; ISLAM, M A; GAN, S H (2014) Toxic compounds in honey. *Journal of Applied Toxicology* 34(7): 733-742. http://dx.doi.org/10.1002/jat.2952

JAWAD, F H; AL-KHALIDI, A; TAWFIQ, N H (1981) Effects of bees honey, zahdi date and its syrup on blood glucose and serum insulin of normal subjects. *Journal of the Faculty of Medicine, Baghdad* 23: 169-180.

JÉANNE, F (1991) Le miel: Sa cristallisation. L'activité de l'eau. *Bulletin Technique Apicole* 76 (18/3): 157-160.

JEDDAR, A; KHARSANY, A; RAMSAROOP, U G; BHAMJEE, A; HAFEJEE, I E; MOOSA, A (1985) The Antibacterial action of honey. *South African Medical Journal* 67(7): 257-258.

JENKINS, D; KENDALL, C; AUGUSTIN, L; FRANCESCHI, S; HAMIDI, M; MARCHIE, A; JENKINS, A; AXELSEN, M (2002) Glycemic index: overview of implications in health and disease. *The American Journal of Clinical Nutrition* 76(1): 266S-273S.

JERKOVIĆ, I; MASTELIC, J; MARIJANOVIC, Z (2006) A variety of volatile compounds as markers in unifloral honey from Dalmatian sage (*Salvia officinalis* L.). *Chemistry and Biodiversity* 3(12): 1307-1316. http://dx.doi.org/10.1002/cbdv.200690134

JERKOVIĆ, I; TUBEROSO, C I G; MARIJANOVIC, Z; JELIC, M; KASUM, A (2009) Headspace, volatile and semi-volatile patterns of *Paliurus spina-christi* unifloral honey as markers of botanical origin. *Food Chemistry* 112(1): 239-245. http://dx.doi.org/10.1016/j.foodchem.2008.05.080

JONES, K C (1987) Honey as an indicator of heavy metal contamination. *Water, Air, and Soil Pollution* 33(1-2): 179-189. http://dx.doi.org/10.1007/BF00191386

JOSEPH, T; AWAH-NDUKUM, J; FONTEH-FLORENCE, A; DELPHINE, N D; JONNAS, P; ANTOINE, M Z (2007) Physico-chemical and microbiological characteristics of honey from the Sudano-Guinean Zone of west Cameron. *African Journal of Biotechnology* 6(7): 908-913.

JUAN-BORRÁS, M; DOMENECH, E; HELLEBRANDOVA, M; ESCRICHE, I (2014) Effect of country origin on physicochemical, sugar and volatile composition of acacia, sunflower and tilia honeys. *Food Research International* 60: 86-94. http://dx.doi.org/10.1016/j.foodres.2013.11.045

JUSZCZAK, L; FORTUNA, T (2006) Rheology of selected polish honeys. *Journal of Food Engineering* 75(1): 43-49. http://dx.doi.org/10.1016/j.jfoodeng.2005.03.049

KABBANI, D; SEPULCRE, F; WEDEKIND, J (2011) Ultrasound-assisted liquefaction of rosemary honey: influence on rheology and crystal content. *Journal of Food Engineering* 107(2): 173-178. http://dx.doi.org/10.1016/j.jfoodeng.2011.06.027

KACANIOVÁ, M; KNAZOVICKA, V; MELICH, M; FIKSELOVA, M; MASSANYI, P; STAWARZ, R; HASCIK, P; PECHOCIAK, T; KUCZKOWSKA, A; PUTAŁA, A (2009) Environmental concentration of selected elements and relation to physicochemical parameters in honey. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering* 44(4): 414-422. http://dx.doi.org/10.1080/10934520802659802

KACANIOVÁ, M; FATRCOVA-SRAMKOVA, K; NOZKOVA, J; MELICH, M; KADASI-HORAKOVA, M; KNAZOVICKA, V; FELSOCIOVA, S; KUNOVA, S; MARIASSYOVA, M (2011) Antiradical activity of natural honeys and antifungal effect against *Penicillium* genera. *Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes* 46(1): 92-96.http://dx.doi.org/10.1080/03601234.2011.534416

KAJIWARA, S; GANDHI, H; USTUNOL, Z (2002) Effect of honey on the growth of and acid production by human intestinal *Bifidobacterium* spp.: An in vitro comparison with commercial oligosaccharides and inulin. *Journal of Food Protection* 65(1): 214-218.

KAO, T K; OU, Y C; RAUNG, S L; LAI, C Y; LIAO, S L; CHEN, C J (2010) Inhibition of nitric oxide production by quercetin in endotoxin/cytokine-stimulated microglia. *Life Sciences* 86(9-10): 315-321. http://dx.doi.org/10.1016/j.lfs.2009.12.014

KAŠKONIENĖ, V; VENSKUTONIS, P R (2010) Floral markers in honey of various botanical and geographic origins: a review. *Comprehensive Reviews in Food Science and Food Safety* 9(6): 620-634. http://dx.doi.org/10.1111/j.1541-4337.2010.00130.x

KASSIM, M; ACHOUI, M; MANSOR, M; YUSOFF, K M (2010a) The inhibitory effects of Gelam honey and its extracts on nitric oxide and prostaglandin E2 in inflammatory tissues. *Fitoterapia* 81(8): 1196-1201. http://dx.doi.org/10.1016/j.fitote.2010.07.024

KASSIM, M; ACHOUI, M; MUSTAFA, M R; MOHD, M A; YUSOFF, K M (2010b) Ellagic acid, phenolic acids, and flavonoids in Malaysian honey extracts demonstrate in vitro anti-inflammatory activity. *Nutrition Research* 30(9): 650-659. http://dx.doi.org/10.1016/j.nutres.2010.08.008

KASSIM, M; MANSOR, M; AL-ABD, N; YUSOFF, K M (2012) Gelam honey has a protective effect against lipopolysaccharide (LPS)-induced organ failure. *International Journal of Molecular Sciences* 13(5): 6370-6381. http://dx.doi.org/10.3390/ijms13056370

KATIRAEE, F; MAHMODI, R; MARDANI, K; BABAEI, E (2014) Antifungal activity of iranian honeybee honey against *Candida, Aspergillus* species and *Trichophyton Rubrum*. *Journal of Food Processing and Preservation* 38(5): 2078-2082. http://dx.doi.org/10.1111/jfpp.12187

KENJERIC, D; MANDIC, M L; PRIMORAC, L; BUBALO, D; PERL, A (2007) Flavonoid profile of Robinia honeys produced in Croatia. *Food Chemistry* 102(3): 683-690.http://dx.doi.org/10.1016/j.foodchem.2006.05.055

KESIC, A; CRNKIC, A; HODZIC, Z; IBRISIMOVIC, N; SESTAN, A (2014) Effects of botanical origin and ageing on HMF content in bee honey. *Journal of Scientific Research & Reports* 3(8): 1057-1066.

KOC, A N; SILICI, S; ERCAL, B D; KASAP, F; HORMET-OZ, H T; MAVUS-BULDU, H (2009) Antifungal activity of turkish honey against *Candida* spp. and *Trichosporon* spp.: an in vitro evaluation. *Medical Mycology* 47(7): 707-712. http://dx.doi.org/10.3109/13693780802572554

KOLAYLI, S; BOUKRAÂ, L; ŞAHIN, H; ABDELLAH, F (2012) Sugars in honey. In *Preedy, V R (Ed). Dietary Sugars: Chemistry, Analysis, Function and Effects*. Royal Society of Chemistry Publishing; Cambridge, UK. pp. 3-15. http://dx.doi.org/10.1039/9781849734929

KOLAYLI, S; YILDIZ, O; SAHIN, H; ALIYAZICIOGLU, R (2014) Biochemistry and physicochemical properties of honey. In *Boukraâ, L (Ed). Honey in Traditional and Modern Medicine.* CRC Press Taylor & Francis Group; Boca Raton (FL), USA. pp. 21-31. http://dx.doi.org/10.1201/b15608-4

KREIDER, R B; RASMUSSEN, C J; LANCASTER, S L; KERKSICK, C; GREENWOOD, M (2002) Honey: an alternative sports gel. *Strength and Conditioning Journal* 24(1): 50-51.

KRELL, R (1996) Value-added products from beekeeping. *Food and Agriculture Services Bulletin* 124. http://www.fao.org/docrep/w0076e/w0076e00.htm#con

KRUSNA, N S A; KOWSALYA, A; RADHA, S; NARAYANAN, R B (2007) Honey as a natural preservative of milk. *Indian Journal of Experimental Biology* 45(5): 459-464.

KÜÇÜK, M; KOLAYLI, S; KARAOGLU, S; ULUSOY, E; BALTACI, C; CANDAN, F (2007) Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chemistry* 100(2): 526-534. http://dx.doi.org/10.1016/j.foodchem.2005.10.010

KWAKMAN, P H S; TE VELDE, A; DE BOER, L; SPEIJER, D; VANDENBROUCKE-GRAULS, C M J E; ZAAT, S A J (2010) How honey kills bacteria. *The FASEB journal* 24(7): 2576-2582. http://dx.doi.org/10.1096/fj.09-150789

KWAKMAN, P H S; TE VELDE, A; DE BOER, L; VANDENBROUCKE-GRAULS, C M J E (2011) Two major medicinal honeys have different mechanisms of bactericidal activity. *Plos One* 6(3): e17709. http://dx.doi.org/10.1371/journal.pone.0017709

LABROPOULOS, A; ANESTIS, S (2012) Honey. In *Varzakas, T; Labropoulos, A; Anestis, S (Eds). Sweeteners: nutritional aspects, applications, and production technology.* CRC Press. Taylor & Francis Group; Boca Ratón (FL), USA. pp. 119-146. http://dx.doi.org/10.1201/b12065-6

LAMBERT, O; PIROUX, M; PUYO, S; THORIN, C; LARHANTEC, M; DELBAC, F; POULIQUEN, H (2012) Bees, honey and pollen as sentinels for lead environmental contamination. *Environmental Pollution* 170: 254-259. http://dx.doi.org/10.1016/j.envpol.2012.07.012

LAVIE, P; GRASSÉ, P P (1963) Sur l'identification des substances antibactériennes présentes dans le miel. *Comptes rendus des Séances de l'Academie des Sciences* 256: 1858-1860.

LAZARIDOU, A; BILIADERIS, C G; BACANDRITSOS, N; SABATINI, A G (2004) Composition, thermal and rheological behaviour of selected Greek honeys. *Journal of Food Engineering* 64(1): 9-21. http://dx.doi.org/10.1016/j.jfoodeng.2003.09.007

LEE, H J; CHUREY, J J; WOROBO, R W (2008a) Antimicrobial activity of bacterial isolates from different floral sources of honey. *International Journal of Food Microbiology* 126(1-2): 240-244. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.04.030

LEE, H J; CHUREY, J J; WOROBO, R W (2008b) Purification and structural characterization of bacillomycin F produced by a bacterial honey isolate active against *Byssochlamys fulva* H2511677722. *Journal of Applied Microbiology* 105(3): 663-673. http://dx.doi.org/10.1111/j.1365-2672.2008.03797.x

LEITA, L; MUHLBACHOVA, G; CESCO, S; BARBATTINI, R; MONDINI, C (1996) Investigation of the use of honey bees and honey bee Products to assess heavy metals contamination. *Environmental Monitoring and Assessment* 43(1): 1-9. http://dx.doi.org/10.1007/BF00399566

LEONG, A G; HERST, P M; HARPER, J L (2012) Indigenous New Zealand honeys exhibit multiple anti-inflammatory activities. *Innate Immunity* 18(3): 459-466. http://dx.doi.org/10.1177/1753425911422263

LEÓN-RUIZ, V; GONZÁLEZ-PORTO, A V; AL-HABSI, N; VERA, S; SAN ANDRES, M P; JAUREGI, P (2013a) Antioxidant, antibacterial and ACE-inhibitory activity of four monofloral honeys in relation to their chemical composition. *Food & Function* 4(11): 1617-1624. http://dx.doi.org/10.1039/c3fo60221d

LEÓN-RUIZ, V; VERA, S; GONZÁLEZ-PORTO, A V; ANDRÉS, M P S (2013b) Analysis of water-soluble vitamins in honey by isocratic RP-HPLC. *Food Analytical Methods* 6(2): 488-496. http://dx.doi.org/10.1007/s12161-012-9477-4

LUCAN, M; SLACANAC, V; HARDI, J; MASTANJEVIC, K; BABIC, J; KRSTANOVIC, V; JUKIC, M (2009) Inhibitory effect of honey-sweetened goat and cow milk fermented with *Bifidobacterium lactis* Bb-12 on the growth of *Listeria monocytogenes*. *Mljekarstvo* 59(2): 96-106.

LUPANO, C E (1997) DSC study of honey granulation stored at various temperatures. *Food Research International* 30(9): 683-688. http://dx.doi.org/10.1016/S0963-9969(98)00030-1

MADDOCKS, S E; JENKINS, R E (2013) Honey: a sweet solution to the growing problem of antimicrobial resistance?. *Future Microbiology* 8(11): 1419-1429. http://dx.doi.org/10.2217/fmb.13.105

MALHAT, F M; HAGGAG, M N; LOUTFY, N M; OSMAN, M A M; AHMED, M T (2015) Residues of organochloride and synthetic pyrethroid pesticides in honey, an indicator of ambient environment, a pilot study. *Chemosphere* 120: 457-461. http://dx.doi.org/10.1016/j.chemosphere.2014.08.032

MANYI-LOH, C E; CLARKE, A M; NDIP, R N (2011a) An overview of honey: Therapeutic properties and contribution in nutrition and human health. *African Journal of Microbiology Research* 5(8): 844-852. http://dx.doi.org/10.5897/AJMR10.008

MANYI-LOH, C E; NDIP, R N; CLARKE, A M (2011b) Volatile compounds in honey: a review on their involvement in aroma, botanical origin determination and potential biomedical activities. *International Journal of Molecular Sciences* 12(12): 9514-9532. http://dx.doi.org/10.3390/ijms12129514

MANZOOR, M; MATHIVANAN, V; SHAH, G N; MIR, G M; SELVISABHANAYAKAM (2013) Physico-chemical analysis of honey of *Apis cerana indica* and *Apis mellifera* from different regions of Anantnag district, Jammu and Kashmir. *International Journal of Pharmacy and Pharmaceutical Sciences* 5(3): 635-638.

MĂRGHITAŞ, L A; DEZMIREAN, D; MOISE, A; BOBIS, O; LASLO, L; BOGDANOV, S (2009) Physico-chemical and bioactive properties of different floral origin honeys from Romania. *Food Chemistry* 112(4): 863-867. http://dx.doi.org/10.1016/j.foodchem.2008.06.055

MARTIN, E C (1958) Some aspects of hygroscopic properties and fermentation of honey. *Bee World* 39 (7): 165-178. http://dx.doi.org/10.1080/0005772X.1958.11095058

MARTOS, I; COSSENTINI, M; FERRERES, F; TOMÁS-BARBERÁN, F A (1997) Flavonoid composition of Tunisian honeys and propolis. *Journal of Agricultural and Food Chemistry* 45(8): 2824-2829. http://dx.doi.org/10.1021/jf9609284

MARTOS, I; FERRERES, F; TOMÁS-BARBERÁN, F A (2000a) Identification of flavonoid markers for the botanical origin of Eucalyptus honey. *Journal of Agricultural and Food Chemistry* 48(5): 1498-1502. http://dx.doi.org/10.1021/jf991166q

MARTOS, I; FERRERES, F; YAO, L; D'ARCY, B; CAFFIN, N; TOMÁS-BARBERÁN, F A (2000b) Flavonoids in monospecific *Eucalyptus* honeys from Australia. *Journal of Agricultural and Food Chemistry* 48(10): 4744-4748. http://dx.doi.org/10.1021/jf000277i

MATO, I; HUIDOBRO, J F; SIMAL-LOZANO, J; SANCHO, M T (2003) Significance of nonaromatic organic acids in honey. *Journal of Food Protection* 66(12): 2371-2376.

MAURIZIO, A (1962) From the raw material to the finished product: honey. *Bee World* 43(3): 66-81. http://dx.doi.org/10.1080/0005772X.1962.11096943

MAVRIC, E; WITTMANN, S; BARTH, G; HENLE, T (2008) Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. *Molecular Nutrition & Food Research* 52(4): 483-489. http://dx.doi.org/10.1002/mnfr.200700282

MCKIBBEN, J; ENGESETH, N J (2002) Honey as a protective agent against lipid oxidation in ground turkey. *Journal of Agricultural and Food Chemistry* 50(3): 592-595. http://dx.doi.org/10.1021/jf010820a

MCLELLAN, M R; KIME, R W; LEE, C Y; LONG, T M (1995) Effect of honey as an antibrowning agent in light raisin processing. *Journal of Food Processing and Preservation* 19(1): 1-8. http://dx.doi.org/10.1111/j.1745-4549.1995.tb00273.x

MENDONÇA, K; MARCHINI, L C; SOUZA, B A; ALMEIDA-ANACLETO, D; MORETI A C C C (2008) Caracterização físico-química de amostras de méis produzidas por *Apis mellifera* L. em fragmento de cerrado no município de Itirapina, São Paulo. *Ciência Rural* 38(6): 1748-1753. http://dx.doi.org/10.1590/S0103-84782008000600040

MERCOSUR. MERCOSUR/GMC/RES. Nº 89/99 of 18 November 1999 that approved the Technical Regulations MERCOSUR of Identity and Quality of Honey. Mercosur's Common Market Group. 8pp.

MOLAN, P C (1992a) The antimicrobial activity of honey 1. The nature of antibacterial activity. *Bee World* 73(1): 5-28.

MOLAN, P C (1992b) The antibacterial activity of honey. 2. Variation in the potency of the antibacterial activity. *Bee World* 73(2): 59-76. http://dx.doi.org/10.1080/0005772X.1992.11099118

MOLAN, P C (1997) Honey as an antimicrobial agent. In *Mizrahi, A; Lensky, Y (eds). Bee Products. Properties, Applications, and Apitherapy.* Symposium Tel Aviv, Israel. pp. 27-37.

MOLAN, P C (1999) The role of honey in the management of wounds. *Journal of Wound Care* 8: 415-418. http://dx.doi.org/10.12968/jowc.1999.8.8.25904

MOLAN, P C (2001) Why honey is effective as a medicine - 2. The scientific explanation of its effects. *Bee World* 82(1): 22-40. http://dx.doi.org/10.1080/0005772X.2001.11099498

MONTENEGRO, G; GÓMEZ, M; PIZARRO, R; CASAUBON, G; PEÑA, R C (2008) Implementación de un panel sensorial para mieles chilenas. *Ciencia e Investigación Agraria* 35(1): 51-58. http://dx.doi.org/10.4067/S0718-16202008000100005

MONTENEGRO, G; SALAS, F; PENA, R C; PIZARRO, R (2009) Antibacterial and antifungic activity of the unifloral honeys of *Quillaja saponaria*, an endemic Chilean species. *Phyton-International Journal of Experimental Botany* 78: 141-146.

MOSSEL, B; BHANDARI, B; D'ARCY, B; CAFFIN, N (2000) Use of Arrhenius model to predict rheological behavior in some Australian honeys. *Lebensmitel wissenhauf und technology* 33: 545-552.

MUNDO, M A; PADILLA-ZAKOUR, O I; WOROBO, R W (2004) Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *International Journal of Food Microbiology* 97(1): 1-8. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.025

MÜNSTEDT, K; BÖHME, M; HAUENSCHILD, A; HRGOVIC, I (2011) Consumption of rapeseed honey leads to higher serum fructose levels compared with analogue glucose/fructose solutions. *European journal of clinical nutrition* 65(1): 77-80. http://dx.doi.org/10.1038/ejcn.2010.186

NAGAI, T; SAKAI, M; INOUE, R; INOUE, H; SUZUKI, N (2001) Antioxidative activities of some commercially honeys, royal jelly, and propolis. *Food Chemistry* 75: 237-240. http://dx.doi.org/10.1016/S0308-8146(01)00193-5

NAGAI, T; INOUE, R; KANAMORI, N; SUZUKI, N; NAGASHIMA, T (2006) Characterization of honey from different floral sources. Its functional properties and effects of honey species on storage of meat. *Food Chemistry 97*(2): 256-262. http://dx.doi.org/10.1016/j.foodchem.2005.03.045

NAGAI, T; TANOUE, Y; KAI, N; SUZUKI, N (2012) Functional property of honey from *Echium vulgare*. *Food and Nutrition Sciences* 3(5): 614-620. http://dx.doi.org/10.4236/fns.2012.35084

NIGUSSIE, K; SUBRAMANIAN, P A; MEBRAHTU G (2012) Physicochemical analysis of Tigray honey: an attempt to determine major quality markers of honey. *Bulletin of the Chemical Society of Ethiopia* 26(1): 127-133. http://dx.doi.org/10.4314/bcse.v26i1.14

NIKAEIN, D; KHOSRAVI, A R; MOOSAVI, Z; SHOKRI, H; ERFANMANESH, A; GHORBANI-CHOBOGHLO, H; BAGHERI, H (2014) Effect of honey as an immunomodulator against invasive aspergillosis in BALB/c mice. *Journal of Apicultural Research* 53(1): 84-90. http://dx.doi.org/10.3896/IBRA.1.53.1.08

NOZAL, M J; BERNAL, J L; TORIBIO, L; ALAMO, M; DIEGO, J C; TAPIA, J (2005) The use of carbohydrate profiles and chemometrics in the characterization of natural honeys of identical geographical origin. *Journal of Agriculture and Food Chemistry* 53(8): 3095-3100. http://dx.doi.org/10.1021/jf0489724

NOZAL-NALDA, M J; BERNAL-YAGÜE, J L; DIEGO-CALVA, J C; MARTÍN-GÓMEZ, M T (2005) Classifying honeys from the Soria Province of Spain via multivariate analysis. *Analytical and Bioanalytical Chemistry* 382(2): 311-319. http://dx.doi.org/10.1007/s00216-005-3161-0

OELSCHLAEGEL, S; GRUNER, M; WANG, P N; BOETTCHER, A; KOELLING-SPEER, I; SPEER, K (2012a) Classification and characterization of manuka honeys based on phenolic compounds and methylglyoxal. *Journal of Agricultural and Food Chemistry* 60(29): 7229-7237. http://dx.doi.org/10.1021/jf300888q

OELSCHLAEGEL, S; PIEPER, L; STAUFENBIEL, R; GRUNER, M; ZEIPPERT, L; PIEPER, B; KOELLING-SPEER, I; SPEER, K (2012b) Floral markers of cornflower (*Centaurea cyanus*) honey and its peroxide antibacterial activity for an alternative treatment of digital dermatitis. *Journal of Agricultural and Food Chemistry* 60(47): 11811-11820. http://dx.doi.org/10.1021/jf303699t

OJEC-OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES (2002). Council Directive 2001/110/EC of 20 December 2001 relating to honey.

OJEDA DE RODRÍGUEZ, G; SULBARÁN DE FERRER, B; FERRER, A; RODRÍGUEZ, B (2004) Characterization of honey produced in Venezuela. *Food Chemistry* 84(4): 499-502. http://dx.doi.org/10.1016/S0308-8146(02)00517-4

OLAITAN, P B; ADELEKE, E O; OLA, O I (2007) Honey: a reservoir for microorganisms and an inhibitory agent for microbes. *African Health Science* 7(3): 159-165. http://dx.doi.org/10.5555/afhs.2007.7.3.159

OLOFSSON, T C; VASQUEZ, A (2008) Detection and identification of a novel lactic acid bacterial flora within the honey stomach of the honeybee *Apis mellifera*. *Current Microbiology* 57(4): 356-363. http://dx.doi.org/10.1007/s00284-008-9202-0

OROIAN, M (2013) Measurement, prediction and correlation of density, viscosity, surface tension and ultrasonic velocity of different honey types at different temperatures. *Journal of Food Engineering* 119(1): 167-172. http://dx.doi.org/10.1016/j.jfoodeng.2013.05.029

ORTIZ-VALBUENA, A; SILVA-LOSADA, M C (1991) Contenido en HMF en las mieles de La Alcarria. *Cuadernos de Apicultura* 10: 8-10.

ORTIZ-VALBUENA, A; FERNÁNDEZ-MAESO, M C; SUBRÁ MUÑOZ DE LA TORRE, E (1996) *Principales características de la miel de La Alcarria*. Consejería de Agricultura y medio ambiente de la junta de comunidades de Castilla-La Mancha; Toledo, España. pp. 26-35.

OSZMIANSKI, J; LEE, C Y (1990) Inhibition of polyphenol oxidase activity and browning by honey. *Journal of Agricultural and Food Chemistry* 38(10): 1892-1895. http://dx.doi.org/10.1021/jf00100a002

OU, B; PRIOR, R L; HUANG, D (2005) The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry* 53(6): 1841-1856. http://dx.doi.org/10.1021/jf030723c

OUCHEMOUKH, S; SCHWEITZER, P; BACHIR BEY, M; DJOUDAD-KADJI, H; LOUAILECHE, H (2010) HPLC sugars profiles of Algerian honeys. *Food Chemistry* 121(2): 561-568. http://dx.doi.org/10.1016/j.foodchem.2009.12.047

OWOYELE, B V; OLADEJO, R O; AJOMALE, K; AHMED, R O; MUSTAPHA, A (2014) Analgesic and anti-inflammatory effects of honey: the involvement of autonomic receptors. *Metabolic Brain Disease* 29(1): 167-173. http://dx.doi.org/10.1007/s11011-013-9458-3

PANSERI, S; CATALANO, A; GIORGI, A; ARIOLI, F; PROCOPIO, A; BRITTI, D; CHIESA, L M (2014) Occurrence of pesticide residues in Italian honey from different areas in relation to its potential contamination sources. *Food Control* 38(1): 150-156.http://dx.doi.org/10.1016/j.foodcont.2013.10.024

PAULUS, H; KWAKMAN, S; ZAAT, S A J (2012) Antibacterial components of honey. *IUBMB Life* 64(1): 48-55. http://dx.doi.org/10.1002/iub.578

PÉREZ, A; SÁNCHEZ, V; BAEZA, R; ZAMORA, M; CHIRIFE, J (2009) Literature review on linear regression equations for relating water activity to moisture content in floral honeys: development of a weighted average equation. *Food and Bioprocess Technology* 2(4): 437-440. http://dx.doi.org/10.1007/s11947-009-0193-z

PÉREZ, R A; IGLESIAS, M T; PUEYO, E; GONZÁLEZ, M; DE LORENZO, C (2007) Amino acid composition and antioxidant capacity of Spanish honeys. *Journal of agricultural and food chemistry* 55(2): 360-365. http://dx.doi.org/10.1021/jf062055b

PERSANO-ODDO, L; BALDI, E; ACCORTI, M (1990) Diastatic activity in some unifloral honeys. *Apidologie* 21(1): 17-24. http://dx.doi.org/10.1051/apido:19900103

PERSANO-ODDO, L; PIAZZA, M G; SABATINI, A G; ACCORTI, M (1995) Characterization of unifloral honeys. *Apidologie* 26(6): 453-465. http://dx.doi.org/10.1051/apido:19950602

PERSANO-ODDO, L; PIAZZA, M G; PULCINI, P (1999) Invertase activity in honey. *Apidologie* 30(1): 57-65. http://dx.doi.org/10.1051/apido:19990107

PIANA, G; RICCIARDELLI D'ALBORE, G; ISOLA, A (1989) *La miel - Alimento de conservación natural: Origen-Recolección-Comercialización*. Ed. Mundi-Prensa; Madrid, Spain. 106 pp.

PIANA, M L; PERSANO ODDO, L; BENTABOL, A; BRUNEAU, E; BOGDANOV, S; GUYOT DECLERCK, C (2004) Sensory analysis applied to honey: state of the art. *Apidologie* 35(1): S26-S37. http://dx.doi.org/10.1051/apido:2004048

PICHICHERO, E; CANUTI, L; CANIN, A (2009) Characterisation of the phenolic and flavonoid fractions and antioxidant power of Italian honeys of different botanical origin. *Journal of the Science of Food and Agriculture* 89(4): 609-616. http://dx.doi.org/10.1002/jsfa.3484

PILJAC-ŽEGARAC, J; STIPCEVIC, T; BELSCAK, A (2009) Antioxidant properties and phenolic content of different floral origin honeys. *Journal of ApiProduct and ApiMedical Science* 1(2): 43-50. http://dx.doi.org/10.3896/IBRA.4.01.2.04

PIRES, J; ESTEVINHO, M L; FEÁS, X; CANTALAPIEDRA, J; IGLESIAS, A (2009) Pollen spectrum and physico-chemical attributes of heather (*Erica* sp.) honeys of north Portugal. *Journal of the Science of Food and Agriculture* 89(11): 1862-1870. http://dx.doi.org/10.1002/jsfa.3663

PIRINI, A; CONTE, L S; FRANCIOSO, O; LERCKER, G (1992) Capillary gas chromatography determination of free amino acids in honey as a mean of discrimination between different botanical sources. *Journal of High Resolution Chromatography* 15(3): 165-170. http://dx.doi.org/10.1002/jhrc.1240150306

PONIKVAR, M; ŠNAJDER, J; SEDEJ, B (2005) Honey as a bioindicator for environmental pollution with SO_2. *Apidologie* 36(3): 403-409. http://dx.doi.org/10.1051/apido:2005027

PONTARA, L P M; CLEMENTE, E; OLIVEIRA, D M; KWIATKOWSKI, A; ROSA, C I L F; SAIA, V E (2012) Physicochemical and microbiological characterization of cassava flower honey samples produced by africanized honeybees. *Ciência e Tecnologia de Alimentos* 32(3): 547-552. http://dx.doi.org/10.1590/S0101-20612012005000066

PONTOH, J; LOW, N H (2002) Purification and characterization of β-glucosidase from honey bees (*Apis mellifera*). *Insect Biochemistry and Molecular Biology* 32(6): 679-690. http://dx.doi.org/10.1016/S0965-1748(01)00147-3

POPA, D; USTUNOL, Z (2011) Influence of sucrose, high fructose corn syrup and honey from different floral sources on growth and acid production by lactic acid bacteria and bifidobacteria. *International Journal of Dairy Technology* 64(2): 247-253. http://dx.doi.org/10.1111/j.1471-0307.2011.00666.x

POPA, M; BOSTAN, R; POPA, D (2013) Honey-marker of environmental pollution. Case study-the Transylvania Region, Romania. *Journal of Environmental Protection and Ecology* 14: 273-280.

POSTMES, T (2001) The treatment of burns and other wounds with honey. In *Munn, P; Jones, R (eds). Honey and healing*. IBRA International Bee Research Association; Cardiff, UK. pp 41-47.

POSTMES, T; VAN DEN BOGAARD, A E; HAZEN, M (1995) The sterilization of honey with cobalt 60 gamma radiation: a study of honey spiked with spores of *Clostridium botulinum* and *Bacillus subtilis*. *Experientia* 51(9-10): 986-989. http://dx.doi.org/10.1007/BF01921753

POULTER, N (2003) Global risk of cardiovascular disease. *Heart* 89(Suppl 2): 2-5. http://dx.doi.org/10.1136/heart.89.suppl_2.ii2

PRIMORAC, L; FLANJAK, I; KENJERIC, D; BUBALO, D; TOPOLNJAK, Z (2011) Specific rotation and carbohydrate profile of Croatian unifloral honeys. *Czech Journal of Food Sciences* 29(5): 515-519.

PYRZYNSKA, K; BIESAGA, M (2009) Analysis of phenolic acids and flavonoids in honey. *Trends in Analytical Chemistry* 28(7): 893-902. http://dx.doi.org/10.1016/j.trac.2009.03.015

RASHED, M N; EL-HATY, M T A; MOHAMED, S M (2009) Bee honey as environmental indicator for pollution with heavy metals. *Toxicological and Environmental Chemistry* 91(3): 389-403. http://dx.doi.org/10.1080/02772240802294870

REN, Z; BIAN, X; LIN, L; BAI, Y; WANG, W (2010) Viscosity and melt fragility in honey-water mixtures. *Journal of Food Engineering* 100(4): 705-710. http://dx.doi.org/10.1016/j.jfoodeng.2010.06.004

RICE-EVANS, C A; MILLER, N J; PAGANGA, G (1997) Antioxidant properties of phenolic compounds. *Trends in plant science* 2(4): 152-159. http://dx.doi.org/10.1016/S1360-1385(97)01018-2

RINAUDO, M T; PONZETTO, C; VIDANO, C; MARLETTO, F (1973) The origin of honey saccharase. *Comparative Biochemistry and Physiology* 46B(2): 245-251. http://dx.doi.org/10.1016/0305-0491(73)90314-3

RISSATO, S R; GALHIANE, M S; DE ALMEIDA, M V; GERENUTTI, M; APON, B M (2007) Multiresidue determination of pesticides in honey samples by gas chromatography-mass spectrometry and application in environmental contamination. *Food Chemistry* 101(4): 1719-1726. http://dx.doi.org/10.1016/j.foodchem.2005.10.034

RODRÍGUEZ-DELGADO, M I (2010) Parámetros descriptores y requisitos para la Denominación de Origen Protegida Mieles de Sierra Morena. PhD Thesis. University of Cordoba (Spain). Advisors: Dr. Salud Serrano Jiménez and Dr. José Luis Ubera Jiménez.

RODRÍGUEZ-GARCÍA, J C; IGLESIAS-RODRÍGUEZ, R; PEÑA CRECENTE, R M; BARCIELA-GARCÍA, J; GARCÍA-MARTÍN, S; HERRERO-LATORRE, C (2006) Preliminary Chemometric Study on the Use of Honey as an Environmental Marker in Galicia (Northwestern Spain). *Journal of Agricultural and Food Chemistry* 54(19): 7206-7212. http://dx.doi.org/10.1021/jf060823t

ROUDAUT, G; DEBEAUFORT, F (2011) Moisture loss, gain and migration in foods. In *Kilcast, D; Subramaniam, P (Eds). Food and Beverage Stability and Shelf Life*. Woodhead Publishing Limited; Cambridge, UK. pp. 63-105.

RUIZ-ARGÜESO, T; RODRÍGUEZ-NAVARRO, A (1973) Gluconic acid-producing bacteria from honeybees and ripening honeys. *Journal of General Microbiology* 76(1): 211-216. http://dx.doi.org/10.1099/00221287-76-1-211

RUIZ-MATUTE, A I; BROKL, M; SORIA, A C; SANZ, M L; MARTÍNEZ-CASTRO, I (2010) Gas chromatographic-mass spectrometric characterization of tri- and tetrasaccharides in honey. *Food Chemistry* 120(2): 637-642. http://dx.doi.org/10.1016/j.foodchem.2009.10.050

RYBAK-CHMIELEWSKA, H (2004) Honey. In *Tomasik, P (Ed). Chemical and functional properties of food saccharides.* CRC Press; Washington, US. pp. 83-89.

SABATINI, A G (2007) Il miele: Origine, composizione e proprietà. In *Sabatini, A G; Botolotti, L; Marcazzan, G L (Eds). Conscere il miele.* Consiglio per la Ricerca e la Sperimentazione in Agricoltora-Istituto Nazionale di Apicoltura (CRA-API). Ed. Avenue media; Bologna-Milano, Italy. pp 3-37.

SÁINZ-LAÍN, C; GÓMEZ-FERRERAS, C (2000) *Mieles Españolas. Características e identificación mediante el análisis de polen*. Ed. Mundi-Prensa; Madrid, Spain. pp. 52-59.

SAJID, M; AZIM, M (2012) Characterization of the nematicidal activity of natural honey. *Journal of agricultural and food chemistry* 60(30): 7428-7434. http://dx.doi.org/10.1021/jf301653n

SÁNCHEZ, M P; HIUDOBRO, J F; MATO, I; MUNIATEGUI, S; SANCHO, M T (2001) Evolution of invertase activity in honey over two years. *Journal of Agricultural and Food Chemistry* 49(1): 416-422. http://dx.doi.org/10.1021/jf0003350

SANCHO, M T; MUNIATEGUI, S; HUIDOBRO, J F; SIMAL, J (1991a) Mieles del País Vasco, X: Tendencia a la granulación. *Anales de Bromatología* XLIII-2/3: 283-292.

SANCHO, M T; MUNIATEGUI, S; SÁNCHEZ, M P; HUIDOBRO, J F; SIMAL, J (1991b) Relationships between electrical conductivity and total and sulphated ash contents in Basque honeys. *Apidologie* 22(5): 487-494. http://dx.doi.org/10.1051/apido:19910501

SANCHO, M T; MUNIATEGUI, S; SÁNCHEZ, M P; HUIDOBRO, J F; SIMAL-LOZANO, J (1992) Evaluating soluble and insoluble ash, alkalinity of soluble and insoluble ash and total alkalinity of ash in honey using electrical conductivity measurements at 20°C. *Apidologie* 23(4): 291-297. http://dx.doi.org/10.1051/apido:19920403

SANCHO, M T; MATO, I; HUIDOBRO, J F; FERNÁNDEZ-MUIÑO, M A; PASCUAL-MATÉ, A (2013) Nonaromatic organic acids of honeys. In *Vit, P; Pedro, S R M; Roubik, D (Eds). Pot honey: A legacy of stingless bees.* Springer Science+Business Media; New York, USA. pp. 447-458. http://dx.doi.org/10.1007/978-1-4614-4960-7_32

SANTOS, F K G; DANTAS-FILHO, A N; LEITE, R H L; AROUCHA, E M M; SANTOS, A G; OLIVEIRA, T A (2014) Rheological and some physicochemical characteristics of selected floral honeys from plants of Caatinga. *Annals of the Brazilian Academy of Sciences* 86(2): 981-994. http://dx.doi.org/10.1590/0001-3765201420130064

SANZ, M L; GONZÁLEZ, M M; MARTÍNEZ-CASTRO, I (2002) Los azúcares de la miel. In *De Lorenzo, C (Ed). La miel de Madrid.* Consejería de Economía e Innovación Tecnológica. Comunidad de Madrid. Instituto Madrileño de investigación Agraria y alimentaria. pp. 95-108. Available at: http://www.madrid.org/bvirtual/BVCM005574.pdf

SANZ, M L; GONZÁLEZ, M; DE LORENZO, C; SANZ, J; MARTÍNEZ-CASTRO, I (2004) Carbohydrate composition and physico chemical properties of artisanal honeys from Madrid (Spain): occurrence of *Echium sp.* honey. *Journal of the Science of Food and Agriculture* 84(12): 1577-1584. http://dx.doi.org/10.1002/jsfa.1823

SANZ, M L; POLEMIS, N; MORALES, V; CORZO, N; DRAKOULARAKOU, A; GIBSON, G R; RASTALL, R A (2005) In vitro investigation into the potential prebiotic activity of honey oligosaccharides. *Journal of agricultural and food chemistry* 53(8): 2914-2921. http://dx.doi.org/10.1021/jf0500684

SANZ, S; GRADILLA, G; JIMENO, F; PÉREZ, C; JUAN, T (1995) Fermentation problems in Spanish north-coast honey. *Journal of Food Protection* 58(5): 515-518.

SARIC, G; MARKOVIC, K; MAJOR, N; KRPAN, M; URSULIN-TRSTENJAK, N; HRUSKAR, M; VAHCIC, N (2012) Changes of antioxidant activity and phenolic content in acacia and multifloral honey during storage. *Food Technology and Biotechnology* 50(4): 434-441.

SCHRAMM, D D; KARIM, M; SCHRADER, H R; HOLT, R R; CARDETTI, M; KEEN, C L (2003) Honey with high levels of antioxidants can provide protection to healthy human subjects. *Journal of agricultural and food chemistry* 51(6): 1732-1735. http://dx.doi.org/10.1021/jf025928k

SERRA-BONVEHÍ, J (1986) La cristallisation du miel: facteurs que l'affectent. *Bulletin Technique Apicole* 54 (13/1): 37-48.

SERRA-BONVEHÍ, J (1989) Estudio de la validez de los índices que predicen la cristalización de la miel. *Revista de Agroquimica y Tecnologia de Alimentos* 29(1): 47-62.

SERRA-BONVEHÍ, J; SOLIVA-TORRENTÓ, M; MUNTANÉ-RAICH, J (2000) Invertase activity in fresh and processed honeys. *Journal of the Science of Food and Agriculture* 80(4): 507-512. http://dx.doi.org/10.1002/(SICI)1097-0010(200003)80:4<507::AID-JSFA558>3.0.CO;2-5

SERRA-BONVEHÍ, J; VENTURA-COLL, F (2003) Flavour index and aroma profiles of fresh and processed honeys. *Journal of the Science of Food and Agriculture* 83(4): 275-282. http://dx.doi.org/10.1002/jsfa.1308

SHAMALA, T R; JYOTHI, Y S; SAIBABA, P (2000) Stimulatory effect of honey on multiplication of lactic acid bacteria under *in vitro* and *in vivo* conditions. *Letters in Applied Microbiology* 30(6): 453-455. http://dx.doi.org/10.1046/j.1472-765x.2000.00746.x

SHERLOCK, O; DOLAN, A; ATHMAN, R; POWER, A; GETHIN, G; COWMAN, S; HUMPHREYS, H (2010) Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. *BMC Complementary and Alternative Medicine* 10: 47. http://dx.doi.org/10.1186/1472-6882-10-47

SHIN, H S; USTUNOL, Z (2005) Carbohydrate composition of honey from different floral sources and their influence on growth of selected intestinal bacteria: An in vitro comparison. *Food Research International* 38(6): 721-728. http://dx.doi.org/10.1016/j.foodres.2005.01.007

SIMAL, J; HUIDOBRO, J; ARAQUISTAIN, J L (1983) Parámetros de calidad de la miel: determinación del contenido en agua. *Offarm* 2(7/8): 243-248.

SIMON, A; TRAYNOR, K; SANTOS, K; BLASER, G; BODE, U; MOLAN, P (2009) Medical honey for wound care-Still the "latest resort"? *Evidence-Based Complementary and Alternative Medicine* 6(2): 165-173. http://dx.doi.org/10.1093/ecam/nem175

SINGER, A J; CLARK, R A (1999) Cutaneous wound healing. *New England Journal of Medicine* 341: 738-746. http://dx.doi.org/10.1056/NEJM199909023411006

SODRÉ, G S; MARCHINI, L C; MORETI, A C C C; OTSUK, I P; CARVALHO, C A L (2011) Physico-chemical characteristics of honey produced by *Apis mellifera* in the Picos region, state of Piauí, Brazil. *Revista Brasileira de Zootecnia* 40(8): 1837-1843. http://dx.doi.org/10.1590/S1516-35982011000800030

SOLER, C; GIL, M I; GARCÍA-VIGUERA, C; TOMÁS-BARBERÁN, F A (1995) Flavonoid patterns of French honeys with different floral origin. *Apidologie* 26(1): 53-60. http://dx.doi.org/10.1051/apido:19950107

SONE, T (1972) *Consistency of foodstuffs*. D. Reidel Publishing Company; Dordrecht, Holland. 188 pp. http://dx.doi.org/10.1007/978-94-010-2876-9

SORIA, A C; MARTÍNEZ-CASTRO, I; SANZ, J (2003) Analysis of volatile composition of honey by solid phase microextraction and gas chromatography-mass spectrometry. *Journal of Separation Science* 26(9-10): 793-801. http://dx.doi.org/10.1002/jssc.200301368

SORIA, A C; GONZÁLEZ, M; DE LORENZO, C; MARTÍNEZ-CASTRO, I (2005) Estimation of the honeydew ratio in honey samples from their physicochemical data and from their volatile composition obtained by SPME and GC-MS. *Journal of the Science of Food and Agriculture* 85(5): 817-824. http://dx.doi.org/10.1002/jsfa.1890

SPANO, N; CASULA, L; PANZANELLI, A; PILO, M I; PIU, P C; SCANU, R; TAPPARO, A; SANNA, G (2006) An RP-HPLC determination of 5-hydroxymethylfurfural in honey. The case of strawberry tree honey. *Talanta* 68(4): 1390-1395. http://dx.doi.org/10.1016/j.talanta.2005.08.003

SPANO, N; CIULU, M; FLORIS, I; PANZANELLI, A; PILO, M I; PIU, P C; SCANU, R; SANNA, G (2008) Chemical characterization of a traditional honey-based Sardinian product: *Abbamele. Food Chemistry* 108(1): 81-85. http://dx.doi.org/10.1016/j.foodchem.2007.10.046

STANDRIDGE, J B (2005) Hypertension and atherosclerosis: Clinical implications from the ALLHAT trial. *Current Atherosclerosis Reports* 7(2): 132-139. http://dx.doi.org/10.1007/s11883-005-0036-y

STEEG, E; MONTAG, A (1988) Quantitative bestimmung Aromatischer Carbonsäuren in Honig. *Zeitschrift für Lebensmittel-untersuchung und -Forschung* 187(2): 115-120. http://dx.doi.org/10.1007/BF01042621

STELMAKIENĖ, A; RAMANAUSKIENĖ, K; BRIEDIS, V; LESKAUSKAITĖ, D (2012) Examination of rheological and physicochemical characteristics in Lithuanian honey. *African Journal of Biotechnology* 11(60): 12406-12414. http://dx.doi.org/10.5897/AJB12.829

SUÁREZ-LUQUE, S; MATO, I; HUIDOBRO, J F; SIMAL-LOZANO, J; SANCHO, M T (2006) Capillary zone electrophoresis method for the determination of inorganic anions and formic acid in honey. *Journal of Agricultural and Food Chemistry* 54(25): 9292-9296. http://dx.doi.org/10.1021/jf061536s

TABOURET, T (1979) Rôle de l'activité de l'eau dans la cristallisation du miel. *Apidologie* 10(4): 341-358. http://dx.doi.org/10.1051/apido:19790403

TANANAKI, C; THRASYVOULOU, A; MENEXES, G (2005) Absorption of volatile compounds in honey from stored spices. *Journal of Apicultural Research* 44 (2): 71-77. http://dx.doi.org/10.1080/00218839.2005.11101152

TAORMINA, P J; NIEMIRA, B A; BEUCHAT, L R (2001) Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. *International Journal of Food Microbiology* 69(3): 217-225. http://dx.doi.org/10.1016/S0168-1605(01)00505-0

TEJPAL, D; GOYAL, N (2009) Effect of inulin, honey and gum acacia on growth of human faecal potential probiotic *Lactobacilli*. *The IUP Journal of Life Sciences* 3(3): 29-34.

TERRAB, A; DÍEZ, M J; HEREDIA, F J (2002) Characterisation of Moroccan unifloral honeys by their physicochemical characteristics. *Food Chemistry* 79(3): 373-379. http://dx.doi.org/10.1016/S0308-8146(02)00189-9

THE NATIONAL HONEY BOARD (2002). Honey-Health and Therapeutic Qualities. The National Honey Board, 390 Lashley Street Longmont, CO 80501-6045, USA. http://www.biologiq.nl/UserFiles/Compendium%20Honey%202002.pdf

TOMÁS-BARBERÁN, F A; FERRERES, F; BLÁZQUEZ, M A; GARCIA-VIGUERA, C; TOMÁS-LORENTE, F (1993a) High-performance liquid chromatography of honey flavonoids. *Journal of Chromatography* 634(7): 41-46. http://dx.doi.org/10.1016/0021-9673(93)80310-5

TOMÁS-BARBERÁN, F A; FERRERES, F; GARCÍA-VIGUERA, C; TOMÁS-LORENTE, F (1993b) Flavonoids in honey of different geographical origin. *Zeitshrift für Lebensmittel-Untersuchung und-Forschung* 196(1): 38-44. http://dx.doi.org/10.1007/BF01192982

TOMÁS-BARBERÁN, F A; MARTOS, I; FERRERES, F; RADOVIC, B S; ANKLAM, E (2001) HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *Journal of the Science of Food and Agriculture* 81(5): 485-496. http://dx.doi.org/10.1002/jsfa.836

TONELLY, D; GATTAVECCHIA, E; GHINI, S; PORRINI, C; CELLI, G; MERCURI, A M (1990) Honey bees and their products as indicators of environmental radioactive pollution. *Journal of Radioanalytical and Nuclear Chemistry* 141(2): 427-436. http://dx.doi.org/10.1007/BF02035809

TONG, Q; ZHANG, X; WU, F; TONG, J; ZHANG, P; ZHANG, J (2010) Effect of honey powder on dough rheology and bread quality. *Food Research International* 43(9): 2284-2288. http://dx.doi.org/10.1016/j.foodres.2010.08.002

TOWNSEND, G F (1979) Processing and storing liquid honey. In *Crane, E (Ed). Honey: A comprehensive survey (2nd Edition).* Heinemann; London, UK. pp. 269-292.

TRACEY, D; KLARESKOG, L; SASSO, E H; SALFELD, J G; TAK, P P (2008) Tumor necrosis factor antagonist mechanisms of action: A comprehensive review. *Pharmacological therapy* 117(2): 244-279. http://dx.doi.org/10.1016/j.pharmthera.2007.10.001

TRUCHADO, P; FERRERES, F; BORTOLOTTI, L; SABATINI, A G; TOMÁS-BARBERÁN, F A (2008) Nectar flavonol rhamnosides are floral markers of acacia (*Robinia pseudacacia*) honey. *Journal of Agricultural and Food Chemistry* 56(19): 8815-8824. http://dx.doi.org/10.1021/jf801625t

TRUCHADO, L; FERRERES, F; TOMÁS-BARBERÁN, F A (2009a) Liquid chromatography-tandem mass spectrometry reveals the widespread occurrence of flavonoid glycosides in honey, and their potential as floral origin markers. *Journal of Chromatography A* 1216(43): 7241-7248. http://dx.doi.org/10.1016/j.chroma.2009.07.057

TRUCHADO, P; LOPEZ-GALVEZ, F; GIL, M I; TOMÁS-BARBERÁN, F A; ALLENDE, A (2009b) Quorum sensing inhibitory and antimicrobial activities of honeys and the relationship with individual phenolics. *Food Chemistry* 115(4): 1337-1344. http://dx.doi.org/10.1016/j.foodchem.2009.01.065

TUBEROSO, C I G; BIFULCO, E; CABONI, P; COTTIGLIA, F; CABRAS, P; FLORIS, I (2010) Floral markers of strawberry tree (*Arbutus unedo* L.) honey. *Journal of Agricultural and Food Chemistry* 58(1): 384-389. http://dx.doi.org/10.1021/jf9024147

TUBEROSO, C I G; JERKOVIĆ, I; SARAIS, G; CONGIU, F; MARIJANOVIĆ, Z; KÚS, P M (2014) Color evaluation of seventeen European unifloral honey types by means of spectrophotometrically determined CIE L* C^*_{ab} h°_{ab} chromaticity coordinates. *Food chemistry* 145: 284-291. http://dx.doi.org/10.1016/j.foodchem.2013.08.032

TURHAN, I; TETIK, N; KARHAN, M; GUREL, F; TAVUKCUOGLU, H R (2008) Quality of honeys influenced by thermal treatment. *LWT-Food Science and Technology* 41(8): 1396-1399. http://dx.doi.org/10.1016/j.lwt.2007.09.008

TURKMEN, N; SARI, F; POYRAZOGLU, E S; VELIOGLU, Y S (2006) Effects of prolonged heating on antioxidant activity and colour of honey. *Food Chemistry* 95(4): 653-657. http://dx.doi.org/10.1016/j.foodchem.2005.02.004

ULSOY, E; KOLAYLI, S; SARIKAYA, A O (2010) Antioxidant and antimicrobial activity of different floral origin honeys from Turkiye. *Journal of Food Biochemistry* 34(S1): 321-335. http://dx.doi.org/10.1111/j.1745-4514.2009.00332.x

USDA - UNITED STATES DEPARTMENT OF AGRICULTURE (2011) National Nutrient Database for Standard Reference: honey. Available at: http://ndb.nal.usda.gov/

USTUNOL, Z (2000) *The effect of honey on the growth of bifidobacteria*. Report for the National honey board: 1-8.

USTUNOL, Z; GANDHI, H (2001) Growth and viability of commercial *Bifidobacterium* spp. in honey-sweetened skim milk. *Journal of Food Protection* 64(11): 1775-1779.

VAL, A; HUIDOBRO, J F; SÁNCHEZ, M P; MUNIATEGUI, S; FERNÁNDEZ-MUIÑO, M A; SANCHO, M T (1998) Enzymatic determination of galactose and lactose in honey. *Journal of Agricultural and Food Chemistry* 46(5): 1381-1385. http://dx.doi.org/10.1021/jf970483z

VELA, L; DE LORENZO, C; PÉREZ, R A (2007) Antioxidant capacity of Spanish honeys and its correlation with polyphenol content and other physicochemical properties. *Journal of the Science of Food and Agriculture* 87(6): 1069-1075. http://dx.doi.org/10.1002/jsfa.2813

VIT, P; BARRERA, M (2002) Intoxicacion con miel de abejas producida en El Limoncito y El Celoso, Venezuela. *Revista de la Facultad de Farmacia* 44(2): 36-42.

WANG, X H; GHELDOF, N; ENGESETH, N J (2004) Effect of processing and storage on antioxidant capacity of honey. *Journal of Food Science* 69(2): C96-C101. http://dx.doi.org/10.1111/j.1365-2621.2004.tb15509.x

WATANABE, K; RAHMASARI, R; MATSUNAGA, A; HARUYAMA, T; KOBAYASHI, N (2014) Anti-influenza viral effects of honey in vitro: potent high activity of manuka honey. *Archives of Medical Research* 45(5): 359-365. http://dx.doi.org/10.1016/j.arcmed.2014.05.006

WHITE, J W JR. (1957) The composition of honey. *Bee World* 38 (3): 57-66. http://dx.doi.org/10.1080/0005772X.1957.11094976

WHITE, J W JR. (1975) La miel. In *Dadant e hijos (Eds). La colmena y la abeja melífera*. Editorial Hemisferio Sur; Hamilton (IL), USA. pp. 397-428.

WHITE, J W JR. (1978) Honey. *Advances in Food Research* 24: 287-374. http://dx.doi.org/10.1016/S0065-2628(08)60160-3

WHITE, J W JR. (1979a) Composition of honey. In *Crane, E (Ed). Honey: A comprehensive survey (2nd Edition)*. Heinemann; London, UK. pp. 157-206.

WHITE, J W JR. (1979b) Physical characteristics of honey. In *Crane, E (Ed). Honey: A comprehensive survey (2nd Edition)*. Heinemann; London, UK. pp. 207-239.

WHITE, J W JR.; MAHER, J (1953) Transglucosidation by honey invertase. *Archives of Biochemistry and Biophysics* 42(2): 360-367. http://dx.doi.org/10.1016/0003-9861(53)90365-8

WHITE, J W JR.; RIETHOF, M L; KUSHNIR, I (1961) Composition of honeys VI: The effect of storage on carbohydrates, acidity and diastase content. *Journal of Food Science* 26(1): 63-71. http://dx.doi.org/10.1111/j.1365-2621.1961.tb00042.x

WHITE, J W JR.; RIETHOF, M L; SUBERS, M H; KUSHNIR, I (1962) *Composition of American honeys*. U.S. Department of Agriculture Technical Bulletin 1261: 1-124.

WHITE, J W JR.; SUBERS, M H; SCHEPARTZ, A J (1963) The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. *Biochimica et Biophysica Acta* 73(1): 57-70. http://dx.doi.org/10.1016/0926-6569(63)90108-1

WHITE, J W JR.; SUBERS, M H (1963) Studies on honey inhibine. 2. A chemical assay. *Journal of Apicultural Research* 2(2): 93-100. http://dx.doi.org/10.1080/00218839.1963.11100066

WHITE, J W JR.; SUBERS M H (1964a) Studies on honey inhibine. 3. Effect of heat. *Journal of Apicultural Research* 3(1): 45-50. http://dx.doi.org/10.1080/00218839.1964.11100082

WHITE, J W JR.; SUBERS M H (1964b) Studies on honey inhibine. 4. Destruction of the peroxide accumulation system by light. *Journal of Food Science* 29(6): 819-828. http://dx.doi.org/10.1111/j.1365-2621.1964.tb00455.x

WHITE, J W JR.; KUSHNIR, I; SUBERS, M H (1964) Effect of storage and processing temperatures on honey quality. *Food Technology* 18(4): 153-156.

WILLERSON, J; RIDKER, P (2004) Inflammation as a cardiovascular risk factor. *Circulation* 109(1): II2-II10. http://dx.doi.org/10.1161/01.CIR.0000129535.04194.38

WITCZAK, M; JUSZCZAK, L; GALKOWSKA, D (2011) Non-newtonian behaviour of heather honey. *Journal of Food Engineering* 104(4): 532-537. http://dx.doi.org/10.1016/j.jfoodeng.2011.01.013

WOLLGAST, J; ANKLAM, E (2002) Review on polyphenols in *Theobroma* cacao: Changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Research International* 33(6): 423-447. http://dx.doi.org/10.1016/S0963-9969(00)00068-5

WOO, K J; JEONG, Y J; INOUE, H; PARK, J W; KWON, T K (2005) Chrysin suppresses lipopolysaccharide-induced cyclooxygenase-2 expression through the inhibition of nuclear factor for IL-6 (NF-IL6) DNA-binding activity. *FEBS Letters* 579(3): 705-711. http://dx.doi.org/10.1016/j.febslet.2004.12.048

WUNDERLIN, D A; PESCE, S F; AMÉ, M V; FAYE, P F (1998) Decomposition of hydroxymethylfurfural in solution and protective effect of fructose. *Journal of Agriculture and Food Chemistry* 46(5): 1855-1863. http://dx.doi.org/10.1021/jf9710140

YANNIOTIS, S; SKALTSI, S; KARABURNIOTI, S (2006) Effect of moisture content on the viscosity of honey at different temperatures. *Journal of Food Engineering* 72(4): 372-377. http://dx.doi.org/10.1016/j.jfoodeng.2004.12.017

YAO, L; DATTA, N; TOMÁS-BARBERÁN, F A; FERRERES, F; MARTOS, I; SINGANUSONG, R (2003) Flavonoids, phenolic acids and abscisic acid in Australian and New Zealand *Leptospermum* honeys. *Food Chemistry* 81(2): 159-168. http://dx.doi.org/10.1016/S0308-8146(02)00388-6

YAO, L; JIANG, Y; SINGANUSONG, R; D'ARCY, B; DATTA, N; CAFFIN, N; RAYMONT, K (2004) Flavonoids in Australian *Melaleuca, Guioa, Lophostemon, Banksia* and *Helianthus* honeys and their potential for floral authentication. *Food Research International* 37(2): 166-174. http://dx.doi.org/10.1016/j.foodres.2003.11.004

YUN, Y W (1996) Fructooligosaccharides-occurrence, preparation and application. *Enzyme and microbial technology* 19(2): 107-117. http://dx.doi.org/10.1016/0141-0229(95)00188-3

ZEINA, B; OTHMAN, O; AL-ASSAD, S (1996) Effect of honey versus thyme on *Rubella* virus survival in vitro. *Journal of Alternative and Complementary Medicine* 2(3): 345-348. http://dx.doi.org/10.1089/acm.1996.2.345

ZEINA, B; ZOHRA, B I; AL ASSAD, S (1997) The effects of honey on *Leishmania* parasites: an in vitro study. *Tropical Doctor* 27 (Suppl 1): 36-38. http://dx.doi.org/10.1177/00494755970270S112

ZHAO, X; ZHOU, Z J; HAN, Y; WANG, Z Z; FAN, J; XIAO, H Z (2013) Isolation and identification of antifungal peptides from *Bacillus* BH072, a novel bacterium isolated from honey. *Microbiological Research* 168(9): 598-606. http://dx.doi.org/10.1016/j.micres.2013.03.001

CHAPTER 3

METHODS OF ANALYSIS FOR HONEY

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ABSTRACT

In this chapter, a thorough updated review for both standardized and most used and novel analytical methods for the analysis of honey has been made. The methodologies applied to honey in the analysis of the physical parameters (electrical conductivity, rheological properties, specific rotation, colour and water activity), the analysis of the properties and the most important components of honey (moisture, sugars, enzymes, HMF, types of acidity and pH, formol index, insoluble solids, organic acids, proteins, amino acids, vitamins, minerals, volatile and semi-volatile compounds and polyphenols), and the antioxidant and antimicrobial activities have been described. Finally, the most applied methods for multicomponent analysis and/or for honey authenticity verification (both the botanical and/or geographical origin honey classification and the detection of honey adulteration) have been described.

1. Introduction

Honey analysis is carried out to verify the quality of this food, its authenticity, as well as to establish, if possible, its botanical and geographical origins. For these purposes, the most common determinations are melissopalinology, sensory, biological and physicochemical. The use of state-of-the art procedures and non-destructive on-line methodologies is nowadays getting increasingly important in food industries, so that currently many laboratories related to bee products are already using modern technology to study honeys.

Several analytical procedures for a variety of quality control parameters and properties of honey have been thoroughly tested, discussed, and published (Anklam, 1998; Bogdanov, 2009; AOAC, 2012; Aissat and Benbarek, 2014; Sarker and Nahar, 2014).

This chapter will focus on summarizing the principles, advantages and disadvantages of the common methods of analysis applied to the most important natural honey characteristics, components and properties. A description of the specific procedures is detailed in the cited references. Melissopalinology, sensory analysis, as well as determination of residues, possible contaminants and honey's adulterations are topics of other chapters.

2. Physical features and properties of honeys

2.1. Electrical conductivity

It represents the capacity of honey to carry the flow of an electric current and mostly depends on its mineral content (Crane, 1975), being different according to the botanical origin of honeys (Codex Alimentarius, 2001; OJEC, 2002). Electrical conductivity of honey is usually

assessed on honey solutions at 20% dry matter, by measuring the electrical resistance with a conductimeter calibrated at a given temperature, currently established at 20ºC (Vorwohl, 1964a, 1964b; Szczesna and Rybak-Chmielewska, 2004; Bogdanov, 2009). However, Bogdanov *et al.* (2004) recommended carrying out electrical conductivity measurements at the international reference temperature of 25ºC. Sancho *et al.* (1991a) found a significant linear relationship between the results of honeys' electrical conductivity measured in humid and in dry matter, so that the method of analysis could be simplified. Relationships were also found between electrical conductivity values, and total, sulphated ash, soluble ash, insoluble ash, and alkalinity of ash (Accorti *et al.*, 1987; Sancho *et al.*, 1991c; Sancho *et al.*, 1992). Electrical conductivity has also been measured by infrared spectroscopy methods such as NIR (near infrared spectroscopy) (Cozzolino and Corbella, 2003), FT-NIR (Fourier transform near infrared spectroscopy) (Ruoff *et al.*, 2007) and FT-MIR (Fourier transform mid-infrared spectroscopy) (Lichtenberg-Kraag *et al.*, 2002; Ruoff *et al.*, 2006a; Almeida-Muradian *et al.*, 2014a). Major *et al.* (2011) determined this parameter by potentiometry.

2.2. Rheological properties

Its measurement in honey is of capital importance for the design of pumping and transport equipment (Trávníček *et al.*, 2012). Several methods have been used to measure rheological properties of honey, among which cone-and-plates, controlled strained and stress methods, double-gap cylinder, dynamic tests, as well as frequency weep assays are commonly employed (Kolayli *et al.*, 2014). Viscosity is the most important rheological property of honey (Kayacier and Karaman, 2008), which depends on water content and temperature (Abu-Jdayl *et al.*, 2002; Yanniotis *et al.*, 2006), and can help classify honey samples by their botanical origins (Wei *et al.*, 2010). Honey viscosity is a key factor for extraction, pumping, setting, filtration, mixing, bottling, and technological applications of this food (Kolayli *et al.*, 2014). The variation in honey viscosity with temperature was expressed by the consistence index (Mossel *et al.*, 2000; Sengul *et al.*, 2005). Even although most honeys were described as Newtonian fluids (Bhandari *et al.*, 1999), ling heather honeys (*Calluna vulgaris*) in particular exhibit thixotropic behaviour and were classified as non-Newtonian fluids, apparently because of containing high molecular weight compounds (Witczak *et al.*, 2011). To measure honeys' rheological properties, dependence of dynamic viscosity on temperature (using the Arrhenius mathematical model), as well as dependence of shear stress on shear rate have been assessed (Trávníček *et al.*, 2012).

2.3. Polarization and specific rotation

It is related to honeys' sugars composition that makes this food rotate the plane of polarized light (Bogdanov *et al.*, 2004). According to Persano-Oddo and Piro (2004), specific rotation could help differentiate blossom and honeydew honeys. The polarimetric method approved for the International Honey Commission (Bogdanov, 2009), measures the angular rotation of

a clear and filtered aqueous honey solution. The procedure was optimized by Serrano *et al.* (2012), using a Plackett-Burman experimental design.

Polarization 920.182 method has been adopted by AOAC (2012).

García-Álvarez *et al.*, 2002 measured polarimetric parameters such as direct polarization, polarization after inversion, polarization due to non-monosaccharides and specific rotation by NIR.

2.4. Colour

It is one of the honey features that influence the consumers' choice. Honeys' colour intensity is usually measured using optical comparators (Fell, 1978; Aubert and Gonnet, 1983), which generally provide with Pfund index grading. Optical comparisons have been established as 985.25 and 960.44 official methods (AOAC, 2012). Honeys' colours according to Pfund scale can also be described by absorbance measurement at different wavelengths such as 560 nm (Kolayli *et al.*, 2014) or 635 nm (Pontis *et al.*, 2014), directly on honeys or in diluted samples. Other researchers measure honey colour by tristimulative methodology, mainly using the C.I.E. (International Lighting Commission) Y, x, y coordinates (Huidobro and Simal, 1984; Mateo-Castro *et al.*, 1992), or L*, a*, b* coordinates (Persano-Oddo *et al.*, 1995; Anupama *et al.*, 2002, among many other authors). For honey characterization several scientists set up reflectance spectroscopy procedures (Negueruela and Pérez-Arquillué, 2000), NIR (Cozzolino and Corbella, 2003), as well as spectroradiometry measurements, that determine colour in the same way as the human eye does (Terrab *et al.*, 2004). The methods based on optical comparison are more subjective than the most recently used instrumental techniques. Terrab *et al.* (2004) claimed that a combination of spectroradiometry and multivariate statistics was particularly suitable to differentiate similar coloured honeys of different botanical origin (thyme and avocado).

2.5. Water activity

It is the amount of water that is available to microorganisms, defined as the ratio of the vapour pressure of water in a material to the vapour pressure of pure water at the same temperature. Between 4ºC and 37ºC, water activity of honey varies between 0.562 and 0.620 (McCarthy, 1995). Honey's water activity is usually determined by the chilled-mirror dewpoint technique. Correlations have been found between honey's moisture and its water activity (Beckh *et al.*, 2004; Cavia *et al.*, 2004).

3. Analysis of the most important constituents

3.1. Moisture

The water content is an important factor that contributes to honey stability against granulation and fermentation during storage (Nanda *et al.*, 2003).

3.1.1. Refractometric method

This is the method proposed by IHC and one of the methods proposed by the AOAC (969.38 method). It is the most used method due to its simplicity and reproducibility. Sugar crystals of honey have to be dissolved previously in a heating bath at 50ºC. Refractive index of the honey is measured at 20ºC with an Abbe or digital refractometer, evaluating moisture percentage by using an empirical formula or a relative conversion table (Bogdanov, 2009; AOAC, 2012).

3.1.2. Direct drying

It is another method proposed by the AOAC (969.38 AOAC method). This method is a gravimetric determination after oven drying at <70ºC under pressure ≤ 50 mm Hg (AOAC, 2012).

3.1.3. Other methods

Infrared spectrometric methods have been used for moisture determination, such as NIR (Ha *et al.*, 1998; Qiu *et al.*, 1999; García-Álvarez *et al.*, 2000; Cho and Ha, 2002; Cozzolino and Corbella, 2003), FT-NIR (Ruoff *et al.*, 2007; Tu *et al.*, 2009) and Fourier transform mid-infrared spectrometry with attenuated total reflectance (FT-MIR-ATR) (Lichtenberg-Kraag *et al.*, 2002; Ruoff *et al.*, 2006a; Pataca *et al.*, 2007; Almeida-Muradian *et al.*, 2014a), in conjunction with multivariate calibration.

Karl Fischer Titration (KFT) technique has been employed by Isengard *et al.* (2001), Isengard and Schultheiß (2003) and Gallina *et al.* (2010). Arida *et al.* (2012) determined moisture by Karl Fisher coulometric method using an automatic potentiometric titrator. Generally, moisture values determined by the refractometry method are lower than those determined by KFT (Isengard *et al.*, 2011; Isengard and Schultheiß, 2003; Bogdanov, 2009; Gallina *et al.*, 2010). Advantages over refractometry method are that pre-treatment liquefaction is not necessary and the empirical formula or relative conversion table, that is not likewise correct for every type of honeys, is not used (Gallina *et al.*, 2010).

Major *et al.* (2011) also measured electrical conductivity by a potentiometric technique.

The ultrasonic shear reflectivity method has been investigated since it is a non-destructive technique, which also gives information on the microstructure of honey (Cereser-Camara and Laux, 2010).

Termogravimetry also can be used as an alternative method for simultaneous determination of moisture and ash in honey samples (Felsner *et al.*, 2004 a,b).

3.2. Sugar analysis

Honey is a supersaturated sugar solution. Sugars are the main constituents of honey accounting for about 95 g/100 g dry matter.

The methods used for the determination of the sugar composition validated by the International Honey Commission (Bogdanov, 2009) are apparent reducing sugars, apparent sucrose, HPLC-IR, HPLC-PAD and GC-FID; and the methods adopted by AOAC (2012) are apparent reducing sugars (920.183 method), apparent sucrose (920.184 method), HPLC-IR (977.20 AOAC method), column chromatography (954.11 and 954.12 methods) and thin layer chromatography for glucose determination (959.12 method). Other methods such as spectroscopy, enzymatic and capillary electrophoresis methods have been also developed.

3.2.1. Determination of total sugars, apparent reducing sugars and apparent sucrose

The method used for quantification of reducing sugars (mainly fructose and glucose) and apparent sucrose in honey is by reducing Soxhlet's modification of Fehling's method (a modification of the Lane and Eynon procedure) (Bogdanov, 2009; AOAC, 2012).

3.2.2. High performance liquid chromatography (HPLC)

HPLC procedure is more welcome than GC, since sample derivatization is normally avoided. For the detection of low molecular weight carbohydrate, several detectors have been used.

- HPLC with refractive index detector (IR)

  It is the most popular method used for sugars identification (Oroian *et al.*, 2014; Silvano *et al.*, 2014). IR detector has several disadvantages such as the lack of sensitivity and selectivity, signal dependence with temperature and with mobile phase flow rate, and the incompatibility with gradient elution (Terol *et al.*, 2012). It requires a simple sample preparation. Silica-based columns of polar aminopropylsilane (-NH2) are the most used, being the main eluent a solution acetonitrile/water (proportion about 80:20 v/v) (Almeida-Muradian *et al.*, 2014b; Bentabol-Manzanares *et al.*, 2014). Identification is carried out by comparing the retention times of the peaks obtained from authentic commercial standards and quantification is normally achieved according to the external calibration method.

- HPLC over a strong anion exchange resin coupled to pulsed amperometric detector (HPAEC-PAD)

  Nowadays, it is the method of choice for carbohydrate analysis (Escuredo *et al.*, 2014; Juan-Borrás *et al.*, 2014), due to its detection limits, much lower than those achieved with IR detector (Nozal-Nalda *et al.*, 2005). HPAEC-PAD takes advantage of the affinity

between the ionized groups of sugars at alkaline pH, being possible a high resolution and highly selective separation of non-derivatized sugars (Bogdanov, 2009). Despite the advantages of HPAEC-PAD, the main problem of this technique is related to the lack of commercial standards for hydrocarbons' chains with high degree of polymerization. Therefore the peak identification could be difficult to be performed (Corradini *et al.*, 2012). HPAEC-PAD columns have to be high pH-resistant polymeric-based strong anion-exchange columns with fast mass transfer and diffusion properties, and good mechanical stability (Bogdanov, 2009). The most common are CarboPac columns manufactured by Dionex (Thermo Fisher Scientific Inc., USA) (Ouchemoukh *et al.*, 2010; Rodríguez-Flores *et al.*, 2014). Sugars are usually eluted under isocratic mode using sodium hydroxide solutions at different concentrations (Juan-Borrás *et al.*, 2014) but also a gradient elution of different mobile phases (water/NaOH or water/NaOH/NaOAc) could be used (Escuredo *et al.*, 2014). Carbohydrates are identified on the basis of their retention times and quantification is made using the external calibration method.

- Other HPLC methods

  Ultra-performance liquid chromatography with an evaporative light scattering detector (UPLC-ELSD) has been also employed for sugars quantification (Zhou *et al.*, 2014a).

3.2.3. Gas chromatography (GC)

GC provides better resolution and sensitivity than HPLC for many important minor carbohydrates in relative short retention times, but it involves a previous derivatization reaction to make sugars volatile compounds (Kaškonienė *et al.*, 2010). Pierce-Pourtallier derivatization is the most common method (Ruiz-Matute *et al.*, 2010), although I.N.A. method has been also used (Pasini *et al.*, 2013). After derivatization process, sugars are determined as their trimethylsilyl (TMS) derivatives (Bogdanov, 2009).

Gas chromatography with flame ionization detector (GC-FID) is the most used GC method. Identification is carried out by comparing their retention times with those of standard compounds and quantification is made with the internal standard method (using sugars not present in honey such as mannitol or phenyl-β-D-glucoside as internal standards, among others) (Sanz *et al.*, 2004; Bogdanov, 2009).

GC mass spectrometry is used, in most cases, to confirm peak identities (De la Fuente *et al.*, 2011), but also for quantitative analysis (Ruiz-Matute *et al.*, 2010). It is a useful technique to characterize complex mixtures of carbohydrates with high degree of polymerisation. The study of the different m/z fragments provides important information about the chemical structure of the molecule (Ruiz-Matute *et al.*, 2010). The most important drawbacks of MS detection are the co-elution of compounds, the similar MS fragmentation pattern obtained for carbohydrates with the same molecular weight (as isomers) and the interferences of other matrix compounds (Sanz *et al.*, 2004). Identification is made by comparison of their mass

spectra data and IT (linear retention index calculated from retention times of the compounds) with those of standard compounds or taking into account data published in the literature (De la Fuente *et al.*, 2011). They are quantified by the internal standard procedure after determination of the total ion current response factors of the individual sugars relative to an internal standard (such as xylose) (Terrab *et al.*, 2002).

3.2.4. Spectroscopy methods

Spectroscopy methods such as NIR (Cho and Hong, 1998; Ha *et al.*, 1998; Qiu *et al.*, 1999; García-Álvarez *et al.*, 2000; Cho and Ha, 2002; Mouazen and Al-Walaan, 2014), FT-NIR-ATR (Fourier transform near infrared spectroscopy method with attenuated total reflectance) (Ruoff *et al.*, 2007; Tu *et al.*, 2009), FT-MIR-ATR (Lichtenberg-Kraag *et al.*, 2002; Tewari and Irudayaraj, 2004; Ruoff *et al.*, 2006a; Pataca *et al.*, 2007; Almeida-Muradian *et al.*, 2014a; Anjos *et al.*, 2015), ^{1}H and ^{13}C NMR (nuclear magnetic resonance spectroscopy) (Jamróz *et al.*, 2014) and Raman Spectroscopy (Özbalzi *et al.*, 2013) in combination with chemometrics, have been applied for sugars analysis in honey. They are quick methods that need minimal sample amount and handling.

3.2.5. Enzymatic determination

Enzymatic methods for determining sugar contents are rapid, sensitive to low sugar concentration and highly specific. They rely on the ability of an enzyme to catalyse a specific reaction (Brummer and Cui, 2005)**.** Through enzymatic commercial kits, UV spectrophotometric determination of glucose, fructose, sucrose and maltose based on the high specificity of the enzymes has been carried out (Gómez-Díaz *et al.*, 2012).

3.2.6. Capillary electrophoresis (CE)

CE technique involves high efficiency, short analysis time, low cost separation with minimum consumption of solvents and minimal sample preparation. Fructose, glucose and sucrose contents have been determined by CE, coupling an amperometric detector (Cheng *et al.*, 2008) and coupling a diode array detector (Biluca *et al.*, 2014).

3.2.7. Electrochemical determination

Potentiometric determination have been used to measured reducing sugars, different sugars (such as glucose, fructose and sucrose) and total sugars by some authors (Papastathopoulos *et al.*, 1977; Gitzapis *et al.*, 1989; Nanos *et al.*, 1991; Basa *et al.*, 2007; Major *et al.*, 2011).

3.2.8. Other non-specific methods

Refractometry can be used to measure ºBrix, directly related with sugar content (Habib *et al.*, 2014b).

Approximate total carbohydrate content (%) can be obtained by difference of the 100% less the addition of ashes, proteins and lipids contents (Almeida-Muradian *et al.*, 2013).

3.2.9. Other methods to detect sugar adulteration

Sugar adulteration of honey with cane or corn can be screened microscopically (Kerkvliet and Meijer, 2000) and verified by determining the $\delta^{13}C$ (a measure of the stable 13C/12C isotopic ratio); due to values originating from C-4 plants (cane plants) are different that from C-3 plants (nectar source plants). The AOAC official methods 998.12, 978.17 and 991.41 have been established for corn and cane sugar adulteration (AOAC, 2012). SCIRA (stable carbon isotope ratio analysis) using an EA-IRMS (Eurovector elemental analyser coupled to an isoprime mass spectrometer) is used as well (Rogers *et al.*, 2014). Guler *et al.* (2014) observed that the indirect adulteration of honey by feeding the bees with C3 plant syrup could not be efficiently detected using the AOAC official methods.

High fructose starch syrup adulteration can be detected by thin layer chromatography, following the AOAC method 979.22 (AOAC, 2012).

3.3. Enzymes

Enzymes are related to the botanical origin of honeys (Persano-Oddo *et al.*, 1990; Anklam, 1998; Persano-Oddo *et al.*, 1999, Persano-Oddo and Piro, 2004), honeys' aging, processing and storage conditions (White *et al.*, 1964; White, 1979a). The main honey enzymes are diastase (amylase), invertase, glucose oxidase (animal origin), as well as catalase and phosphatase (vegetal origin). Minimum honeys' diastase values are included within honeys' standards (Codex Alimentarius, 2001; OJEC, 2002).

3.3.1. Diastase

Several methods for diastase activity determination have been proposed. The first of them was set up by Schade *et al.* (1958), and is based on the measurement, at intervals, of the blue colour developed by iodine and a standard solution of starch hydrolysed by honeys' diastase. This procedure, with further modifications (White and Pairent, 1959; Hadorn and Zürcher, 1972) has been included in the official and harmonized methods of honey analysis, such as Codex Alimentarius (2001), Bogdanov (2009) and the procedure number 958.09 of the AOAC (2012). Siegenthaler (1975), proposed the "Phadebas method" photometric procedure, in which an insoluble blue dyed cross-linked type of starch is used as the substrate. This assay proved to be considerably simpler and faster than the "Schade method". Bogdanov (1984) modified the "Phadebas method", and Persano-Oddo and Pulcini (1999) proposed formula for the diastase assay by "Phadebas" in honeys with low enzyme content. "Phadebas procedure" was included within the harmonized methods of the International Honey Commission (Bogdanov, 2009).

Sakač and Sak-Bosnar (2012) and Sak-Bosnar and Sakač (2012) set up a fast, cheap and simple potentiometric analysis for honey's diastase activity, whose results correlated with those by classic "Schade method" and commercial "Phadebas method". The analysis is based on a direct potentiometric measurement of triiodide ion, which is proportional to the diastase activity of the honey sample.

3.3.2. Invertase

Hadorn and Zürcher (1966) proposed a polarimetric method for the analysis of this honey enzyme. Invertase activity is usually determined by Siegenthaler procedure (Siegenthaler, 1977), based on the spectrophotometric measurement of 4-nitrophenol formed after the reaction of honey invertase with 4-nitrophenyl-α-D-glucopyranoside, used as substrate. The harmonized methods of the International Honey Commission include this method (Bogdanov, 2009). Lichtenberg-Kraag *et al.* (2002) predicted invertase by FT-MIR-ATR. Von der Ohe *et al.* (1999), proposed the international units (U/kg) for expressing the results of invertase.

3.3.3. β-glucosidase

The method of analysis of this enzyme in honey was proposed by Low *et al.* (1986), and consists of a modification of Siegenthaler's technique (Siegenthaler, 1977), using 4-nitrophenyl-β-D-glucopyranoside as substrate.

3.3.4. Glucose-oxidase

Dold and Witzenhausen (1955) proposed a microbiologic method to analyse glucose-oxidase in honey that consisted of checking the effect of honey on the growth of *Staphylococcus aureus* strains. Hydrogen peroxide production was estimated after adding o-dianisidine and peroxidase. Salashinski and Bazhenova (1979) determined glucose-oxidase by a titrimetric assay, in which honey was previously buffered to pH 5.7, and the production of hydrogen peroxide was determined by iodine titration. White and Subers (1963) developed a colorimetric assay to determine the hydrogen peroxide released from diluted honey. Schepartz and Subers (1964) proposed a similar and sensitive colorimetric procedure, but with a previous dialysis of the honey samples, because these researchers verified that such honey aminoacids as proline interfered. The method consisted of the measurement of the absorbance, at 402 nm, of a coloured compound formed after the reaction of hydrogen peroxide with o-dianisidine and peroxidase. Wenhan and Luqing (1993) proposed another colorimetric procedure, in which hydrogen peroxide oxidized potassium iodide.

3.3.5. Catalase

Most published methods for the determination of honey catalase are based on the analysis of the concentration of hydrogen peroxide, before and after a period of incubation (Huidobro *et al.*, 2005). However, all methods previous to the proposal of Schepartz and Subers (1966)

were not appropriate for honey, due to the fact that reagents reacted with other constituents of this foodstuff. The assay of Schepartz and Subers (1966) consisted on the determination of hydrogen peroxide in a previously dialyzed honey solution within a test system containing o-dianisidine and peroxidase. The method was modified by Huidobro *et al.* (2005), dramatically improving the dialysis experimental conditions, reagents preparation and precision. Recently, Franchini *et al.* (2011) set up a method for catalase amperometric detection consisting of a sensor in association with flow injection analysis and a tubular reactor containing Amberlite IRA-743 resin.

3.3.6. Acid phosphatase

Giri (1938) developed a method of analysis for honey's acid phosphatase, determining inorganic phosphorous from β-glycerol phosphate after samples incubation. The incubation time was reduced and the pH of the assay was modified (Günther and Burckhart, 1967), as well as the concentration of substrate, buffer composition, and catalyst agent (Ivanov, 1978). Sánchez *et al.* (2005) and Alonso-Torre *et al.* (2006) verified that the 50% honey solutions employed by Günther and Burckhart (1967) were too concentrated for dark samples and the absorbances too high for a reliable determination, so that Sánchez *et al.* (2005) and Alonso-Torre *et al.* (2006), modified the method of Günther and Burckhart (1967) using 20% (w/v) honey solutions, thus improving the precision.

3.4. Hydroxymethylfurfural

HMF content is an indicator of honey freshness, which is an important criterion for the evaluation of heat damage and/or aging of the product (Bogdanov *et al.*, 2004). There are several methods validated for the determination of HMF in honey. Bogdanov (2009) found small differences between them at very low levels, being repeatability and reproducibility better applying White (1979b) and high performance liquid chromatography methods than applying Winkler (1955) assay. Truzzi *et al.* (2012) found that HPLC method seemed to be more appropriate for the determination of low levels of HMF. HMF results are expressed in mg/kg.

3.4.1. Determination of HMF after Winkler

The absorbance of a filtered honey solution, after clarification with carrez solutions and addition of p-toluidine and barbituric acid solutions, is measured at 550 nm against a blank in the maximum colour intensity (3-4 minutes after the barbituric acid addition) (Winkler, 1955; Bogdanov, 2009). As the Winkler method uses p-toluidine that could be carcinogenic, it should be used only if one of the other methods are not available.

Salinas *et al.*, 1991, proposed a semiautomatic photometric flow injection method for HMF determination on the basis of the Winkler's determination with a good sensibility, reproducibility and precision.

3.4.2. Determination of HMF after White

The absorbance of a filtered honey solution, after clarification with carrez solutions, is measured at 284 and 336 nm against the reference solution (the same solution after addition of sodium bisulphite) (White, 1979b). Apart from having been validated by the IHC (Bogdanov, 2009), this procedure is official as 980.23 in AOAC (2012).

3.4.3. Determination of HMF by HPLC with ultraviolet detector

HMF is determined in a filtered aqueous honey solution. The signal obtained using a reverse phase HPLC with UV detection (UV or DAD detector) at 285 nm is compared with the signal of a standard. A water-methanol mixture is used as mobile phase (Jeuring and Kuppers, 1980; Kahoun *et al.*, 2008; Bogdanov, 2009; Spano *et al.*, 2009).

3.4.4. Determination of HMF by capillary electrophoresis

Capillary electrophoresis with diode array detector is a fast, low cost and simple method that has showed good results for linearity, precision and accuracy (Teixidó *et al.*, 2011; Rizelio *et al.*, 2012b; Wong *et al.*, 2012; Biluca *et al.*, 2014). Micellar electrokinetic chromatography (MEKC) is the most used method. Teixidó *et al.* (2011) and Rizelio *et al.* (2012b) did not observed significant differences when compared the results with LC-MS/MS method.

3.4.5. Other methods

NIR and FT-NIR methods have been investigated by different authors (Cho and Hong, 1998; Ha *et al.*, 1998; Qiu *et al.*, 1999; Cozzolino and Corvella, 2003; Ruoff *et al.*, 2006a; Ruoff *et al.*, 2007; Almeida-Muradian *et al.*, 2012), but the prediction accuracies were low and unreliable. Lichtenberg-Kraag *et al.* (2002) predicted HMF with an acceptable calibration by FT-MIR-ATR.

Teixidó *et al.* (2006) proposed a simple and selective method by gas chromatography coupled to mass spectrometry (GC-MS) after a SPME-derivatization step.

3.5. pH, free acidity, lactonic acidity, total acidity and formol number

Both the methods proposed by the IHC (Bogdanov, 2009) and the method 962.19 defined by the AOAC (AOAC, 2012) are based in titrimetric methods.

3.5.1. Determination of pH

The low pH of honey inhibits the growth of microorganisms (Gomes *et al.*, 2011). pH is measured directly on a water solution of the honey sample using a pH-meter. Usually, this honey solution is 10 g honey in 100 ml water or 10 g honey in 75 ml water (Bogdanov, 2009; AOAC, 2012). Cozzolino and Corbella (2003) measured pH by NIR, Ruoff *et al.* (2007) by FT-NIR and Lichtenberg-Kraag *et al.* (2002), Ruoff *et al.* (2006a) and Almeida-Muradian *et al.* (2014a) by FT-MIR-ATR.

3.5.2. Determination of acidity

The honey acidity is due to the presence of organic acids in balance with their lactones and some inorganic ions (Nanda *et al.*, 2003). Acidity content is expressed in milliequivalents/kg honey. Total acidity is the sum of both free and lactone acidities.

- Determination of free acidity by endpoint titration to pH 8.3 (IHC method)

  A honey solution is titrated with 0.1M NaOH to pH 8.30 in less than 2 minutes, through a burette or an automatic titrator (Bogdanov, 2009).

- Determination of free acidity and lactones by equivalence point titration (IHC method)

  Equivalence point titration is a more correct method than endpoint titration for honey acidity determination, where the equivalence point is fixed for each honey (Bogdanov, 2009).

  The free acidity is measured by titration of a honey solution with 0.05M NaOH up to the equivalence point determined by plotting the neutralization curve.

  The lactone acidity is measured after addition of an excess of NaOH and back titration with 0.025M sulphuric acid up to the second equivalence point.

- Determination of free acidity and lactones by endpoint titration to pH 8.5 and pH 8.3 respectively (AOAC method)

  Free acidity is measured by titration of a honey solution with 0.05 M NaOH to pH 8.50 at rate of 5.0 ml/min.

  Lactone acidity is measured after back titration with 0.05 M HCl to pH 8.30 after addition of 10 ml NaOH.

- Other methods

  Klofutar *et al.* (1990) and Major *et al. (*2011) measured acidity by a potentiometric titration technique.

  Qiu *et al.* (1999) predicted free acidity and lactone by NIR, being the prediction accuracy poor and unreliable, while Cho and Ha (2002) determined acidity, being considered the results sufficient for practical use. Ruoff *et al.* (2007) measured free acidity by FT-NIR and Lichtenberg-Kraag *et al.* (2002) and Ruoff *et al.* (2006a) by FT-MIR. Acidity was also determined by this last method (Pataca *et al.*, 2007; Almeida-Muradian *et al.*, 2014a). Almeida-Muradian *et al.* (2012) also predicted pH using FT-IR-ATR.

3.5.3. Determination of formol number

Formol number gives a measure of total amino acids. It is a parameter important to honey authentication and to detect adulterations (Sancho *et al.*, 1991b). Zürcher (1963) carried out a simultaneous determination of the formol number, pH, free acids and lactone content. In the

Manuel Suisse des Denrées Alimentaires (1974), formol number is measured by titration to pH 8.00. Simal and Huidobro (1984) determined formol number by titration with NaOH 0.05M to pH 8.30 after lactonic acid titration, adding 15 ml of neutralized formaldehyde to the honey solution.

3.6. Insoluble matter

Insoluble matter is an important parameter to detect honey impurities such as bee wax, other honeycomb debris, and bee and filthy particles. The harmonized gravimetric method has a very low reproducibility. A solution of honey in water is collected on a crucible and dried in the oven at 135ºC. The residue is weighed after being washed free of soluble material (Bogdanov, 2009).

3.7. Organic acids

Non-aromatic organic acids have been widely analysed to characterize honey samples from different botanical and geographical origins (Mato *et al.*, 2003; Oelschlägel *et al.*, 2011). In general, the most applied methods have been enzymatic assays, chromatography, and electrophoresis (Mato *et al.*, 2006b; Sancho *et al.*, 2013).

3.7.1. Enzymatic assays

Enzymatic assays are usually the most precise, specific and accurate methods, which require simple equipment, so that many scientists have used them to analyse non-aromatic organic acids of honeys (Tourn *et al.*, 1980; Stoya *et al.*, 1986; Stoya *et al.*, 1987; Hansen and Guldborg, 1988; Talpay, 1988; Talpay, 1989; Sabatini *et al.*, 1994; Mato *et al.*, 1997; Mutinelli *et al.*, 1997; Mato *et al.*, 1998a, 1998b; Cossu and Alamanni, 1999; Alamanni *et al.*, 2000; Bogdanov *et al.*, 2002; Gheldof *et al.*, 2002; Pulcini *et al.*, 2004; Persano-Oddo *et al.*, 2008; Vit *et al.*, 2009). These methods are based on spectrophotometric measurements of specific compounds, after reacting organic acids and specific enzymes. Nevertheless, enzymatic assays allow the analysis of only one organic acid each time, they are usually time-consuming and the enzymatic reagents do not last very long.

3.7.2. Chromatography

Chromatography is also a very useful technique to analyse honeys' nonaromatic organic acids. This technique allows the simultaneous determination of several organic acids.

Stinson *et al.* (1960) employed paper and on-column ion exchange chromatography.

Gas Chromatography with flame ionization or mass spectrometry detectors was also used to separate, identify and quantify honey nonaromatic organic acids. Most procedures required a previous derivatization (Echigo and Takenaka, 1974; Wilkins *et al.*, 1995; Horváth and Molnár-Perl, 1998; Pilz-Güther and Speer, 2004; Sanz *et al.*, 2005), but Jurado-Sánchez *et al.*

(2011) set up a method based on continuous solid-phase extraction without prior derivatization.

High performance liquid chromatography has proved to be one of the best methods to analyse honey non-aromatic organic acids. The most used detectors have been ultraviolet (Cherchi *et al.*, 1994; Cherchi *et al.*, 1995; Del Nozal *et al.*, 1998; Alamanni *et al.*, 2000; Suárez-Luque *et al.*, 2002a, 2002b; Nozal *et al.*, 2003a; Nozal *et al.*, 2003b; Serra-Bonvehí *et al.*, 2004; Hrobonová *et al.*, 2007; Zhu *et al.*, 2010; Beretta *et al.*, 2012), conductivity (Pérez-Cerrada *et al.*, 1989; Defilippi *et al.*, 1995; Del Nozal *et al.*, 2000), and electrochemical detectors (Casella and Gatta, 2001; Daniele *et al.*, 2012). High performance liquid chromatography has good reproducibility and sensitivity, but interferences must be removed.

3.7.3. Capillary electrophoresis

Capillary electrophoresis has been more recently employed to analyse honeys' nonaromatic organic acids in honeys (Boden *et al.*, 2000; Navarrete *et al.*, 2005; Mato *et al.*, 2006a; Suárez-Luque *et al.*, 2006; Tezcan *et al.*, 2011), being less sensitive and precise than other methods, but with the important advantages being low cost, very fast, and allowing the simultaneous determination of several non-aromatic organic acids with a simple sample preparation.

3.8. Protein and protein-related compounds

Protein and protein-related compounds have been analysed in honeys mainly to botanically and/or geographically characterize them, to research the freshness, authenticity and maturity of honeys, as well as to determine possible honeys adulterations. In general, most research has been focused on the separation and quantification of amino acids, in particular, proline, and on the determination of enzymatic activities.

3.8.1. Proteins

Bergner and Diemair (1975) analysed honey proteins by ionic exchange chromatography. Different immunoassays were also used to characterize honeys' proteins (Baroni *et al.*, 2002; Hayashi *et al.*, 2011). Nisbet *et al.* (2009) separated honey proteins by sodium dodecyl sulphate polyacrylamide gel electrophoresis, concluding that protein profile can be used in differentiation between pure and adulterated honeys. Although Kjeldahl method is used for total nitrogen quantification, being related with proteins, this method should only be employed in foods where proteins are the main nitrogen compounds (Iglesias *et al.*, 2002). Nevertheless, a Micro-Kjeldahl procedure was set up and proposed for honey (Bera and Almeida-Muradian, 2005).

3.8.2. Amino acids

Their analysis has been proposed as a potentially useful tool to mainly discriminate the geographical origin of honeys (Bogdanov *et al.*, 2004), because amino acid composition is very variable and depends more on the bees than on the plants (Bergner and Hahn, 1972; Gilbert *et al.*, 1981; Davies and Harris, 1982). Cotte *et al.* (2004) pointed out that the amino acid composition of honey could be a suitable indicator of honey adulteration, as well as Meda *et al.* (2005), who also claimed that the amino acid composition could be a useful criterion to estimate the maturity of honey. Studies about honeys' amino acid composition have researched the amounts of individual amino acids, and, in most papers, multivariate statistical techniques have been used, as well (Kanematsu *et al.*, 1982; Speer and Montag, 1986; Pérez and Herrera, 1987; Cometto *et al.*, 2003; Cotte *et al.*, 2004; Seif-Eldin and Elfadil, 2010; among others).

- Proline

  It is the main honey amino acid, representing 50-80% of the total honeys' amino acids (Kolayli *et al.*, 2014), its quantity being considered as a criterion of honey ripeness (Von der Ohe *et al.*, 1991). Analysis of proline is usually carried out colorimetrically, measuring the coloured complex of proline and ninhydrin following the official procedures (Bogdanov, 2009; AOAC, 2012). Truzzi *et al.* (2014) compared the official methods for the analysis of proline in honey, concluding that the AOAC 979.20 method is better in terms of accuracy and time saving.

  FT-NIR (Ruoff *et al.*, 2007) and FT-MIR (Lichtenberg-Kraag *et al.*, 2002; Ruoff *et al.*, 2006a) have also been used for proline determination.

- Amino acid composition

  Apart from specific colorimetric methods for some amino acids in particular, honey's amino acid profile was studied by several chromatographic procedures. Petrov (1974) and Davies (1975) analysed the amino acid profiles of honeys from several countries using an automatic amino acid analyser. Ionic exchange chromatography was the technique used by Bergner and Hahn (1972) and Bergner and Diemair (1975). Gas-chromatography after extraction, purification and derivatization, usually employing a flame ionization detector, was a method used by Bosi and Battaglini (1978), Gilbert *et al.* (1981), Pirini *et al.* (1992) and Conte *et al.* (1998). High-performance liquid chromatography, commonly with fluorescence detection, was a procedure used by Pawlowska and Armstrong (1994), Hermosín *et al.* (2003), Cotte *et al.* (2004), Iglesias *et al.* (2004), González-Paramás *et al.* (2006), Senyuva *et al.* (2009) and Carratù *et al.* (2011), among others.

3.9. Vitamins

As honey is a supersaturated sugar solution with very low lipid content, vitamins in honey are mainly water soluble.

3.9.1. Vitamin C or Ascorbic acid

Ascorbic acid is very important by its antioxidant character (Castro *et al.*, 2001; Gheldof *et al.*, 2002; León-Ruiz *et al.*, 2011; León-Ruiz *et al.*, 2013a). For this reason a large number of methods to measure antioxidant capacity express their results in acid equivalent antioxidant content (AEAC).

- Volumetric method

  The 2,6-dichloroindophenol titrimetric method 967.21 proposed for juices by AOAC (2012) has sometimes been applied to honey (Guler *et al.*, 2007; Escuredo *et al.*, 2013b). A solution of honey in acetic acid and metaphosphoric acid is titrated with 2,6-dichloroindophenol. Some modifications are developed by some authors, such as the use of oxalic acid instead of acetic acid-metaphosphoric acid solution (León-Ruiz *et al.*, 2011). Results are expressed in milligrams of vitamin C per 100 g honey.

- Spectrophotometric method

  Ascorbic acid is extracted with metaphosphoric acid and filtered. The filtrate is mixed with 2,6-dichlorophenolindophenol and the absorbance is measured within 30 min at 515 nm against a blank. Ascorbic acid content is calculated on the basis of a standard calibration curve. Results are expressed in mg of vitamin C per kg of honey (Ferreira *et al.*, 2009; Islam *et al.*, 2012; Khalil *et al.*, 2012; Moniruzzaman *et al.*, 2013).

- HPLC method

  Reverse phase HPLC method (RP-HPLC) with UV detector for determination of vitamin C provides low detection, quantification limits and good precision and reproducibility (Ciulu *et al.*, 2011). It has been a method widely used because is faster and simpler than volumetric method (Castro *et al.*, 2001; Gheldof *et al.*, 2002; Álvarez-Suárez *et al.*, 2010a; Álvarez-Suárez *et al.*, 2010b; Aazza *et al.*, 2013; León-Ruiz *et al.*, 2013a). León-Ruiz *et al.* (2011) compared volumetric and RP-HPLC methods concluding there were not statistically significant differences between them.

- Other methods

  Da Silva *et al.* (2012), developed an amperometric method for the specific determination of ascorbic acid in honey using a flow injection analysis (FIA) system and a tubular reactor containing the enzyme ascorbate oxidase. This method is more sensitive, economical, practical and less time consuming and there is a high correlation of the results obtained with this method and the volumetric ones. Escuredo *et al.* (2013b) predicted vitamin C by NIR.

3.9.2. Other vitamins

Colorimetric, spectrophotometric, spectrofluorimetric and microbiological methods were employed in the past (Haydak *et al.*, 1942; Kitzes *et al.*, 1943). In recent years, the most used method is reversed-phase HPLC with or without previous extraction step and coupled with different detectors. Viñas *et al.* (2012) quantified thiamine (vitamin B1) by dispersive liquid-liquid microextraction (DLLME) procedure coupled to LC with fluorimetric detection. A derivatization step was needed. Daneshva-Tarigh and Shemirani (2014) developed a similar method with some modifications, using ultrasound-assisted dispersive magnetic solid phase extraction spectrofluorimetry (USA-DMSPE-FL), increasing the extraction efficiency and doing an in situ derivatization in a short period of time.

Tuberoso *et al.* (2012) performed riboflavin (vitamin B2) quantification by LC-DAD without sample purification. They found low limits of detection and quantification and good precision.

3.9.3. Vitamin profile

Different water-soluble B group vitamins (B1, B2, B3, B5, B6, B9, B12), vitamin B-related compounds (such as vitamin B2, B3 and B6 vitamers) and vitamin C have been quantified at the same time using RP-HPLC coupled to UV/VIS detector (Ciulu *et al.*, 2011; Aazza *et al.*, 2013; León-Ruiz *et al.*, 2013b), to PDA detector (Chua *et al.*, 2013) or to fluorescence detector (Viñas *et al.*, 2004a, 2004b).

3.10. Mineral composition

Honey mineral composition is related to the botanical and geographical origin of this food (Tuzen *et al.*, 2007; Kolayli *et al.*, 2008, 2014; Pasquini *et al.*, 2014), to the procedures employing during its processing (Pisani *et al.*, 2008), as well as to the environmental conditions surrounding the hives (Anklam, 1998; Przybylowski and Wilczyńska, 2001).

As a whole, mineral content of honey can be assessed by the gravimetric method of ash quantification proposed by the International Honey Commission (IHC) and by the AOAC (method 920.181), in which honey is ashed at temperatures close to 600ºC (Sancho *et al.*, 1991c; Sancho *et al.*, 1992; Bogdanov, 2009; AOAC, 2012). However, nowadays the tedious determinations of honey ash content has been replaced by electrical conductivity measurement, which is considerably simpler and faster (Bogdanov *et al.*, 2004), since the higher the mineral content is, the higher the electrical conductivity is. Although ash determination takes into account water soluble and insoluble material, while electrical conductivity only allows soluble material determination (Ortiz *et al.*, 1996), high statistically significant linear correlations (r values up to 0.99) have been reported between the electrical conductivity and ash content of honey by several authors (Pires *et al.*, 2009; Silva *et al.*, 2009; Feás *et al.*, 2010a; Feás *et al.*, 2010b). In fact, some authors calculated ash content depending on the electrical conductivity (Gomes *et al.*, 2010; Alqarni *et al.*, 2012; Ferrauto and Pavone,

2013), according to different formulas reported by other authors (Piazza *et al.*, 1991; Sancho *et al.*, 1992).

Mineral elements are minority honey compounds, and their specific determination is very time consuming, being necessary to remove such interference components as sugars (Pohl *et al.*, 2012). A number of procedures have been developed to identify and quantify honey mineral compounds.

3.10.1. Gravimetric, titrimetric, colorimetric and turbidimetric procedures

Ehrhardt and Liebig (1965) used a combination of gravimetry and complexometry to quantify chloride and phosphate in dried honey samples. For specific anions, titrimetric and colorimetric methods with such different reagents as molybdate vanadate or barium sulphate (Rodríguez-Otero *et al.*, 1992; González-Paramás *et al.*, 2000) were used. Azeredo *et al.* (1998) determined potassium by precipitation with sodium tetraphenylborate, followed by ionic exchange column separation, and eventual titrimetry. Other cations (Ca, Mg, Fe, Mn), were quantified by titrimetric and colorimetric assays (Rodríguez-Otero *et al.*, 1992; González-Paramás *et al.*, 2000).

Spectroscopic procedures are the most employed methods for the analysis of mineral compounds in honey. The main drawback of these assays is that they take very long time, due to the fact that samples must be previously ashed to release the mineral elements that will be later dissolved and analysed by other procedures (Terrab and Heredia, 2004; Ioannidou *et al.*, 2005; Nozal-Nalda *et al.*, 2005; Mendes *et al.*, 2006; Osman *et al.*, 2007; Pohl, 2009; Özcan *et al.*, 2012).

3.10.2. Atomic absorption spectroscopy

The method is based on the radiation absorption from a light source by free atoms in the gaseous state. According to the atomization procedure, atomic absorption spectroscopy could be divided into flame atomic absorption spectroscopy and electrothermal absorption spectroscopy (Miller and Rutzke, 2003). Flame atomic absorption spectrometry is routinely used for honey analysis, and is mostly focused on the content of alkali and alkaline earth metals (Pohl *et al.*, 2012), being relatively laborious but low cost. Electrothermal atomic absorption spectrometry can be used without sample pre-treatment stage and has been recently employed by Ajtony *et al.* (2007), Tuzen *et al.* (2007), Bilandžić *et al.* (2014) and De Andrade *et al.* (2014). among others.

3.10.3. Atomic emission spectroscopy

This method is based on the measurement of the intensity of the emitted light (usually from a flame or plasma), that is proportional to the number of excited atoms or ions of a particular element. Pohl *et al.* (2012) compared flame atomic absorption and emission spectrometry procedures applied to honey, concluding that prior mineralization of samples is tedious and

there is a risk of sample contamination and elements losses because of volatilization, so that further research of non-destructive analyses of honeys by these procedures is necessary.

3.10.4. Inductively coupled plasma-mass spectrometry (ICP-MS)

It is a reliable technique to determine mineral elements at very low concentrations (about parts per trillion). It consists of sample ionization, followed by separation and quantification of ions with a mass spectrometer. This method is fast, precise and sensitive (Sarker and Nahar, 2014). Inductively coupled plasma-mass spectrometry was first used for honey analysis by Caroli *et al.* (1999). Recently, this method was used combined with chemometrics (Chudzinska and Baralkiewicz, 2011; Chen *et al.*, 2014) for honey characterization. Döker *et al.* (2014) claimed that microwave-assisted digestion procedures using diluted reagents were the most promising ones for their application to honey samples.

3.10.5. X- Ray fluorescence spectroscopy

In respect of honey analysis, this method is fast, simple and enough precise, but its main drawback is its lack of sensitivity for mineral elements whose concentrations are lower than 0.1 ppm (Kump *et al.*, 1996). Total reflection X-ray spectrometry in combination with chemometrics was used by Necemer *et al.* (2009) and Kropf *et al.* (2010), among others, to characterize several honey samples.

3.10.6. Chromatographic methods

The most employed method to determine mineral content of honey is ionic chromatography with conductivity or electrochemical detectors (Pérez-Cerrada *et al.*, 1989; Poiana *et al.*, 1996). Radovic *et al.* (2001b) determined honey anions and cations using two ionic chromatographic methods combined with statistical procedures, concluding that those procedures could be potentially useful for floral honey characterization. However, chromatographic methods usually need a previous solid-phase extraction procedure and are very time consuming (Suárez-Luque *et al.*, 2006).

3.10.7. Capillary zone electrophoresis

It is a very fast, precise, sensitive and reliable procedure to determine honey cations, anions and formic acid without any preparation of sample apart from dilution and filtration (Suárez-Luque *et al.*, 2005, 2006), so it has been recommended as a suitable method for routine analysis. Rizelio *et al.* (2012a) and Shi *et al.* (2012) also applied capillary zone electrophoresis to determine mineral content of honeys.

3.10.8. Other methods

Other procedures to quantify honey mineral elements with advantages in relation with simplicity regarding samples pre-treatment have been mid-infrared, near-infrared, instrumental neutron activation analysis, voltammetry, potentiometry and Raman

spectroscopy (Li *et al.*, 1995; Sanna *et al.*, 2000; Buldini *et al.*, 2001; Muñoz and Palmero, 2006; Pellerano *et al.*, 2012; Aissat and Benbarek, 2014; Honório *et al.*, 2014; Meng *et al.*, 2014; Escuredo *et al.*, 2015).

3.11. Analysis of volatile and semivolatile compounds

The analysis of volatile and semi volatile compounds in honey has demonstrated being a powerful tool for honey characterization by using general profiles as fingerprints or some individual components as floral markers.

Aroma compounds are present in honey at very low concentrations as complex mixtures of volatile components of different functionality and relatively low molecular weight (Cuevas-Glory *et al.*, 2007). One matter of paramount importance is to obtain representative extracts. This is very complicated since the range of the extracted compounds highly depends on the isolation technique employed to remove water, sugars and other interferents and on the detection modes.

The most frequently used technique applied to final analysis of aroma-related compounds in honey is Gas Chromatography with Mass Spectrometry (GC-MS) that combines high separation efficiency and sensitivity, also providing the compounds MS-spectra what is a useful tool for identification. Some advances in GC applied to honey's volatiles include the use of comprehensive gas chromatography (GCxGC) and the time-on flight MS and MS-MS detectors. Some specific aroma-related compounds such as some acids or phenolic compounds have been analysed by UV-VIS Spectroscopy, Infrared Spectroscopy, Fluorescence Spectroscopy, GC, or HPLC. Nuclear Magnetic Resonance (NRM) and Sniffing Olfactometry, a technique that requires trained experts to sniff components usually eluted from a gas chromatograph, can greatly contribute to the identification of compounds and has been applied in recent years in the characterization of aromatic constituents of honey being powerful tools for identification of individual components. Eventually, different kinds of electronic noses (MSE-Nose; zNose^{TM} and i-PENMOD AIRSENSE Analytics GmbH), that analyse the volatile fraction as a whole providing aroma fingerprints of the products, can be used for honeys characterization (Ampuero *et al.*, 2004; Benedetti *et al.*, 2004; Kenjerić *et al.*, 2009; Dymerski *et al.*, 2014).

The most applied isolation techniques can be classified as: Solvent extraction methods, Distillation methods, Headspace methods and Sorption methods. Some comparative studies on different extraction techniques have been developed (Ampuero *et al.*, 2004; Alissandrakis *et al.*, 2005; Moreira and De Maria, 2005; Jerković *et al.*, 2007; Jerković and Marijanovic, 2009; Prosen *et al.*, 2010; Jerković *et al.*, 2011), detecting their pros and cons and concluding that none of this methods can be considered as an ideal and absolutely representative; all of them possess advantages and disadvantages which may have bigger or smaller impact on the identification and interpretation of the compositional peculiarities of volatile compounds

(Kaškonienė and Venskutonis, 2010). Extraction techniques can be subdivided according to the use of solvents since solvent free techniques avoid both the use of expensive and toxic organic solvents and the need of solvent disposal.

3.11.1. Extraction techniques that employ Solvents

- Solvent Extraction and column extraction

  Solvent Extraction has been widely used for honey characterization (Rowland *et al.*, 1995; D'Arcy *et al.*, 1997; Serra-Bonvehí and Ventura-Coll, 2003), extracting a wide range of volatile and some semi-volatile compounds that can also be useful for honey's characterization. Other additional advantages are that the formation of thermally produced artifacts is minimized since most methods avoid heating and the use of non-expensive equipment. One important disadvantage is that some analytes can be masked by the solvent front. Another problem is the loss of volatiles and formation of new compounds during the solvent evaporation step (Kaškonienė *et al.*, 2008). Other cons are the large volumes of solvents employed and the long extraction time. One last problem associated with Solvent Extraction is that some extracted compounds can contaminate the injection port of the chromatograph.

  One related technique is Column Extraction. The use of polymers can be helpful to isolate major groups of volatiles but as it uses solvents to recover the analytes but it has the same limitations of the solvent extraction techniques (Cuevas-Glory *et al.*, 2007).

  In the last decade Ultrasound-Assisted Solvent Extraction (USE) was proposed as a good method to isolate aroma-related compounds in honey (Alissandrakis *et al.,* 2009; Jerković *et al.,* 2009). This extraction technique uses low amounts of organic solvents and reduces extraction times by extracting the sample in a water bath with ultrasound assistance, what greatly improves the extraction efficiency. Ultrasound mechanical effect provides a greater penetration of solvent into matrix *via* cavitation effects (Jerković *et al.*, 2009).

- Simultaneous Steam Distillation Extraction (Likens-Nickerson)

  Likens-Nickerson has been successfully applied to honey aroma-related compounds analysis (Bouseta and Collin, 1995; Guyot *et al.*, 1998; Castro-Vázquez *et al.*, 2008) but is time-consuming, requires rather high quantity of the sample and provides results that are not easily compared (Verzera and Condurso, 2012). Isolation of low molecular weight and higher boiling temperature compounds is limited. The use of high temperatures leads the formation of thermally-produced artifacts. This problem can be minimized by working under vacuum conditions. Most methods applied to honey combine acetone extraction or, most recently, dichloromethane extraction under an inert atmosphere followed by Simultaneous Steam Distillation and Solvent Extraction. In the last years micro scale methods have been developed, that minimizes the quantity of sample and solvents required for extraction.

3.11.2. Solvent-free extraction techniques

- Static and Dynamic Headspace

  The main advantage of head space is that the profiles of headspace are more closely associated with sensory perception (Kaškonienė *et al.*, 2008). Static Headspace has rarely been applied to the analysis of the volatile fraction of honey due to low concentrations of volatiles in this foodstuff and the low recoveries of semi-volatile components (Rowland *et al.*, 1995; Cuevas-Glory *et al.*, 2007), but Dynamic Headspace Purge and Trap (Tananaki *et al.*, 2007; Soria *et al.*, 2008; Escriche *et al.*, 2011; Juan-Borrás *et al.*, 2014) allows analysing a wide range of both volatile and semi-volatile organic compounds.

- Solid-Phase Micro Extraction (SPME) and Head Solid Phase Micro Extraction (HD-SPME)

  The SPME, and the most used nowadays HD-SPME, are fast and inexpensive methods and have been widely applied in recent years for analysis of aroma components of many honeys due to its simplicity, high sensitivity and low cost (Piasenzotto *et al.*, 2003; Baroni *et al.*, 2006; Alissandrakis *et al.*, 2007; Kaškonienė *et al.,* 2008; Senyuva *et al.*, 2009; Stanimirova *et al.*, 2010; Cuevas-Glory *et al.*, 2012; Pasini *et al.,* 2013; Karabagias *et al.*, 2014a; Moniruzzaman *et al.*, 2014). Volatiles are concentrated in a fiber, being the most employed ones: PDMS (polydimethylsiloxane), PDMS/DVB (polydimethylsiloxane/ divinylbenzene), CAR/PDMS (carboxene/ polydimethylsiloxane), DVB/CAR/PDMS (divinylbenzene/ carboxene/ polydimethylsiloxane), CW/DVB (carbowax/ divinylbenzene) and PA (polyacrilate). Each one extracts a different range of compounds. There are many factors implied in the extraction efficiency including the polarity of fiber coatings and the fiber coating film thickness and many operating conditions such as vial size, sample volume, salt addition, pH, temperature, magnetic stirring, equilibrium and extraction time, and GC- split/splitless desorption time (Verzera and Condurso, 2012). To date, it has not been specified which of the commercial fibers are best suited for honey volatile analyses.

3.12. Polyphenols analysis

Polyphenols or phenolic compounds are phytochemicals with important antioxidant activity in honey. They are natural products of secondary plant metabolism, being phenolic acids and flavonoids the most important classes (Gómez-Caravaca *et al.*, 2006).

3.12.1. Flavonoids profile

The profile of flavonoids and phenolic acids and/or the identification of these compounds as chemical markers, represent an important tool for the characterization of the botanical and/or geographical origin of honeys. The main steps of the analytical procedure for the determination of phenolic compounds are extraction, separation, identification and quantification.

- Isolation of phenolic compounds

The isolation of phenolic compounds from the complex honey matrix is a critical step, where interfering components, such as sugars, need to be removed (Pyrzynska and Biesaga, 2009). Some authors omitted the isolation step, time-consuming and with loss of some trace analytes (Biesaga and Pyrzynska, 2009) or carried out a minimum sample preparation using ultrasonic extraction (Gambacorta *et al.*, 2014). Other authors combined two extraction techniques (Silici *et al.*, 2013). The most important extraction techniques are liquid-liquid extraction (LLE) and solid phase extraction (SPE) using non polar resins or commercial cartridges.

a) Liquid-liquid extraction (LLE)

LLE using mainly ethyl acetate as solvent has been widely used (Trautvetter *et al.*, 2009; Karabagias *et al.*, 2014b). The inconvenients of this extraction technique are the interphase formation, which avoids the complete recovery of phenolic compounds (Ferreres *et al.*, 1994), and the high volume of solvents used. IDLLME (inverse dispersive liquid liquid microextraction) and DLLME are simpler, cheaper, faster and more environmental friendly than the classical method (Campone *et al.*, 2014; Campillo *et al.*, 2015).

b) Solid-Phase Extraction (SPE)

- Filtration through the non-ionic polymeric resin Amberlite XAD-2, carried out for the first time in honey by Ferreres *et al.* (1991), has been the most used method for polyphenols extraction (Cavazza *et al.*, 2013; Escriche *et al.*, 2014). Phenolic compounds are adsorbed by the resin, remaining in the column, while polar compounds as sugars are eluted with the aqueous solvent (acidified water followed by distilled water), being the phenolic fraction eluted with methanol (Tomás-Barberán *et al.*, 2001). Sometimes, a clean-up step through a Sephadex LH-20 column (Silva *et al.*, 2013b) or using LLE with diethyl ether is made (Escriche *et al.*, 2014). The disadvantages are the low affinity of some phenolic compounds and polar glycosides flavonoids with the resin and the high amounts of honey sample and organic solvent used, with the important environmental impact implied, being as well a time-consuming method (Michalkiewicz *et al.*, 2008).
- Reversed-phase SPE procedures with commercial cartridges packed with different sorbents seem to be the simplest and the most effective way to phenolic compounds extraction. They are faster and less expensive methods compared to Amberlite XAD-2 resin (Bertoncelj *et al.*, 2011), needing small quantities of sample and low consumption of organic solvents, being more appropriate for flavonoids glycosides extraction. The main SPE cartridges used are hydrophobic reverse silica-based bonded phase cartridges (C18 cartridges) (Gašić *et al.*, 2014; Silici *et al.*, 2014), styrene-divinylbenzene copolymers cartridges (such as Strata-X cartridge)

(Bertoncelj *et al.*, 2011; Sergiel *et al.*, 2014) and divinylbenzene and N-vinylpyrrolidone copolymers (such as Oasis HLB cartridge) (Zhou *et al.*, 2014b).

- Instrumental analysis

Liquid chromatography (LC) is considered to be the most useful separation technique for the analysis of polyphenols, although gas chromatography (GC) or capillary electrophoresis (CE) have been also used.

a) High performance liquid chromatography (HPLC)

HPLC using a reversed-phase C18 column is the most used method (Perna *et al.*, 2013; Zhou *et al.*, 2014b). Gradient elution is used, being phase A formic acid or acetic acid and phase B methanol or acetonitrile (Habib *et al.*, 2014a; Silici *et al.*, 2014).

- HPLC coupled with UV detectors, such as photodiode array detector (DAD), is the most common method used to analyse phenolic compounds in honey (Tuberoso *et al.*, 2013; Habib *et al.*, 2014a). However, the UV detection is not sensitive enough to compounds presents in low quantities, the detection limit of substances with low UV-sensitivity is very poor, and identification can be difficult due to some components showing similar UV spectra or co-eluting at the same time (Liang *et al.*, 2009; Bertoncelj *et al.*, 2011). For these reasons, electrospray ionisation mass spectrometry (ESI-MS) detector (Pasini *et al.*, 2013) and/or NMR (Silva *et al.*, 2013b) are connected in series as a complementary technique for identification purposes. Phenolic compounds exhibit the maximum absorbance between 260 nm and 370 nm in the UV region, being identified by comparison with standards, the UV spectra and the retention times. Polyphenols quantification through external standard calibration method is used.
- HPLC-ESI-MS has been also employed for quantification purposes (Gašić *et al.*, 2014). This method enables high selectivity and sensitivity in the analysis of honey polyphenols, providing precise structural information of the compounds. MS system can be used alone (Campillo *et al.*, 2015) or in tandem (MSn) (Zhou *et al.*, 2014b) increasing the sensitivity and selectivity of the method and obtaining additional structural information. Phenolic compounds are identified according to the corresponding spectral characteristics: mass spectra, accurate mass, characteristic fragmentation, and retention time. The quantification is done using calibration curves of standard compounds.
- Other HPLC methods coupling electrochemical detectors such as coulometric electrode array detector (CEAD) (Petrus *et al.*, 2011) or coupling a fluorescence detector (FD) (Michalkiewicz *et al.*, 2008) have been employed.

b) Other methods

- Capillary electrophoresis with UV or MS detection has the advantage of high separation efficiency and shorter analysis time in comparison to HPLC (Delgado *et*

al., 1994). Nevertheless, it is difficult to separate all flavonoids in one run, being necessary to wash the capillary after each analysis in order to obtain a better reproducibility (Petrus *et al.*, 2011). Micellar electrokinetic capillary chromatography (MECC) (Delgado *et al.*, 1994) and capillary zone electrophoresis (CZE) (Arráez-Román *et al.*, 2006) have been used.

- A reduced number of GC methods have been developed for flavonoids determination in honey, due to their low volatility makes necessary a derivatization step. Volatile and semivolatile phenolic compounds can be determined by GC-MS without derivatization (Verzera and Condurso, 2012).

3.12.2. Polyphenol content

Modifications of the Folin-Ciocalteu method (Singleton *et al.*, 1999) are the most used methods to determine total phenolic content. The aqueous honey solution is mix with Folin-Ciocalteu reagent and sodium carbonate. After an incubation period, absorbance of the sample is measured between 725-765 nm, using gallic acid as standard (Wilczyńska, 2014; Rodríguez-Flores *et al.*, 2015). Escuredo *et al.* (2013b) predicted total phenol content by NIR.

3.12.3. Flavonoid content

The most commonly method applied is the Dowd method adapted by Arvouet-Grand *et al.* (1994), based on the formation of aluminium-flavonoid complexes in neutral media. It is used to determine flavonols and luteolin content (Pekal and Pyrzynska, 2014). The measurements are done between 415-425 nm, with quercetin as standard compound for quantitative analysis (Meda *et al.*, 2005; Sant'ana *et al.*, 2014). Sancho *et al.* (2016) showed that this procedure must be carried out on honey extracts after sugars removal, because honey sugars interfere. In addition, a sample colour correction is compulsory, because at these wavelengths (415-425), there is also an interference of the colour of the honey extract that must be subtracted.

The second most used method is based on aluminium complex formation but in alkaline medium (in the presence of $NaNO_2$), specific for rutin, luteolin and catechin analysis (Pekal and Pyrzynska, 2014). The measurements are done normally at 510 nm, using catechin as reference compound (Khalil *et al.*, 2012).

4. Honey properties

4.1. Antioxidant activity

Honey antioxidant activity has been determined by several procedures (Álvarez-Suárez *et al.*, 2009) that can be grouped by the mechanism of action into hydrogen atom transfer based reactions methods, and single electron transfer based reactions methods (Huang *et al.*, 2005; Prior *et al.*, 2005). The most applied assays to measure antioxidant activity of honey are mentioned below.

4.1.1. 2,2-Diphenyl-1-picrylhydrazyl "DPPH"

The method is based on the determination of the reducing capacity of antioxidants toward 2,2-diphenyl-1-picrylhydrazyl radical. Despite being a simple, fast and low cost procedure, the assay is one of the less reliable methods to assess antioxidant activity, due to the interference of such compounds as carotenoids (Noruma *et al.*, 1997), the steric inaccessibility of some antioxidant substances, and the high possibility of inaccurate interpretations of antioxidant activity, among other important drawbacks (Prior *et al.*, 2005). Nevertheless, due to its simplicity, DPPH assay has been the most used method to measure honey's antioxidant activity (Aljadi and Kamaruddin, 2004; Beretta *et al.*, 2005; Ferreira *et al.*, 2009; Socha *et al.*, 2009; Jerković and Marijanovic, 2010; Saxena *et al.*, 2010; Silici *et al.*, 2010; Isla *et al.*, 2011; Serem and Bester, 2012; Escuredo *et al.*, 2013a; Liu *et al.*, 2013; Silva *et al.*, 2013b; Noor *et al.*, 2014; Kuś *et al.*, 2014a, 2014b; Gašic *et al.*, 2014; Rodríguez-Flores *et al.*, 2015). Escuredo *et al.* (2013b) predicted DPPH by NIR.

4.1.2. Oxygen radical absorbance capacity "ORAC"

It determines, by fluorescence, the antioxidant inhibition of peroxyl radical induced oxidations, usually expressing the results as Trolox equivalents (Prior *et al.*, 2005). After a comprehensive review of the methodology applied to antioxidant activity determination, with all the pros and cons thoroughly reviewed (Huang *et al.*, 2005; Prior *et al.*, 2005; Tabart *et al.*, 2009), the ORAC procedure was proposed as one of the most interesting procedures of antioxidant activity determination for further study and validation. This method has been applied to assess antioxidant activity of honeys by Gheldof and Engeseth (2002), Gheldof *et al.* (2002), Wang *et al.* (2004), Álvarez-Suárez *et al.* (2010a), Serem and Bester (2012), Aazza *et al.* (2013), Gorjanović *et al.* (2013) and Spilioti *et al.* (2014).

4.1.3. Trolox equivalent antioxidant capacity "TEAC"

Antioxidant activity is measured as the ability of a given antioxidant to decrease the colour of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation produced by oxidants (Prior *et al.*, 2005). In honey, this procedure has been applied by Baltrušaityté *et al.* (2007), Socha *et al.* (2009), Vit *et al.* (2009), Álvarez-Suárez *et al.* (2010a, 2010b), Lachman *et al.* (2010), Sant'Ana *et al.* (2012), Serem and Bester (2012), Silva *et al.* (2013a), Gorjanović *et al.* (2013), Kowalski (2013), Tuberoso *et al.* (2013), Escriche *et al.* (2014), Habib *et al.* (2014a), Wilczyńska (2014) and Sancho *et al.* (2016). As well as "ORAC", "TEAC" procedure was proposed as one of the methods that should be standardized for antioxidant activity determination (Prior *et al.*, 2005). After researching TEAC method applied to honeys and honeys' extracts Sancho *et al.* (2016) found significant relationships between TEAC values of honeys and honeys' extracts and between TEAC results measuring the absorbance at different times, so that it was possible to reliably calculate TEAC at end-point from the value of absorbance at 6 minutes.

4.1.4. Ferric reducing antioxidant power "FRAP" and copper reduction assay "CUPRAC"

FRAP measures the reduction of ferric 2,4,6-tripyridyl-*s*-triazine to a coloured product (Prior *et al.*, 2005). This method has been employed to determine antioxidant activity of honeys by Aljadi and Kamaruddin (2004), Beretta *et al.* (2005), Blasa *et al.* (2006), Bertoncelj *et al.* (2007), Blasa *et al.* (2007), Kücük *et al.* (2007), Estevinho *et al.* (2008), Jerković and Marijanovic (2010), Saxena *et al.* (2010), Rosa *et al.* (2011), Rodríguez *et al.* (2012), Ciappini and Stoppani (2014), Kuś *et al.* (2014a, 2014b), Noor *et al.* (2014) and Bolanos de la Torre *et al.* (2015). CUPRAC is a variant of the FRAP assay, employing Cu instead of Fe (Prior *et al.*, 2005). For honeys, it has been used by Ulusoy *et al.* (2010). However, according to Prior *et al.* (2005), with such a complex mixture of antioxidants as honey, selecting an appropriate reaction time is of paramount importance, both for "FRAP" and for "CUPRAC" procedures.

4.1.5. Chemiluminescence "CL"

The method is based on the reaction of oxidants with marker compounds to produce species that emit chemiluminescence, measuring the time of depressed light emission after adding the antioxidant (Prior *et al.*, 2005). In honey, it has been applied by Schramm *et al.* (2003) and Nasuti *et al.* (2006).

4.1.6. The croton or β-carotene bleaching

The assay is based on the inhibition by antioxidants, of the croton or β-carotene bleaching by oxidation (Prior *et al.*, 2005). It has been applied to honey by Ferreira *et al.* (2009) and Guerrini *et al.* (2009).

4.1.7. Thiobarbituric Acid Reactive Substances (TBARS)

The method is based on the inhibition of lipid peroxidation by measurement of thiobarbituric reactive substances, and has been applied to honey by Ferreira *et al.* (2009), Idris *et al.* (2011) and Štajner *et al.* (2014).

4.1.8. Folin-Ciocalteu to determine total phenolics and measurement of reducing power

Folin-Ciocalteu method (shown in 3.12.2) is based on a redox reaction and therefore it can be considered as another reliable procedure to measure antioxidant activity (Huang *et al.*, 2005; Prior *et al.*, 2005). This colorimetric assay was used by practically all the researchers who investigated honeys' antioxidant activity with the previously described methods, and in all the studies a positive correlation was obtained between total phenolics and the antioxidant activity measured by other procedures. Ferreira *et al.* (2009) also measured Portuguese honeys' reducing power, whose values were correlated to total phenolics' results.

4.1.9. Other methods to measure honeys' antioxidant activity

Apart from the procedures mentioned above, different researchers measured antioxidant activity of honeys using a linoleic acid system (Nagai *et al.*, 2001), or determining linoleic acid oxidation (Silva *et al.*, 2013b).

Honey has shown to be able to scavenge particular free radicals such as hydroxyl (Henriques *et al.*, 2006; Pérez *et al.*, 2006; Álvarez-Suárez *et al.*, 2010a; Dong *et al.*, 2013; Liu *et al.*, 2013; Čanadanović-Brunet *et al.*, 2014; Ciappini and Stoppani, 2014; Štajner *et al.*, 2014), superoxide (Nagai *et al.*, 2001; Kücük *et al.*, 2007; Kishore *et al.*, 2011; Dong *et al.*, 2013; Liu *et al.*, 2013; Čanadanović-Brunet *et al.*, 2014), nitric oxide (Aazza *et al.*, 2013; Habib *et al.*, 2014a), peroxynitrite (Kücük *et al.*, 2007; Kishore *et al.*, 2011) and peroxide (Noor *et al.*, 2014).

4.2. Antimicrobial activity

Different authors have described the antimicrobial activity of honey. All the authors that study honey's antimicrobial activity use in vitro *endpoint methods* (those in which a microorganism is challenged for an arbitrary period and the results reflect the inhibitory power of a compound only for the time specified) such as agar diffusion, agar and broth dilution or gradient plates (López-Malo Vigil *et al.*, 2005). There are several methods to assess the antimicrobial activity of honey; method selection depends on the aim of analysis, but also on time equipment and economical reasons.

4.2.1. Agar diffusion method

Agar diffusion (wells or discs) method is probably the most widely used test for determination of antimicrobial activity. This assay is used as a preliminary screening of susceptibility of bacteria to honey (Al Somal *et al.*, 1994; Estrada *et al.*, 2005; Pérez-Martin *et al.*, 2008; Sgariglia *et al.*, 2010; Fidaleo *et al.*, 2011; Voidarou *et al.*, 2011). In this method, honey is added to an agar plate on a paper disk or in a well. The compound diffuses through the agar, resulting in a concentration gradient that is inversely proportional to the distance from the disk or well. To run the test, petri dishes are prepared to contain a nonselective medium, which is surface or depth inoculated with a suspension containing approximately 5-6 logs CFU/ml of the test microorganism (Piddock, 1990). Allen *et al.* (1991) and Irish *et al.* (2008) included a phenol standard curve, where the mean diameter of the clear zone around each phenol standard was calculated and squared. A standard graph was then plotted of % phenol against the square of the mean diameter of the clear zone. A best-fit straight line was plotted and the equation of this line was used to calculate the activity of each honey sample. The antimicrobial activity is expressed as the equivalent phenol concentration.

4.2.2. Dilution assay

Dilution assay (broth and agar) is the next more used method. This method is used when quantitative data are required. With this test, minimum inhibition concentration (MIC) is obtained. MIC is defined as the lowest concentration of honey preventing visible growth (Melliou and Chinou, 2011) or the lowest concentration of test honey which results in 100% inhibition of growth of the test organism (Sherlock *et al.*, 2010).

Both methods, broth and agar dilution assays, are highly appreciated because they are used to determine the minimal inhibitory concentration, can be used for anaerobic and microaerophilic microorganisms, large number of strains may be tested at once, and contamination is easily detected. Among them the microassay is the preferred by researchers because it is quick, reliable, large numbers of honey samples and large range of concentrations can be assayed simultaneously.

- Broth dilution assay

  Broth dilution assay can be performed in 96-wells microtiter plates (Lin *et al.*, 2009; Sherlock *et al.*, 2010; Melliou and Chinou, 2011; Isidorov *et al.*, 2015) or in tubes (Bogdanov, 1997; Hegazi, 2011). In both, honey is serially diluted and a single concentration is added to nonselective broth medium. Later on, tubes or wells are inoculated with the test microorganism (5-6 logs CFU/ml). A negative control (without honey and culture) and positive control (without honey) should be prepared. Plates or tubes are incubated at the optimum temperature for the test microorganism during 24 hours. Results are expressed as absence of visible growth (Lin *et al.*, 2009; Melliou and Chinou, 2011) or absence of turbidity, measuring the optical density (OD) before (T0) and after (T24) the incubation period (Sherlock *et al.*, 2010). The OD for each replicate at T0 was subtracted from the OD for each replicate at T24. The adjusted OD for each control well was then assigned a value of 100% growth. The percent inhibition of growth was thus determined using the formula: Percent Inhibition=1-(OD test well/OD of corresponding control well)x100.

  Broth dilution assay may be used to determine the lethality of the test microorganism. To establish lethality or minimum bactericidal concentration (MBC) after incubation, 10-100 μL medium from the last tubes or wells in which the microorganism exhibits growth as well as all tubes or wells showing no growth (no turbidity) are plated on a nonselective agar. The lowest concentration of antimicrobial that produces killing of the test microorganism is termed the minimum bactericidal concentration or minimum lethal concentration (NCCLS, 2002; Lin *et al.*, 2009).

- Agar dilution assay

  Agar dilution method or agar incorporation technique has been used by Al Somal *et al.* (1994), French *et al.* (2005), Fidaleo *et al.* (2011) and Carnwath *et al.* (2014). To perform

this method, double-strength nutrient agar solution is prepared, sterilized and held at 50ºC in a water bath. Honey solutions at different concentrations are prepared in sterile distilled water using aseptic techniques and mixed with an equal volume of double-strength nutrient agar. A final volume (i.e. 20 ml) is poured into each labelled petri dishes, which are then left to dry. Samples (10 µl) of each culture are inoculated into the agar plates. Finally, the inoculated plates are incubated and then the growth is recorded.

4.2.3. Standard plate method

Other method less used to assess the antimicrobial activity of honey is standard plate count method (Elbanna *et al.*, 2014). For this method, an inoculum of the test microorganism, containing a known initial count, is mixed with honey at different concentrations or different conditions. These tubes are incubated and the percentage of survived viable counts of tested microorganism is determined by applying 1 mL of each tube onto sterile Petri dish (with agar media). The plates were incubated at the optimum temperature for each bacterial strain. The inhibition is expressed as decreasing percentage of initial counts.

4.2.4. Other methods

Rapid methods for measuring the antimicrobial activity of honey are under evaluation. Black (2011) published that a promising NIR calibration can be developed for total antimicrobial and for non-peroxide antimicrobial activity of honey, and Sultanbawa *et al.* (2015) described the MIR as a rapid tool to detect methylglyoxal (2-oxopropanal), reported as one of the key compounds that contribute to the non-peroxide antimicrobial activity in New Zealand manuka honey and antibacterial activity in Australian honeys.

5. Multicomponent analysis or/and honey authenticity

5.1. Infrared spectroscopy (IR)

Spectroscopic techniques in the infrared (IR) region of the electromagnetic spectrum are reliable, accurate, rapid, non-destructive and low cost. In addition, they require little or no sample preparation (Cozzolino *et al.*, 2011; Cozzolino *et al.*, 2012). IR in combination with multivariate analysis, has been applied for the simultaneous quantitative analysis of the most important honey physicochemical parameters, honey authentication and detection of adulteration with sugar.

5.1.1. Near infrared spectroscopy (NIR)

NIR, or its modification FT-NIR (Fourier transform near infrared spectroscopy), can be carried out by reflectance (Dvash *et al.*, 2002; Cozzolino and Corbella, 2003; Chen *et al.*, 2011, Chen *et al.*, 2012), transmittance (Qiu *et al.*, 1999) and transflectance (García-Álvarez *et al.*, 2000; García-Álvarez *et al.*, 2002; Downey *et al.*, 2003; Kelly *et al.*, 2006b).

Transmittance spectra have sharper peaks, better resolution and calibration tests are better than those of reflectance (Qiu *et al.*, 1999). Transflectance is a combination of reflectance and transmission measurements that provides a more reliable determination of absorbance than transmission techniques (García-Álvarez *et al.*, 2000).

The calibration obtained from the samples of the honey was successfully applied to the simultaneous prediction of components, being a procedure with high predictive ability and accuracy. Ha *et al.* (1998), García-Álvarez *et al.* (2000) and Tu *et al.* (2009) measured moisture and different sugars at the same time. Qiu *et al.* (1999) and Cho and Ha (2002) simultaneously determined moisture, sugars, acidity and HMF, being the prediction accuracy unsatisfactory for the last two parameters. Ruoff *et al.* (2007) found satisfying accuracies for the determination of moisture, fructose, glucose and sucrose, but the prediction accuracy for HMF, proline, pH, electrical conductivity, free acidity and some minor sugars was poor and unreliable. Cozzolino and Corbella (2003) analysed moisture, pH, electrical conductivity, colour and HMF, but the prediction accuracy was not good enough for the last parameter. García-Álvarez *et al.* (2002) determined different polarimetric parameters in honey and Escuredo *et al.* (2013b) determined phenols, flavonoids, vitamin C, DPPH, oxidation index and copper.

NIR has been applied for botanical and geographical discrimination classification of honey, being NIR signal a fingerprint for honey characterization (Davies *et al.*, 2002; Ruoff *et al.*, 2006b; Woodcock *et al.*, 2007; Chen *et al.*, 2008; Woodcock *et al.*, 2009; Hennessy *et al.*, 2010; Chen *et al.*, 2012; Herrero-Latorre *et al.*, 2013).

Also NIR has been used to detect sugar adulteration, such as addition of fructose and glucose (Downey *et al.*, 2003), beet invert syrup and high fructose corn syrup (Kelly *et al.*, 2006b; Chen *et al.*, 2011; Tu *et al.*, 2011).

5.1.2. Visible and Near infrared spectroscopy (Vis-NIR)

The Vis-NIR combination has been applied for the discrimination of honey origins (Corbella and Cozzolino, 2005; Shao *et al.*, 2008; Zhao *et al.*, 2011) or for the detection of honey adulteration with sugar (Mouazen and Al-Walaan, 2014).

5.1.3. Mid-infrared spectroscopy (MIR)

Mid-infrared spectroscopy using the attenuated total reflectance accessory of a Fourier transform infrared spectrometer (FT-MIR-ATR) has been widely employed. Modifications of the methods are FT-MIR with microattenuated total reflectance (mATR) (Tewari and Irudayaraj, 2004, 2005), diffuse reflectance (DR) (Bertelli *et al.*, 2007) or horizontal attenuated reflectance (HATR) (Wang *et al.*, 2010).

Lichtenberg-Kraag *et al.* (2002) determined at the same time different sugars, moisture, HMF, electrical conductivity, pH, free acidity, proline and invertase, with good calibration models.

Ruoff *et al.* (2006a) validated a procedure for the simultaneous determination of several parameters. Satisfactory accuracies were found for different sugars, moisture, electrical conductivity, pH and free acidity. On the contrary, accuracy for HMF, proline and other minor sugars was rather poor. Pataca *et al.* (2007) determined reducing sugars, moisture and acidity with satisfactory results of the calibration models. Almeida-Muradian *et al.* (2012, 2014a) measured different sugars, moisture, electrical conductivity, pH and acidity.

FT-MIR-ATR has been also used for honey botanical and geographical discrimination (Tewari and Irudayaraj, 2005; Ruoff *et al.*, 2006c; Bertelli *et al.*, 2007; Etzold and Lichtenberg-Kraag, 2008). Anscombe (2006) compared FT-NIR-ATR and FT-MIR-ATR and found that ATR-MIR spectroscopy was the most promising method.

Finally, different authors used this technique to detect sugar adulteration with corn, cane sugar, sugar mixtures and invert sugars mixtures (Irudayaraj and Sivakesava, 2001; Sivakesava and Irudayaraj; 2001a, 2001b; Sivakesava and Irudayaraj; 2002; Irudayaraj *et al.*, 2003; Kelly *et al.*, 2004; Kelly *et al.*, 2006a; Gallardo *et al.*, 2009).

5.2. Nuclear magnetic resonance (NMR)

A modern and non-destructive promising method for honey analysis is nuclear magnetic resonance (Sarker and Nahar, 2014). This technique was used to rapidly analyse methylglyoxal in manuka honey samples (Donarski *et al.*, 2010). Nuclear magnetic resonance, coupled with chemometrics and combined with HPLC-DAD-ESI-MS was successfully used for the determination of honeys' quinoline alkaloids (Beretta *et al.*, 2009). 1D nuclear magnetic resonance spectra and multivariate analysis proved to be a simple and rapid procedure for detecting adulterations of honey with sugar (Bertelli *et al.*, 2010). Nowadays, another field in which nuclear magnetic resonance is having an important impact, is honeys' characterization. Diffusion Ordered Spectroscopy nuclear magnetic resonance was applied for the analysis of key components of manuka honey, concluding that nuclear magnetic resonance was a suitable technique for ppm level quantification of proton-bearing organic compounds (Gresley *et al.*, 2012). Different honeydew honeys and blossom honeys were distinguished by analysing quercitol quantities by nuclear magnetic resonance (Simova *et al.*, 2012). Low field nuclear magnetic resonance was successfully employed to classify Brasilian honeys by their botanical origins (Ribeiro *et al.*, 2014). Nuclear magnetic resonance combined with multivariate data processing methods provides with a fingerprint for each honey botanical source (Beretta *et al.*, 2008; Lolli *et al.*, 2008; Schievano *et al.*, 2010; Boffo *et al.*, 2012; Consonni *et al.*, 2012; Schievano *et al.*, 2012; Jamróz *et al.*, 2014). Nevertheless, nuclear magnetic resonance is still an expensive technique to be used in routine laboratories.

5.3. Electrochemical methods

Electrochemical tongues composed by different sensors (chemical of metallic working electrodes), based on potentiometric or voltammetric determinations, have been used with many purposes: multicomponent determination, origin discrimination and adulteration detection.

Potentiometric tongue has been used for the simultaneous determination of different sugars, moisture, electrical conductivity and acidity (Major *et al.*, 2011) and botanical and geographical honey classification (Dias *et al.*, 2008; Wei *et al.*, 2009; Major *et al.*, 2011; Zakaria *et al.*, 2011; Escriche *et al.*, 2012; Sousa *et al.*, 2014; Wei and Wang, 2014).

Different authors used voltammetric tongue for honey authentication (Wei and Wang, 2011; Tiwari *et al.*, 2013; Wei and Wang, 2014) and adulteration detection (Cai *et al.*, 2013).

5.4. Pyrolysis-mass spectroscopy

Radovic *et al.* (2001a) discriminated honeys according their botanical origin by pyrolysis-mass spectroscopy (Py-MS) in combination with chemometrics. However, this method proved not to be useful for geographical origin separation of honeys. By this method, the sample is heated, being the organic material quickly decomposed. The resulting low molecular weight products provide a characteristic profile of the sample according to its chemical composition. Although it is a very fast and sensitive fingerprint technique, the instrumentation is very expensive, so it is not commonly used for honey discrimination (Bogdanov *et al.*, 2004).

REFERENCES

AAZZA, S; LYOUSSI, B; ANTUNES, D; MIGUEL, M G (2013) Physicochemical characterization and antioxidant activity of commercial Portuguese honeys. *Journal of Food Science* 78(8): C1159-C1165. http://dx.doi.org/10.1111/1750-3841.12201

ABU-JDAYL, B; GHZAWI, A A; AL-MALAH, K I M; ZAITOUN, S (2002) Heat effect on rheology of light and dark coloured honey. *Journal of Food Engineering* 51(1): 33-38. http://dx.doi.org/10.1016/S0260-8774(01)00034-6

ACCORTI, M; PIAZZA, M G; PERSANO-ODDO, L (1987) La conductivité électrique et le contenu en cendre du miel. *Apiacta* 22: 19-20.

AISSAT, S; BENBAREK, H (2014) Importance of botanical origin of honeys. In *Boudraâ, L (Ed)*. *Honey in Traditional and Modern Medicine.* CRC Press Taylor & Francis Group; Boca Ratón, Florida, USA. pp. 59-111.

AJTONY, Z; BENCS, L; HARASZI, R; SZIGETI, J; SZOBOSZLAI, N (2007) Study on the simultaneous determination of some essential and toxic trace elements in honey by multi-element graphite furnance atomic absorption spectrometry. *Talanta* 71(2): 683-690. http://dx.doi.org/10.1016/j.talanta.2006.05.023

AL SOMAL, N; COLEY, K E; MOLAN, P C; HANCOCK, B M (1994) Susceptibility of *Helicobacter pylori* to the antibacterial activity of manuka honey. *Journal of the Royal Society of Medicine* 87(1): 9-12.

ALAMANNI, M C; COSSU, M; SANNA, F (2000) Un metodo HPLC per il dosaggio degli acidi ossalico, lattico e formico quali acaricidi e componenti naturali nel miele. *La Rivista di Scienza dell'Alimentazione* 29(2): 139-147.

ALISSANDRAKIS, E; TARANTILIS, P A; HARIZANIS, P C; POLISSIOU, M (2005) Evaluation of four isolation techniques for honey aroma compounds. *Journal of the Science of Food and Agriculture* 85(1): 91-97. http://dx.doi.org/10.1002/jsfa.1934

ALISSANDRAKIS, E; TARANTILIS, P A; HARIZANIS, P C; POLISSIOU, M (2007) Aroma investigation of unifloral Greek citrus honey using solid-phase microextraction coupled to gas chromatographic-mass spectrometric analysis. *Food Chemistry* 100(1): 396-404. http://dx.doi.org/10.1016/j.foodchem.2005.09.015

ALISSANDRAKIS, E; TARANTILIS, P A; PAPPAS, C;HARIZANIS, P C; POLISSIOU, M (2009) Ultrasound-assisted extraction gas chromatography-mass spectrometry analysis of volatile compounds in unifloral thyme honey from Greece. *European Food Research and Technology* 229(3): 365-373. http://dx.doi.org/10.1007/s00217-009-1046-8

ALJADI, A M; KAMARUDDIN, M Y (2004) Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chemistry* 85(4): 513-518. http://dx.doi.org/10.1016/S0308-8146(02)00596-4

ALLEN, K L; MOLAN, P C; REID, G M (1991) A survey of the antibacterial activity of some New Zealand honeys. *Journal of Pharmacy and Pharmacology* 43(12): 817-822. http://dx.doi.org/10.1111/j.2042-7158.1991.tb03186.x

ALMEIDA-MURADIAN, L B; LUGINBÜH, L W; BADERTSCHER R.; GALLMANN, P (2012) Generalizability of PLS calibrations with FT-IR ATR spectrometry for the prediction of some physicochemical measurands of honey. *ALP Science* 541(March 2012): 1-20. Available at: http://www.agroscope.admin.ch/publikationen/einzelpublikation/index.html?aid=28839&la

ALMEIDA-MURADIAN, L B; STRAMM, M K; HORITA, A; BARTH, O M; DE FREITAS, A D S; ESTEVINHO, L M (2013) Comparative study of the physicochemical and palynological characteristics of honey from *Melipona subnitida* and *Apis mellifera*. *International Journal of Food Science and Technology* 48(8): 1698-1706. http://dx.doi.org/10.1111/ijfs.12140

ALMEIDA-MURADIAN, L B; SOUSA, R J; BARTH, O M; GALLMANN, P (2014a) Preliminary data on Brazilian monofloral honey from the northeast region using FT-IR ATR spectroscopic, palynological, and color analysis. *Quimica Nova* 37(4): 716-719. http://dx.doi.org/10.5935/0100-4042.20140115

ALMEDIA-MURADIAN, L B; STRAMM, M K; ESTEVINHO, L M (2014b) Efficiency of the FT-IR ATR spectrometry for the prediction of the physicochemical characteristics of *Melipona subnitida* honey and study of the temperature's effect on those properties. *International Journal of Food Science and Technology* 49(1): 188-195. http://dx.doi.org/10.1111/ijfs.12297

ALONSO-TORRE,, S R; CAVIA, M M; FERNÁNDEZ-MUIÑO, M A; MORENO, G; HUIDOBRO, J F; SANCHO, M T (2006) Evolution of acid phosphatase activity of honeys from different climates. *Food Chemistry* 97(4): 750-755. http://dx.doi.org/10.1016/j.foodchem.2005.06.010

ALQARNI, A S; OWAYSS, A A; MAHMOUD, A A; HANNAN, M A (2014) Mineral content and physical properties of local and imported honeys in Saudi Arabia. *Journal of Saudi Chemical Society* 18(5): 618-625. http://dx.doi.org/10.1016/j.jscs.2012.11.009

ÁLVAREZ-SUARÉZ, J M; TULIPANI, S; ROMANDINI, S; VIDAL, A; BATTINO, M (2009) Methodological aspects about determination of phenolic compounds and in vitro evaluation of antioxidant capacity in the honey: a review. *Current Analytical Chemistry* 5(4): 293-302. http://dx.doi.org/10.2174/157341109789077768

ÁLVAREZ-SUÁREZ, J M; GONZÁLEZ-PARAMÁS, A M; SANTOS-BUELGA, C; BATTINO, M (2010a) Antioxidant characterization of native monofloral Cuban honeys. *Journal of Agricultural and Food Chemistry* 58(17): 9817-9824. http://dx.doi.org/10.1021/jf1018164

ÁLVAREZ-SUÁREZ, J M; TULIPANI, S; DÍAZ, D; ESTEVEZ, Y; ROMANDINI, S; GIAMPIERI, F; DAMIANI, E; ASTOLFI, P; BOMPADRE, S; BATTINO, M (2010b) Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. *Food and Chemical Toxicology* 48(8-9): 2490-2499. http://dx.doi.org/10.1016/j.fct.2010.06.021

AMPUERO, S; BOGDANOV, S; BOSSET, J O (2004) Classification of unifloral honeys with an MS-based electronic nose using different sampling modes: SHS; SPME and Index. *European Food Research and Technology* 218(2): 198-207. http://dx.doi.org/10.1007/s00217-003-0834-9

ANJOS, O; CAMPOS, M G; RUIZ, P C; ANTUNES, P (2015) Application of FTIR-ATR spectroscopy to the quantification of sugar in honey. *Food Chemistry* 169: 218-223. http://dx.doi.org/10.1016/j.foodchem.2014.07.138

ANKLAM, E (1998) A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry* 63(4): 549-562. http://dx.doi.org/10.1016/S0308-8146(98)00057-0

ANSCOMBE, N (2006) Spectroscopy identifies botanical and geographic origins of honey. *Photonics Spectra* 40(11): 34-36.

ANUPAMA, D; BHAT, K; SAPNA, V (2002) Sensory and physicochemical properties of commercial samples of honey. *Food Research International* 36(2): 183-191. http://dx.doi.org/10.1016/S0963-9969(02)00135-7

AOAC-ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (2012) Official Methods of Analysis of AOAC International. *Latimer J W (Ed)*. Gaithersburg, Maryland, USA.

ARIDA, H; HASSAN, R; EL-NAGGAR, A (2012) Quality assessment of honey using modern analytical tools. *Analytical Letters* 45(11): 1526-1536. http://dx.doi.org/10.1080/00032719.2012.675492

ARRÁEZ-ROMÁN, D; GÓMEZ-CARAVACA, A M; GÓMEZ-ROMERO M; SEGURA-CARRETERO, A; FERNÁNDEZ-GUTÍERREZ, A (2006) Identification of phenolic compounds in rosemary honey using solid-phase extraction by capillary electrophoresis-electrospray ionization-mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* 41(5): 1648-1656. http://dx.doi.org/10.1016/j.jpba.2006.02.035

ARVOUET-GRAND, A; VENNAT, B; POURRAT, A; LEGRET, P (1994) Standardisation d'un extrait de propolis et identification des principaux constituants. *Journal de Pharmacie de Belgique* 49(6): 462-468.

AUBERT, S; GONNET, M (1983) Mesure de la couleur des miels. *Apidologie* 14: 105-118.

AZEREDO, M; DA CUNHA, L; DE CASTRO, J (1998) Determination of potassium in honey after precipitation with sodium tetraphenylborate and separation in ion exchanger column. *Quimica Nova* 21(5): 651-654. http://dx.doi.org/10.1590/S0100-40421998000500018

BALTRUŠAITYTÉ, V; VENSKUTONIS, P R; ČEKSTERYTÉ, V (2007) Radical scavenging activity of different floral origin honey and beebread phenolic extracts. *Food Chemistry* 101(2): 502-514. http://dx.doi.org/10.1016/j.foodchem.2006.02.007

BARONI, M V; CHIABRANDO, G A; COSTA, C; WUNDERLIN, D A (2002) Assessment of the floral origin of honey by SDS-page immunoblot techniques. *Journal of Agricultural and Food Chemistry* 50(6): 1362-1367. http://dx.doi.org/10.1021/jf011214i

BARONI, M V; NORES, M L; DÍAZ, M P; CHIABRANDO, G A; FASSANO, J P; COSTA, C; WUNDERLIN, D A (2006) Determination of volatile organic compound patterns characteristic of five unifloral honey by solid-phase microextraction-gas chromatography-mass spectrometry coupled to chemometrics. *Journal of Agricultural and Food Chemistry* 54(19): 7235-7241. http://dx.doi.org/10.1021/jf061080e

BASA, A; MAGNUSZEWSKA, J; KROGULEC, T; BARANSKI, A S (2007) Cyclic chronopotentiometric determination of sugars at Au and Pt microelectrodes in flowing solutions. *Journal of Chromatography A* 1150(1-2): 312-319. http://dx.doi.org/10.1016/j.chroma.2006.08.076

BECKH, G; WESSEL, P; LÜLLMANN, C (2004) Naturliche bestandteile des Honigs: Hefen und deren Stoffwechselprodukte - Teil 2: Der Wassergehalt und die Wasseraktivität als Qualitätsparameter mit Bezug zum Hefewachstum. *Deutsche Lebensmittel-Rundschau* 100(1): 14-17.

BENEDETTI, S; MANNINO, S; SABATINI, A G; MARCAZZAN, G L (2004) Electronic nose and neural network use for the classification of honey. *Apidologie* 35(4): 397-402. http://dx.doi.org/10.1051/apido:2004025

BENTABOL-MANZANARES, A; HERNÁNDEZ, Z; RODRÍGUEZ, B; RODRÍGUEZ, E; DÍAZ, C (2014) Physicochemical characteristics of minor monofloral honeys from Tenerife, Spain. *LWT - Food Science and Technology* 55(2): 572-578. http://dx.doi.org/10.1016/j.lwt.2013.09.024

BERA, A; ALMEIDA-MURADIAN, L B (2005) Mel com propolis: considerações sobre a composiÇão e rotulagem. *Revista do Instituto Adolfo Lutz (Impresso) 64* (1): 117-121.

BERETTA, G; GRANATA, P; FERRERO, M; ORIOLI, M; FACINO, R M (2005) Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytica Chimica Acta* 533(2): 185-191. http://dx.doi.org/10.1016/j.aca.2004.11.010

BERETTA, G; CANEVA, E; REGAZZONI, L; BAKHTYARI, N G; FACINO, R M (2008) A solid-phase extraction procedure coupled to 1H NMR, with chemometric analysis, to seek reliable markers of the botanical origin of honey. *Analytica Chimica Acta* 620(1-2): 176-182. http://dx.doi.org/10.1016/j.aca.2008.05.025

BERETTA, G; ARTALI, R; CANEVA, E; ORLANDINI, S; CENTINI, M; FACINO, R M (2009) Quinoline alkaloids in honey: Further analytical (HPLC-DAD-ESI-MS, multidimensional diffusion-ordered NMR spectroscopy), theoretical and chemometric studies. *Journal of Pharmaceutical and Biomedical Analysis* 50(3): 432-439. http://dx.doi.org/10.1016/j.jpba.2009.05.029

BERETTA, G; FERMO, P; FACINO, R M (2012) Simple and rapid simultaneous profiling of minor components of honey by size exclusion chromatography (SEC) coupled to ultraviolet diode array detection (UV-DAD), combined with chemometric methods. *Journal of Pharmaceutical and Biomedical Analysis* 58: 193-199. http://dx.doi.org/10.1016/j.jpba.2011.09.006

BERGNER, K G; HAHN, H (1972) Zum Vorkommen und zur Herkunft der freien Aminosäuren in Honig. *Apidologie* 3(1): 5-34. http://dx.doi.org/10.1051/apido:19720101

BERGNER, K G; DIEMAIR, S (1975) Proteine des Bienen- honigs. I. Abtrennung und Konzentrierung der Proteine des Honigs. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* 157: 1-6.

BERTELLI, D; PLESSI, M; SABATINI, A G; LOLLI, M; GRILLENZONI, F (2007) Classification of Italian honeys by mid-infrared diffuse reflectance spectroscopy (DRIFTS). *Food Chemistry* 101(4): 1565-1570. http://dx.doi.org/10.1016/j.foodchem.2006.04.010

BERTELLI, D; LOLLI, M; PAPOTTI, G; BORTOLOTTI, L; SERRA, G; PLESSI, M (2010) Detection of honey adulteration by sugar syrups using one-dimensional and two-dimensional high-resolution nuclear magnetic resonance. *Journal of Agricultural and Food Chemistry* 58(15): 8495-8501. http://dx.doi.org/10.1021/jf101460t

BERTONCELJ, J; DOBERŠEK, U; JAMNIK, M; GOLOB, T (2007) Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chemistry* 105(2): 822-828. http://dx.doi.org/10.1016/j.foodchem.2007.01.060

BERTONCELJ, J; POLAK, T; KROPF, U; KOROŠEC, M; GOLOB, T (2011) LC-DAD-ESI/MS analysis of flavonoids and abscisic acid with chemometric approach for the classification of Slovenian honey. *Food Chemistry* 127(1): 296-302. http://dx.doi.org/10.1016/j.foodchem.2011.01.003

BHANDARI, B; D'ARCY B; CHOW, S (1999) A research note: rheology of selected Australian honeys. *Journal of Food Engineering* 41(1): 65-68. http://dx.doi.org/10.1016/S0260-8774(99)00078-3

BIESAGA, M; PYRZYNSKA, K (2009) Liquid chromatography/tandem mass spectrometry studies of the phenolic compounds in honey. *Journal of Chromatography A* 1216(38): 6620-6626. http://dx.doi.org/10.1016/j.chroma.2009.07.066

BILANDŽIĆ, N; GAČIĆ, M; ĐOKIĆ, M; SEDAK, M; ŠIPUŠIĆ, Đ I; KONČURAT, A; GAJGER, I T (2014) Major and trace elements levels in multifloral and unifloral honeys in Croatia. *Journal of Food Composition and Analysis* 33(2): 132-138. http://dx.doi.org/10.1016/j.jfca.2013.12.002

BILUCA, F C; DELLA BETTA, F; DE OLIVEIRA, G P; PEREIRA, L M; GONZAGA, L V; COSTA, A C O; FET, R (2014) 5-HMF and carbohydrates content in stingless bee honey by CE before and after thermal treatment. *Food Chemistry* 159: 244-249. http://dx.doi.org/10.1016/j.foodchem.2014.03.016

BLACK, J (2011) Rapid Method for measuring the antimicrobial activity of honey. *Rural Industries Research and Development Corporation. Australian*. Government. ISBN 978-1-74254-202-7.

BLASA, M; CANDIRACCI, M; ACCORSI, A; PIACENTINI, M P; ALBERTINI, M C; PIATTI, E (2006) Raw Millefiori honey is packed full of antioxidants. *Food Chemistry* 97(2): 217-222. http://dx.doi.org/10.1016/j.foodchem.2005.03.039

BLASA, M; CANDIRACCI, M; ACCORSI, A; PIACENTINI, M P; PIATTI, E (2007) Honey flavonoids as protection agents against oxidative damage to human red blood cells. *Food Chemistry* 104(4): 1635-1640. http://dx.doi.org/10.1016/j.foodchem.2007.03.014

BODEN, J; HAUMANN, I; MAINKA, A (2000) Anwendung der Kapillarelektrophorese in der Lebensmittelanalytik. *GIT Labor-Fachzeitschrift* 44: 924-927.

BOFFO, E F; TAVARES, L A; TOBIAS, A C T; FERREIRA, M M C; FERREIRA, A G (2012) Identification of components of Brazilian honey by H-1 NMR and classification of its botanical origin by chemometric methods. *LWT-Food Science and Technology* 49(1): 55-63. http://dx.doi.org/10.1016/j.lwt.2012.04.024

BOGDANOV, S (1984) Honigdiastase, Gegenüberstellung verschiedener Bestimmungsmethoden. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene* 75: 214-220.

BOGDANOV, S (1997) Nature and origin of the antibacterial substances in honey. *LWT-Food Science and Technology* 30(7): 748-753. http://dx.doi.org/10.1006/fstl.1997.0259

BOGDANOV, S (2009) Harmonised methods of the International Honey Commission. http://www.ihc-platform.net/ihcmethods2009.pdf (Accessed 21/12/2014)

BOGDANOV, S; CHARRIÈRE, J D; IMDORF, A; KILCHENMANN, V; FLURI, P (2002) Determination of residues in honey after treatments with formic and oxalic acid under field conditions. *Apidologie* 33(4): 399-409. http://dx.doi.org/10.1051/apido:2002029

BOGDANOV, S; RUOFF, K; PERSANO-ODDO, L (2004) Physico-chemical methods for the characterization of unifloral honeys: a review. *Apidologie* 35(Suppl. 1): S4-S17. http://dx.doi.org/10.1051/apido:2004047

BOLANOS DE LA TORRE, A A S; HENDERSON, T; NIGAM, P S; OWUSU-APENTEN, R K (2015) A universally calibrated microplate ferric reducing antioxidant power (FRAP) assay for foods and applications to Manuka honey. *Food Chemistry* 174: 119-123. http://dx.doi.org/10.1016/j.foodchem.2014.11.009

BOSI, G; BATTAGLINI, M (1978) Gas analysis of free and protein amino acids in some unifloral honeys. *Journal of Apicultural Research* 17: 152-166.

BOUSETA, A; COLLIN, S (1995) Flavor and free aminoacid composition of lavender and eucalyptus honeys. *Journal of Food Science* 61(4): 683-687. http://dx.doi.org/10.1111/j.1365-2621.1996.tb12181.x

BRUMMER, Y; CUI, S W (2005) Understanding carbohydrate analysis. In *Cui, S W (Ed). Food carbohydrates: chemistry, physical properties, and applications.* Taylor and Francis; Boca Ratón, Florida, USA. pp. 67-104.

BULDINI, P L; CAVALLI, S; MEVOLI, A; SHARMA, J L (2001) Ion chromatographic and voltammetric determination of heavy and transition metals in honey. *Food Chemistry* 73(4): 487-495. http://dx.doi.org/10.1016/S0308-8146(01)00132-7

CAI, J; WU, X; YUAN, L; HAN, E; ZHOU, L; ZHOU, A (2013) Determination of Chinese *Angelica* honey adulterated with rice syrup by an electrochemical sensor and chemometrics. *Analytical Methods* 5(9): 2324-2328. http://dx.doi.org/10.1039/C3AY00041A

CAMPILLO, N; VIÑAS, P; FÉREZ-MELGAREJO, G; HERNÁNDEZ-CÓRDOBA, M (2015) Dispersive liquid-liquid microextraction for the determination of flavonoid aglycone compounds in honey using liquid chromatography with diode array detection and time-of-flight mass spectrometry. *Talanta* 131: 185-191. http://dx.doi.org/10.1016/j.talanta.2014.07.083

CAMPONE, L; PICCINELLI, A L; PAGANO, I; CARABETTA, S; DI SANZO, R; RUSSO, M; RASTRELLI, L (2014) Determination of phenolic compounds in honey using dispersive liquid-liquid microextraction. *Journal of Chromatography A* 1334: 9-15. http://dx.doi.org/10.1016/j.chroma.2014.01.081

ČANADANOVIĆ-BRUNET, J; ĆETKOVIĆ, G; ŠAPONJAC, V T; STAJČIĆ, S; VULIĆ, J; DJILAS, S; ŠTAJNER, D; POPOVIĆ, B (2014) Evaluation of phenolic content, antioxidant activity and sensory characteristics of Serbian honey-based product. *Industrial Crops and Products* 62: 1-7. http://dx.doi.org/10.1016/j.indcrop.2014.08.009

CARNWATH, R; GRAHAM, E M; REYNOLDS, K; POLLOCK, P J (2014) The antimicrobial activity of honey against common equine wound bacterial isolates. *The Veterinary Journal* 199(1): 110-114. http://dx.doi.org/10.1016/j.tvjl.2013.07.003

CAROLI, S; FORTE, G; LAMICELI, A L (1999) Determination of essential and potentially toxic trace elements in honey by inductively coupled plasma-based techniques. *Talanta* 50(2): 327-336. http://dx.doi.org/10.1016/s0039-9140(99)00025-9

CARRATÙ, B; CIARROCCHI, M; MOSCA, M; SANZINI, E (2011) Free amino acids, oxalate and sulphate for honey characterization. *Journal of ApiProduct and ApiMedical Science* 3(2): 81-88. http://dx.doi.org/10.3896/IBRA.4.03.2.03

CASELLA, I G; GATTA, M (2001) Determination of electroactive organic acids by anion-exchange chromatography using a copper modified electrode. *Journal of Chromatography A* 912(2): 223-233. http://dx.doi.org/10.1016/S0021-9673(01)00590-8

CASTRO, R N; AZEREDO, L C; AZEREDO, M A A; DE SAMPAIO, C S T (2001) HPLC assay for the determination of ascorbic acid in honey samples. *Journal of Liquid Chromatography and Related Technologies* 24(7): 1015-1020. http://dx.doi.org/10.1081/JLC-100103427

CASTRO-VÁZQUEZ, L; DÍAZ-MAROTO, M C; GONZÁLEZ-VIÑAS, M A; DE LA FUENTE, E; PÉREZ-COELLO, M S (2008) Influence of storage conditions on chemical composition and sensory properties of citrus honey. *Journal of Agricultural and Food Chemistry* 56(6): 1999-2006. http://dx.doi.org/10.1021/jf072227k

CAVAZZA, A; CORRADINI, C; MUSCI, M; SALVADEO, P (2013) High-performance liquid chromatographic phenolic compound fingerprint for authenticity assessment of honey. *Journal of the Science of Food and Agriculture* 93(5):1169-1175. http://dx.doi.org/10.1002/jsfa.5869

CAVIA, M M; FERNÁNDEZ-MUIÑO, M A; HUIDOBRO, J F; SANCHO, M T (2004) Correlation between moisture and water activity of honeys harvested in different years. *Journal of Food Science* 69(5): 368-370. http://dx.doi.org/10.1111/j.1365-2621.2004.tb10699.x

CERESER-CAMARA, V; LAUX, D (2010) Moisture content in honey determination with a shear ultrasonic reflectometer. *Journal of Food Engineering* 96(1): 93-96. http://dx.doi.org/10.1016/j.jfoodeng.2009.06.049

CHEN, H; FAN, C; CHANG, Q; PANG, G; HU, X; LU, M; WANG, W (2014) Chemometric determination of the botanical origin for Chinese honeys on the basis of mineral elements determined by ICP-MS. *Journal of Agricultural and Food Chemistry* 62(11): 2443-2448. http://dx.doi.org/10.1021/jf405045q

CHEN, L Z; ZHAO, J; YE, Z H; ZHONG, Y P (2008) Determination of adulteration in honey using near-infrared spectroscopy. *Guang Pu Xue Yu Guang Pu Fen Xi/Spectroscopy and Spectral Analysis* 28(11): 2565-2568. http://dx.doi.org/10.3964/j.issn.1000-0593(2008)11-2565-04

CHEN, L; XUE, X; YE, Z; ZHOU, J; CHEN, F; ZHAO, J (2011) Determination of Chinese honey adulterated with high fructose corn syrup by near infrared spectroscopy. *Food Chemistry* 128(4): 1110-1114. http://dx.doi.org/10.1016/j.foodchem.2010.10.027

CHEN, L; WANG, J; YE, Z; ZHAO, J; XUE, X; VANDER HEYDEN, Y H; SUN, Q (2012) Classification of Chinese honeys according to their floral origin by near infrared spectroscopy. *Food Chemistry* 135(2): 338-342. http://dx.doi.org/10.1016/j.foodchem.2012.02.156

CHENG, X; ZHANG, S; ZHANG, H; WANG, Q; HE, P; FANG, Y (2008) Determination of carbohydrates by capillary zone electrophoresis with amperometric detection at a nano-nickel oxide modified carbon paste electrode. *Food Chemistry* 106(2): 830-835. http://dx.doi.org/10.1016/j.foodchem.2007.06.063

CHERCHI, A; SPANEDDA, L; TUBEROSO, C; CABRAS, P (1994) Solid-phase extraction and high-performance liquid chromatographic determination of organic acids in honey. *Journal of Chromatography A* 669(1-2): 59-64. http://dx.doi.org/10.1016/0021-9673(94)80336-6

CHERCHI, A; PORCU, M; SPANEDDA, L; TUBEROSO, C I G; COSENTINO, S; PALMAS, F (1995) Individuazione di parametri utili per la caratterizzazione e la valorizzazione di mieli tipici della Sardegna: asfódelo, cardo e corbezzolo. *La Rivista di Scienza dell'Alimentazione* 24(4): 523-534.

CHO, H J; HONG, S H (1998) Acacia honey quality measurement by near-infrared spectroscopy. *Journal of Near Infrared Spectroscopy* 6(1): A329-A331. http://dx.doi.org/10.1255/jnirs.217

CHO, H J; HA, Y L (2002) Determination of honey quality by Near Infrared Spectroscopy. *Korean Journal of Food Science and Technology* 34(3): 356-360.

CHUA, L S; RAHAMAN, N L A; ADNAN, N A; TAN, T T EE (2013) Antioxidant activity of three honey samples in relation with their biochemical components. *Journal of Analytical Methods in Chemistry,* Vol. 2013, Article ID 313798, 8 pp. http://dx.doi.org/10.1155/2013/313798

CHUDZINSKA, M; BARALKIEWICZ, D (2011) Application of ICP-MS method of determination of 15 elements in honey with chemometric approach for the verification of their authenticity. *Food and Chemical Toxicology* 49(11): 2741-2749. http://dx.doi.org/10.1016/j.fct.2011.08.014

CIAPPINI, M C; STOPPANI, F S (2014) Determination of antioxidant capacity, flavonoids, and total phenolic content in eucalyptus and clover honeys. *Journal of Apicultural Science* 58(1): 103-111. http://dx.doi.org/10.2478/jas-2014-0010

CIULU, M; SOLINAS, S; FLORES, I; PANZANELLI, A; PILO, M I; PIU, P C; SPANO, N; SANNA, G (2011) RP-HPLC determination of water-soluble vitamins in honey. *Talanta* 83(3): 924-929. http://dx.doi.org/10.1016/j.talanta.2010.10.059

CODEX ALIMENTARIUS STANDARD FOR HONEY (2001) Codex standard for honey 12-1981. Revised Codex Standard for Honey. Standards and Standard Methods, volume 11. http://www.codexalimentarius.net (Accessed 21/12/2014)

COMETTO, P M; FAYE, P F; DI PAOLA NARANJO, R; RUBIO, M A; ALDAO M A JJ (2003) Comparison of free amino acids profile in honey from three Argentinian regions. *Journal of Agricultural and Food Chemistry* 51(17): 5079-5087. http://dx.doi.org/10.1021/jf021081g

CONSONNI, R; CAGLIANI, L R; COGLIATI, C (2012) NMR characterization of saccharides in Italian honeys of different floral sources. *Journal of Agricultural and Food Chemistry* 60(18): 4526-4534. http://dx.doi.org/10.1021/jf3008713

CONTE, L S; MIORINI, M; GIOMO, A; BERTACCO, G; ZIRONI, R (1998) Evaluation of some fixed components for unifloral honey characterization. *Journal of Agricultural Food Chemistry* 46(5): 1844-1849. http://dx.doi.org/10.1021/jf970837m

CORBELLA, E; COZZOLINO, D (2005) The use of visible and near infrared spectroscopy to classify the floral origin of honey samples produced in Uruguay. *Journal of Near Infrared Spectroscopy* 13(1): 63-68. http://dx.doi.org/10.1255/jnirs.458

CORRADINI, C; CAVAZZA, A; BIGNARDI, C (2012) High-performance anion-exchange chromatography coupled with pulsed electrochemical detection as a powerful tool to evaluate carbohydrates of food interest: principles and applications. *International Journal of Carbohydrate Chemistry*, Vol. 2012, Article ID 487564, 13 pp. http://dx.doi.org/10.1155/2012/487564

COSSU, M; ALAMANNI, M C (1999) Possibilità di impiego di una metodica enzimatica per la determinazione dell'acido ossalico in campioni di miele della Sardegna. *La Rivista di Scienza dell'Alimentazione* 28: 315-319.

COTTE, J F; CASABIANCA, H; GIROUD, B; ALBERT, M; LHERITIER, J; GRENIER-LOUSTALOT, M F (2004) Characterization of honey amino acid profiles using high-pressure liquid chromatography to control authenticity. *Analytical and Bioanalytical Chemistry* 378(5): 1342-1350. http://dx.doi.org/10.1007/s00216-003-2430-z

COZZOLINO, D; CORBELLA, E (2003) Determination of honey quality components by near infrared reflectance spectroscopy. *Journal of Apicultural Research* 42(1-2): 16-20. http://dx.doi.org/10.1080/00218839.2003.11101081

COZZOLINO, D; CORBELLA, E; SMYTH, H E (2011) Quality control of honey using infrared spectroscopy: a review. *Applied Spectroscopy Reviews* 46(7): 523-538. http://dx.doi.org/10.1080/05704928.2011.587857

COZZOLINO, D; CORBELLA, E; SMYTH, H (2012) Quality control of honey using spectroscopic methods. In *Bondurand, G; Bosch, H (Eds). Honey: production, consumption and health benefits.* Nova Science Publishers, Inc.; Hauppauge, New York, USA. pp. 113-131.

CRANE, E (1975) Honey: A Comprehensive Survey. Heinemann; Newnes, Oxford, UK.

CUEVAS-GLORY, L F; PINO, J A; SANTIAGO, L S; SAURI-DUCH, E (2007) A review of volatile analytical methods for determining the botanical origin of Honey. *Food Chemistry* 103(3): 1032-1043. http://dx.doi.org/10.1016/j.foodchem.2006.07.068

CUEVAS-GLORY, L F; ORTIZ-VÁZQUEZ, E; PINO, J A; SAURI-DUCH, E (2012) Floral classification of Yucatan Peninsula honeys by PCA and HS-SPME/GC-MS of volatile compounds. *International Journal of Food Science and Technology* 47(7): 1378-1383. http://dx.doi.org/10.1111/j.1365-2621.2012.02983.x

DA SILVA, V L; CERQUEIRA, M R F; LOWINSOHN, D; MATOS, M A C; MATOS, R C (2012) Amperometric detection of ascorbic acid in honey using ascorbate oxidase immobilised on amberlite IRA-743. *Food Chemistry* 133(3): 1050-1054. http://dx.doi.org/10.1016/j.foodchem.2012.01.066

DANESHVA-TARIGH, G; SHEMIRANI, F (2014) Simultaneous in situ derivatization and ultrasound-assisted dispersive magnetic solid phase extraction for thiamine determination by spectrofluorimetry. *Talanta* 123: 71-77. http://dx.doi.org/10.1016/j.talanta.2014.01.045

DANIELE, G; MAITRE, D; CASABIANCA, H (2012) Identification, quantification and carbon stable isotopes determinations of organic acids in monofloral honeys. A powerful tool for botanical and authenticity control. *Rapid Communications in Mass Spectrometry* 26(17): 1993-1998. http://dx.doi.org/10.1002/rcm.6310

D'ARCY, B R; RINTOUL, G B; ROWLAND, C Y; BLACKMAN, A J (1997) Composition of Australian honey extractives. 1. Norisoprenoids, monoterpenes, and other natural volatiles from blue gum (*Eucalyptus leucoxylon*) and yellow box (*Eucalyptus melliodora*) honeys. *Journal of Agricultural and Food Chemistry* 45(5): 1834-1843. http://dx.doi.org/10.1021/jf960625

DAVIES, A M C (1975) Amino acid analysis of honeys from eleven countries. *Journal of Apicultural Research* 14(1): 29-39. http://dx.doi.org/10.1080/00218839.1975.11099798

DAVIES, A M C; HARRIS, R G (1982) Free amino acid analysis of honeys from England and Wales: application to the determination of the geographical origin of honeys. *Journal of Apicultural Research* 21(3): 168-173. http://dx.doi.org/10.1080/00218839.1982.11100536

DAVIES, A M C; RADOVIC, B; FEARN, T; ANKLAM, E (2002) A preliminary study on the characterisation of honey by near infrared spectroscopy. *Journal of Near Infrared Spectroscopy* 10: 121-135. http://dx.doi.org/10.1255/jnirs.329

DE ANDRADE, C K; DOS ANJOS, V E; FELSNER, M L; TORRES, Y R; QUINÁIA, S P (2014) Direct determination of Cd, Pb and Cr in honey by slurry sampling electrothermal atomic absorption spectrometry. *Food Chemistry* 146: 166-173. http://dx.doi.org/10.1016/j.foodchem.2013.09.065

DE LA FUENTE, E; RUIZ-MATUTE, A I; VALENCIA-BARRERA, R M; SANZ, J; MARTINEZ CASTRO I (2011) Carbohydrate composition of Spanish unifloral honeys. *Food Chemistry* 129(4): 1483-1489. http://dx.doi.org/10.1016/j.foodchem.2011.05.121

DEFILIPPI, A; PIANCONE, G; PRANDTATTER, A; TIBALDI, G (1995) Honey quality: Ion chromatographic determination of formic acid. *Industrie Alimentari* 34: 495-497.

DEL NOZAL, M J; BERNAL, J L; MARINERO, P; DIEGO, J C; FERCHILLA, J I; HIGES, M; LLORENTE, J (1998) High performance liquid chromatographic determination of organic acids in honey from different botanical origin. *Journal of Liquid Chromatography and related Technologies* 21(20): 3197-3214. http://dx.doi.org/10.1080/10826079808001268

DEL NOZAL, M Z A; BERNAL, J L; DIEGO, J C; GÓMEZ, L A; RUIZ, J M; HIGES, M (2000) Determination of oxalate, sulphate and nitrate in honey and honeydew by ion-chromatography. *Journal of Chromatography A* 881(1-2): 629-638. http://dx.doi.org/10.1016/S0021-9673(00)00271-5

DELGADO, C; TOMÁS-BARBERÁN, F A; TALOU, T; GASET, A (1994) Capillary electrophoresis as an alternative to HPLC for determination of honey flavonoids. *Chromatographia* 38(1): 71-78. http://dx.doi.org/10.1007/BF02275729

DIAS, L A; PERES, A M; VILAS-BOAS, M; ROCHA, M A; ESTEVINHO, L; MACHADO, A A S C (2008) An electronic tongue for honey classification. *Microchimica Acta* 163(1): 97-102. http://dx.doi.org/10.1007/s00604-007-0923-8

DÖKER, S; AYDEMIR, O; USLU, M (2014) Evaluation of digestion procedures for trace element analysis of Cankiri, Turkey honey by inductively coupled plasma mass spectrometry. *Analytical Letters* 47(12): 2080-2094. http://dx.doi.org/10.1080/00032719.2014.895908

DOLD, H; WITZENHAUSEN, R (1955) Ein verfahren zur beurteilung der örtlichen inhibitorischen (keimvermehrungshemmenden) Wirkung von Honigsorten verschiedener herkunft. *Zeitschrift für Hygiene und Infektionskrankheiten* 141(4): 333-347. http://dx.doi.org/10.1007/BF02149974

DONARSKI, J A; ROBERTS, D P T; CHARLTON, A (2010) Quantitative NMR spectroscopy for the rapid measurement of methylglyoxal in manuka honey. *Analytical Methods* 2(10): 1479-1483. http://dx.doi.org/10.1039/C0AY00125B

DONG, R; ZHENG, Y; XU, B (2013) Phenolic profiles and antioxidant capacities of Chinese unifloral honeys from different botanical and geographical sources. *Food and Bioprocess Technology* 6(3): 762-770. http://dx.doi.org/10.1007/s11947-011-0726-0

DOWNEY, G; FOURATIER, V; KELLY, J D (2003) Detection of honey adulteration by addition of fructose and glucose using near infrared transflectance spectroscopy. *Journal of Near Infrared Spectroscopy* 11(6): 447-456. http://dx.doi.org/10.1255/jnirs.395

DVASH, L; AFIC, O; SCHAFFER, A; YESELSON, Y; DAG, A; LANDAU, S (2002) Determination by near-infrared spectroscopy of perseitol used as a marker for the botanical origin of avocado (*Persea americana* Mill.) honey. *Journal of Agricultural and Food Chemistry* 50(19): 5283-5287.http://dx.doi.org/10.1021/jf020329z

DYMERSKI, T; GEBICKI, J; WARDENCKI, W; NAMIESNIK, J (2014) Application of an electronic nose instrument to fast classification of polish honey types. *Sensors* 14(6): 10709-10724. http://dx.doi.org/10.3390/s140610709

ECHIGO, T; TAKENAKA, T (1974) Production of organic acids in honey by honeybees. *Nippon Nogei Kagaku Kaishi* 48(4): 225-230. http://dx.doi.org/10.1271/nogeikagaku1924.48.225

EHRHARDT, P; LIEBIG, J (1965) Inorganic constituents of the honeydew of *Megoura vicia*. *Experientia* 21: 472-473.

ELBANNA, K; ATTALLA K; ELBADRY, M; ABDELTAWAB, A; GAMAL-ELDIN, H; RAMADAN, M F (2014) Impact of floral sources and processing on the antimicrobial activities of different unifloral honeys. *Asian Pacific Journal of Tropical Disease* 4(3): 194-200. http://dx.doi.org/10.1016/S2222-1808(14)60504-1

ESCRICHE, I; KADAR, M; JUAN-BORRÁS, M; DOMENECH, E (2011) Using flavonoids, phenolic compounds and headspace volatile profile for botanical authentication of lemon and orange honeys. *Food Research International* 44(5): 1504-1513. http://dx.doi.org/10.1016/j.foodres.2011.03.049

ESCRICHE, I; KADAR, M; DOMENECH, E; GIL-SÁNCHEZ, L (2012) A potentiometric electronic tongue for the discrimination of honey according to the botanical origin. Comparison with traditional methodologies: physicochemical parameters and volatile profile. *Journal of Food Engineering* 109(3): 449-456. http://dx.doi.org/10.1016/j.jfoodeng.2011.10.036

ESCRICHE, I; KADAR, M; JUAN-BORRAS, M; DOMENECH, E (2014) Suitability of antioxidant capacity, flavonoids and phenolic acids for floral authentication of honey. Impact of industrial thermal treatment. *Food Chemistry* 142: 135-143. http://dx.doi.org/10.1016/j.foodchem.2013.07.033

ESCUREDO, O; MÍGUEZ, M; FERNÁNDEZ-GONZÁLEZ, M; SEIJO, M C (2013a) Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. *Food Chemistry* 138(2-3): 851-856. http://dx.doi.org/10.1016/j.foodchem.2012.11.015

ESCUREDO, O; SEIJO, M C; SALVADOR, J; GONZÁLEZ-MARTÍN, M I (2013b) Near infrared spectroscopy for prediction of antioxidant compounds in the honey. *Food Chemistry* 141(4): 3409-3414. http://dx.doi.org/10.1016/j.foodchem.2013.06.066

ESCUREDO, O; DOBRE, I; FERNÁNDEZ-GONZÁLEZ, M; SEIJO, M C (2014) Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. *Food Chemistry* 149: 84-90. http://dx.doi.org/10.1016/j.foodchem.2013.10.097

ESCUREDO, O; GONZÁLEZ-MARTÍN, M I; RODRÍGUEZ-FLORES, M S; SEIJO, M C (2015) Near infrared spectroscopy applied to the rapid prediction of the floral origin and mineral content of honeys. *Food Chemistry* 170: 47-54. http://dx.doi.org/10.1016/j.foodchem.2014.08.061

ESTEVINHO, L; PEREIRA, A P; MOREIRA, L; DIAS, L G; PEREIRA, E (2008) Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food and Chemical Toxicology* 46(12): 3774-3779. http://dx.doi.org/10.1016/j.fct.2008.09.062

ESTRADA, H; GAMBOA, M M; CHAVES, C; ARIAS, M L (2005) Evaluación de la actividad antimicrobiana de la miel de abeja contra *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, Escherichia coli, *Salmonella enteritidis*, *Listeria monocytogenes* y *Aspergillus niger*. Evaluación de su carga microbiológica. *Archivos Latinoamericanos de Nutrición* 55(2): 127-135.

ETZOLD, E; LICHTENBERG-KRAAG, B (2008) Determination of the botanical origin of honey by Fourier-transformed infrared spectroscopy: an approach for routine analysis. *European Food Research and Technology* 227(2): 579-586. http://dx.doi.org/10.1007/s00217-007-0759-9

FEÁS, X; PIRES, J; ESTEVINHO, M L; IGLESIAS, A; PINTO DE ARAUJO, J P (2010a) Palynological and physicochemical data characterisation of honeys produced in the Entre-Douro e Minho region of Portugal. *International Journal of Food Science & Technology* 45(6): 1255-1262. http://dx.doi.org/10.1111/j.1365-2621.2010.02268.x

FEÁS, X; PIRES, J; IGLESIAS, A; ESTEVINHO, M L (2010b) Characterization of artisanal honey produced on the Northwest of Portugal by melissopalynological and physico-chemical data. *Food and Chemical Toxicology* 48(12): 3462-3470. http://dx.doi.org/10.1016/j.fct.2010.09.024

FELL, R D (1978) The color grading of honey. *American Bee Journal* 18: 782-789.

FELSNER, M L; CANO, C B; BRUNS, R E; WATANABE, H M; ALMEIDA-MURADIAN, L B; MATOS, J R (2004a) Characterization of monofloral honeys by ash contents through a hierarchical design. *Journal of Food Composition and Analysis* 17(6): 737-747. http://dx.doi.org/10.1016/j.jfca.2003.11.001

FELSNER, M L; CANO, C B; MATOS, J R; ALMEIDA-MURADIAN, L B; BRUNS, R E (2004b) Optimization of thermogravimetric analysis of ash content in honey. *Journal of the Brazilian Chemical Society* 15(6): 797-802. http://dx.doi.org/10.1590/S0103-50532004000600002

FERRAUTO, G; PAVONE, P (2013) Palynological, physico-chemical and organoleptic characteristics of carob tree (*Ceratonia siliqua* L.) honey from Sicily. *International Journal of Food Science and Technology* 48(8): 1596-1602. http://dx.doi.org/10.1111/ijfs.12129

FERREIRA, I C F R; AIRES, E; BARREIRA, J C M; ESTEVINHO, L M (2009) Antioxidant activity of Portuguese honey samples: different contributions of the entire honey and phenolic extract. *Food Chemistry* 114(4): 1438-1443. http://dx.doi.org/10.1016/j.foodchem.2008.11.028

FERRERES, F; TOMÁS-BARBERÁN, F A; GIL, M; TOMÁS-LORENTE, F (1991) An HPLC technique for flavonoid analysis in honey. *Journal of the Science of Food and Agriculture* 56(1): 49-56. http://dx.doi.org/10.1002/jsfa.2740560106

FERRERES, F; TOMÁS-BARBERÁN, F A; SOLER, C; GARCÍA-VIGUERA, C; ORTIZ, A; TOMÁS-LORENTE, F (1994) A simple extractive technique for honey flavonoid HPLC analysis. *Apidologie* 25(1): 21-30. http://dx.doi.org/10.1051/apido:19940103

FIDALEO, M; ZUORRO, A; LAVECCHIA R (2011) Antimicrobial activity of some Italian honey against pathogenic bacteria. *Chemical Engineering Transactions*, 24: 1015-1020. http://dx.doi.org/10.3303/CET1124170

FRANCHINI, R A D; MATOS, M A C; MATOS, R C (2011) Amperometric determination of catalase in Brazilian commercial honeys. *Analytical Letters* 44(1-3): 232-240. http://dx.doi.org/10.1080/00032719.2010.500758

FRENCH, V M; COOPER, R A; MOLAN, P C (2005) The antibacterial activity of honey against coagulase-negative staphylococci. *Journal of Antimicrobial Chemotherapy* 56(1): 228-231. http://dx.doi.org/10.1093/jac/dki193

GALLARDO-VELÁZQUEZ, T; OSORIO-REVILLA, G; ZUÑIGA-DE LOA, M; RIVERA-ESPINOZA, Y (2009) Application of FTIR-HATR spectroscopy and multivariate analysis to the quantification of adulterants in Mexican honeys. *Food Research International* 42(3): 313-318. http://dx.doi.org/10.1016/j.foodres.2008.11.010

GALLINA, A; STOCCO, N; MUTINELLI, F (2010) Karl Fischer Titration to determine moisture in honey: a new simplified approach. *Food Control* 21(6): 942-944. http://dx.doi.org/10.1016/j.foodcont.2009.11.008

GAMBACORTA, E; SIMONETTI, A; GARRISI, N; INTAGLIETTA, I; PERNA, M (2014) Antioxidant properties and phenolic content of sulla (*Hedysarum* spp.) honeys from Southern Italy. *International Journal of Food Science and Technology* 49(10): 2260-2268. http://dx.doi.org/doi:10.1111/ijfs.12541

GARCÍA-ÁLVAREZ, M; HUIDOBRO, J F; HERMIDA, M; RODRÍGUEZ-OTERO, J L (2000) Major components of honey analysis by near-infrared transflectance spectroscopy. *Journal of Agricultural and Food Chemistry* 48(11): 5154-5158. http://dx.doi.org/10.1021/jf000170v

GARCÍA-ÁLVAREZ, M; CERESUELA, S; HUIDOBRO, J F; HERMIDA, M; RODRÍGUEZ-OTERO, J L (2002) Determination of polarimetric parameters of honey by near-infrared transflectance spectroscopy. *Journal of Agricultural and Food Chemistry* 50(3): 419-425. http://dx.doi.org/10.1021/jf0105438

GAŠIC, U; KEČKEŠ, S; DABIĆ, D; TRIFKOVIĆ, J; MILOJKOVIĆ-OPSENICA, D; NATIĆ, M; TEŠIC, Ž (2014) Phenolic profile and antioxidant activity of Serbian polyfloral honeys. *Food Chemistry* 145: 599-607. http://dx.doi.org/10.1016/j.foodchem.2013.08.088

GHELDOF, N; ENGESETH, N J (2002) Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *Journal of Agricultural and Food Chemistry* 50(10): 3050-3055. http://dx.doi.org/10.1021/jf0114637

GHELDOF, N; WANG, X H; ENGESETH, N J (2002) Identification and quantification of antioxidant components of honeys from various floral sources. *Journal of Agricultural and Food Chemistry* 50(21): 5870-5877. http://dx.doi.org/10.1021/jf0256135

GILBERT, J; SHEPHERD, M J; WALLWORK, M A; HARRIS, R G (1981) Determination of the geographical origin of honeys by multivariate analysis of gas chromatographic data on their free amino acid content. *Journal of Apicultural Research* 20(2): 125-135.

GIRI, K V (1938) The chemical composition and enzyme content of Indian honey. *Madras Agricultural Journal* XXVI(2): 68-72.

GITZAPIS, P; TIMOTHEOU-POTAMIA, M (1989) Determination of reducing sugars with a 2,4-dinitrophenolate-selective membrane electrode. *Analytica Chimica Acta* 218: 37-46. http://dx.doi.org/10.1016/S0003-2670(00)80280-9

GOMES, S; DIAS, L G; MOREIRA, L L; RODRIGUES, P; ESTEVINHO, L (2010) Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. *Food and Chemical Toxicology* 48(2): 544-548. http://dx.doi.org/10.1016/j.fct.2009.11.029

GOMES, T; FEÁS, X; IGLESIAS, A; ESTEVINHO, L M (2011) Study of organic honey from the Northeast of Portugal. *Molecules* 16(7): 5274-5286. http://dx.doi.org/10.3390/molecules16075374

GÓMEZ-CARAVACA, A M; GÓMEZ-ROMERO, M; ARRÁEZ-ROMÁN, D; SEGURA-CARRETERO, A; FERNÁNDEZ-GUTIÉRREZ, A (2006) Advances in the analysis of phenolic compounds in products derived from bees. *Journal of Pharmaceutical and Biomedical Analysis* 41(4): 1220-1234. http://dx.doi.org/10.1016/j.jpba.2006.03.002

GÓMEZ-DÍAZ, D; NAVAZA, J M; QUINTÁNS-RIVEIRO L C (2012) Physicochemical characterization of Galician honeys. *International Journal of Food Properties* 15(2): 292-300. http://dx.doi.org/10.1080/10942912.2010.483616

GONZÁLEZ-PARAMÁS, A M; BÁREZ, J A G; GARCÍA-VILANOVA, R J; PALA, T R; ALBAJAR, R A; SÁNCHEZ, J S (2000) Geographical discrimination of honeys by using mineral composition and common chemical quality parameters. *Journal of the Science of Food and Agriculture* 80(1): 157-165. http://dx.doi.org/10.1002/(SICI)1097-0010(20000101)80:1<157::AID-JSFA506>3.0.CO;2-B

GONZÁLEZ-PARAMÁS, A M; GÓMEZ, J A; CORDÓN, C; GARCÍA-VILLANOVA, R J; SÁNCHEZ, J (2006) HPLC-fluorimetric method for analysis of amino acids in products of the hive (honey and bee-pollen). *Food Chemistry* 95: 148-156. http://dx.doi.org/10.1016/j.foodchem.2005.02.008

GORJANOVIĆ, S Ž; ÁLVAREZ-SUÁREZ, J M; NOVAKOVIĆ, M M; PASTOR, F T; PEZO, L; BATTINO, M; SUŽNJEVIĆ, D Ž (2013) Comparative analysis of antioxidant activity of honey of different floral sources using recently developed polarographic and various spectrophotometric assays. *Journal of Food Composition and Analysis* 30(1): 13-18. http://dx.doi.org/10.1016/j.jfca.2012.12.004

GRESLEY, A L; KENNY, J; CASSAR, C; KELLY, A; SINCLAIR, A; FIELDER, M D (2012) The application of high resolution diffusion NMR to the analysis of manuka honey. *Food Chemistry* 135(4): 2879-2886. http://dx.doi.org/10.1016/j.foodchem.2012.07.072

GUERRINI, A; BRUNI, R; MAIETTI, S; POLI, F; ROSSI, D; PAGANETTO, G; MUZZOLI, M; SCALVENZI, L; SACCHETTI, G (2009) Ecuadorian stingless bee (Meliponinae) honey: A chemical and functional profile of an ancient health product. *Food Chemistry* 114(4): 1413-1420. http://dx.doi.org/10.1016/j.foodchem.2008.11.023

GULER, A; BAKAN, A; NISBET, C; YAVUZ, O (2007) Determination of important biochemical properties of honey to discriminate pure and adulterated honey with sucrose (*Saccharum officinarum* L.) syrup. *Food Chemistry* 105(3): 1119-1125. http://dx.doi.org/10.1016/j.foodchem.2007.02.024

GULER, A; KOCAOKUTGEN, H; GARIPOGU, A V; ONDER, H; EKINCI, D; BIYIK, S (2014) Detection of adulterated honey produced by honeybee (*Apis mellifera* L.) colonies fed with different levels of commercial industrial sugar (C3 and C4 plants) syrups by the carbon isotope ratio analysis. *Food Chemistry* 155: 155-160. http://dx.doi.org/10.1016/j.foodchem.2014.01.033

GÜNTHER, F; BURCKHART, O (1967) Bestimmung der Sauren Gesamtphosphatase in Honig. *Deutsche Lebensmittel-Rundschau* 63(2): 41-44.

GUYOT, C., BOUSETA, A., SCHEIRMAN, V., COLLIN, S (1998) Floral origin markers of chestnut and lime tree honeys. *Journal of Agricultural and Food Chemistry* 46(2): 625-633. http://dx.doi.org/10.1021/jf970510l

HA, J; KOO, M; OK, H (1998) Determination of the constituents of honey by near infrared spectroscopy. *Journal of Near Infrared Spectroscopy* 6(1): A367-A369. http://dx.doi.org/10.1255/jnirs.226

HABIB, H M; AL MEQBALI, F T; KAMAL, H; SOUKA, U D; IBRAHIM, W H (2014a) Bioactive components, antioxidant and DNA damage inhibitory activities of honeys from arid regions. *Food Chemistry* 153: 28-34. http://dx.doi.org/10.1016/j.foodchem.2013.12.044

HABIB, H M; AL MEQBALI, F T; KAMAL, H; SOUKA, U D; IBRAHIM, W H (2014b) Physicochemical and biochemical properties of honeys from arid regions. *Food Chemistry* 153: 35-43. http://dx.doi.org/10.1016/j.foodchem.2013.12.048

HADORN, H; ZÜRCHER, K (1963): "Formozahl von Honig. Gleichzeitige Bestimmung von Formozahl, pH, freier Säure und Lactongehalt in Honig. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene* 54: 304-321.

HADORN, H; ZÜRCHER, K (1966) Eine verbesserte polarimetrische Methode zur Saccharasezahlbestimmung im Honig. *Deutsche Lebensmittel Rundschau* 62: 195-201.

HADORN, H; ZÜRCHER, K (1972) Eine einfache kinetische Methode zur Bestimmung der Diastasezahl in Honig. *Deutsche Lebensmittel Rundschau* 68: 209-216.

HANSEN, H; GULDBORG, M (1988) Residues in honey and wax after treatment of bee colonies with formic acid. *Tidsskrift for Planteavl* 92: 1-10.

HAYASHI, T; TAKAMATSU, N; NAKASHIMA, T; ARITA, T (2011) Immunological characterization of honey proteins and identification of MRJP 1 as an IgE-binding protein. *Bioscience, Biotechnology and Biochemistry* 75(3): 556-560. http://dx.doi.org/10.1271/bbb.100778

HAYDAK, M H; PALMER, L S; TANQUARY, M C; VIVINO, A E (1942) Vitamin content of honeys. *The Journal of Nutrition* 23: 581-588.

HEGAZI, A G (2011) Antimicrobial activity of different Egyptian honeys as comparison of Saudi Arabia honey. *Research Journal of Microbiology* 6(5): 488-495. http://dx.doi.org/10.3923/jm.2011.488.495

HENNESSY, S; DOWNEY, G; O'DONNELL, C P (2010) Attempted confirmation of the provenance of Corsican PDO honey using FT-IR spectroscopy and multivariate data analysis. *Journal of Agricultural and Food Chemistry* 58(17): 9401-9406. http://dx.doi.org/10.1021/jf101500n

HENRIQUES, A; JACKSON, S; COOPER, R; BURTON, N (2006) Free radical production and quenching in honeys with wound healing potential. *Journal of Antimicrobial Chemotherapy* 58(4): 773-777. http://dx.doi.org/10.1093/jac/dkl336

HERMOSÍN, I; CHICÓN, R M; CABEZUDO, M D (2003) Free amino acid composition and botanical origin of honey. *Food Chemistry* 83(2): 263-268. http://dx.doi.org/10.1016/S0308-8146(03)00089-X

HERRERO-LATORRE, C; PEÑA-CRECENTE, R M; GARCÍA-MARTÍN, S; BARCIELA-GARCÍA, J (2013) A fast chemometric procedure based on NIR data for authentication of honey with protected geographical indication. *Food Chemistry* 141(4): 3559-3565. http://dx.doi.org/10.1016/j.foodchem.2013.06.022

HONÓRIO, G G; AZEVEDO, G C; MATOS, M A C; DE OLIVEIRA, M A L; MATOS, R C (2014) Use of boron-doped diamond electrode pre-treated cathodically for the determination of trace metals in honey by differential pulse voltammetry. *Food Control* 36(1): 42-48. http://dx.doi.org/10.1016/j.foodcont.2013.08.004

HORVÁTH, K; MOLNÁR-PERL, I (1998) Simultaneous GC-MS quantitation of o-phosphoric, aliphatic and aromatic carboxylic acids, proline, hydroxyethylfurfural and sugars as their TMS derivatives in honeys. *Chromatographia* 48(1): 120-126. http://dx.doi.org/10.1007/BF02467527

HROBONOVÁ, K; LEHOTAY, J; CIZMÁRIK, J (2007) Determination of quinic and shikimic acids in products derived from bees and their preparates by HPLC. *Journal of Liquid Chromatography and Related Technologies* 30(17): 2635-2644. http://dx.doi.org/10.1080/10826070701540654

HUANG, D; OU, B; PRIOR, R L (2005) The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry* 53(6): 1841-1856. http://dx.doi.org/10.1021/jf030723c

HUIDOBRO, J F; SIMAL, J (1984) Determinación del color y turbidez en mieles. *Anales de Bromatología* XXXVI: 225-245.

HUIDOBRO, J F; SÁNCHEZ, M P; MUNIATEGUI, S; SANCHO, M T (2005) Precise method for the measurement of catalase activity in honey. *Journal of AOAC International* 88(3): 800-804.

IDRIS, Y M A; MARIOD, A A; HAMAD, S I (2011) Physicochemical properties, phenolic contents and antioxidant activity of Sudanese honey. *International Journal of Food Properties* 14(2): 450-458. http://dx.doi.org/10.1080/10942910903243673

IGLESIAS, M T; PUEYO, E; POLO, M C (2002) Los componentes nitrogenados. In *De Lorenzo, C (Ed). La miel de Madrid*. Consejería de Economía e Innovación Tecnológica. Comunidad de Madrid. Instituto Madrileño de investigación Agraria y alimentaria. pp. 109-120. Available at: http://www.madrid.org/bvirtual/BVCM005574.pdf (Accessed 21/12/2014)

IGLESIAS, M T; DE LORENZO, C; POLO, M D; MARTIN-ALVEREZ, P J; PUEYO, E (2004) Usefulness of amino acid composition to discriminate between honeydew and floral honeys. Application to honeys from a small geographic area. *Journal of Agricultural and Food Chemistry* 52(1): 84-89. http://dx.doi.org/10.1021/jf030454q

IOANNIDOU, M D; ZACHARIADIS, G A; ANTHEMIDIS, A N; STRATIS, J A (2005) Direct determination of toxic trace metals in honey and sugars using inductively coupled plasma atomic emission spectrometry. *Talanta* 65(1): 92-97. http://dx.doi.org/10.1016/j.talanta.2004.05.018

IRISH, J; BLAIR, S; CARTER, D A (2008) The antibacterial activity of honey derived from Australian flora. *PLoS ONE*, 6(3): e18229. http://dx.doi.org/10.1371/journal.pone.0018229

IRUDAYARAJ, J; SIVAKESAVA, S (2001) Detection of adulteration in honey by discriminant analysis using FTIR spectroscopy. *Transactions of the American Society of Agricultural Engineers* 44(3): 643-650.

IRUDAYARAJ, J; XU, F; TEWARI, J (2003) Rapid determination of invert cane sugar adulteration in honey using FTIR spectroscopy and multivariate analysis. *Journal of Food Science* 68(6): 2040-2045. http://dx.doi.org/10.1111/j.1365-2621.2003.tb07015.x

ISENGARD, H D; SCHULTHEIß, D; RADOVIC, B; ANKLAM, E (2001) Alternatives to official analytical methods used for the water determination in honey. *Food Control* 12(7): 459-466. http://dx.doi.org/10.1016/S0956-7135(01)00044-5

ISENGARD, H D; SCHULTHEIß, D (2003) Water determination in honey - Karl Fischer Titration, an alternative to refractive index measurements? *Food Chemistry* 82(1): 151-154. http://dx.doi.org/10.1016/S0308-8146(02)00543-5

ISIDOROV, V A; BAGAN, R; BAKIER, S; SWIECICKA, I (2015) Chemical composition and antimicrobial activity of Polish herbhoneys. *Food Chemistry* 171:84-88. http://dx.doi.org/10.1016/j.foodchem.2014.08.112

ISLA, M I; CRAIG, A; ORDOÑEZ, R; ZAMPINI, C; SAYAGO, J; BEDASCARRASBURE, E; ÁLVAREZ, A; SALOMÓN, V; MALDONADO, L (2011) Physico chemical and bioactive properties of honeys from Northwestern Argentina. *LWT-Food Science and Technology* 44(9): 1922-1930. http://dx.doi.org/10.1016/j.lwt.2011.04.003

ISLAM, A; KHALIL, I; ISLAM, N; MONIRUZZAMAN, M; MOTTALIB, A; SULAIMAN, S A; GAN, S H (2012) Physicochemical and antioxidant properties of Bangladeshi honeys stored for more than one year. *BMC Complementary and Alternative Medicine* 12: 177-187. http://dx.doi.org/10.1186/1472-6882-12-177

IVANOV, T (1978) Study of invertase, acid and alkaline phosphatase and esterase activities in bee honey. *Zhivonovud Nauk.* 15(3): 103-112.

JAMRÓZ, M K; PARADOWSKA, K; ZAWADA, K; MAKAROVA, K; KÁZMIERSKI, S; WAWER, I (2014) ^{1}H and ^{13}C NMR-based sugar profiling with chemometric analysis and antioxidant activity of herbhoneys and honeys. *Journal of the Science of Food and Agriculture* 94(2): 246-255. http://dx.doi.org/10.1002/jsfa.6241

JERKOVIĆ, I; MASTELIC, J; MARIJANOVIC, Z; KLEIN, Z; JELIC, M (2007) Comparison of hydrodistillation and ultrasonic extraction for the isolation of volatile compounds from two unifloral honeys from *Robinia pseudoacacia* L. and *Castanea sativa* L. *Ultrasonics Sonochemistry* 14(6): 750-756. http://dx.doi.org/10.1016/j.ultsonch.2006.12.014

JERKOVIĆ, I; MARIJANOVIĆ, Z (2009) A short review of headspace extraction and ultrasonic solvent extraction for honey volatiles fingerprinting. *Croatian Journal of Food Science and Technology* 1(2): 28-34.

JERKOVIĆ, I; TUBEROSO C I G; MARIJANOVIĆ, Z; JELIĆ, M; KASUM, A (2009) Headspace, volatile and semi-volatile patterns of *Paliurus spina-christi* unifloral honey as markers of botanical origin. *Food Chemistry* 112(1): 239-245. http://dx.doi.org/10.1016/j.foodchem.2008.05.080

JERKOVIĆ, I; MARIJANOVIC, Z (2010) Oak (*Quercus frainetto* Ten.) honeydew honey-Approach to screening of volatile organic composition and antioxidant capacity (DPPH and FRAP assay). *Molecules* 15(5): 3744-3756. http://dx.doi.org/10.3390/molecules15053744

JERKOVIĆ I; MASTELIC, J; MARIJANOVIC, Z (2011) Volatile compounds of *Asphodelus microcarpus* Salzm et Viv. honey obtained by HS-SPME and USE analyzed by GC/MS. *Chemistry and Biodiversity* 8(4): 587-598. http://dx.doi.org/10.1002/cbdv.201000205

JEURING, J; KUPPERS, F (1980) High performance liquid chromatography of furfural and hydroxymethylfurfural in spirits and honey. *Journal of the Association of Official Analytical Chemists* 63(6): 1215-1218.

JUAN-BORRÁS, M; DOMENECH, E; HELLEBRANDOVA, M; ESCRICHE, I (2014) Effect of country origin on physicochemical, sugar and volatile composition of acacia, sunflower and tilia honeys. *Food Research International* 60: 86-94. http://dx.doi.org/10.1016/j.foodres.2013.11.045

JURADO-SÁNCHEZ, B; BALLESTEROS, E; GALLEGO, M (2011) Gas chromatographic determination of 29 organic acids in foodstuffs after continuous solid-phase extraction. *Talanta* 84(3): 924-930. http://dx.doi.org/10.1016/j.talanta.2011.02.031

KAHOUN, D; ŘEZKOVÁ, S; VEŠKRNOVÁ, K; KRÁLOVSKÝ, J; HOLČAPEK, M (2008) Determination of phenolic compounds and hydroxymethylfurfural in meads using high performance liquid chromatography with coulometric-array and UV detection. *Journal of Chromatography A* 1202(1): 19-33. http://dx.doi.org/10.1016/j.chroma.2008.06.016

KANEMATSU, H; AOYAMA, M; MARUYAMA, T; NIIYA, I (1982) Amino acid analysis of honeys with different geographical and floral origin. *Journal of Japan Society of Food Nutrition* 35(4): 297-303.

KARABAGIAS, I K; BADEKA, A; KONTAKOS, S; KARABOURNIOTI, S; KONTOMINAS, M G (2014a) Characterisation and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical parameters and chemometrics. *Food Chemistry* 146: 548-557. http://dx.doi.org/10.1016/j.foodchem.2013.09.105

KARABAGIAS, I K; VAVOURA, M V; NIKOLAOU, C; BADEKA, A V; KONTAKOS, S; KONTOMINAS, M G (2014b) Floral authentication of Greek unifloral honeys based on the combination of phenolic compounds, physicochemical parameters and chemometrics. *Food Research International* 62: 753-760. http://dx.doi.org/10.1016/j.foodres.2014.04.015

KAŠKONIENĖ, V; VENSKUTONIS, P R; ČEKSTERYTĖ, V (2008) Composition of volatile compounds of honey of various floral origin and beebread collected in Lithuania. *Food Chemistry* 111(4): 988-997. http://dx.doi.org/10.1016/j.foodchem.2008.05.021

KAŠKONIENĖ V; VENSKUTONIS, P R (2010) Floral markers in honey of various botanical and geographic origins: a review. *Comprehensive reviews in Food Science and Food Safety* 9: 620-634. http://dx.doi.org/10.1111/j.1541-4337.2010.00130.x

KAŠKONIENĖ, V; VENSKUTONIS, P R; ČEKSTERYTĖ, V (2010) Carbohydrate composition and electrical conductivity of different origin honeys from Lithuania. *LWT- Food Science and Technology* 43(5): 801-807. http://dx.doi.org/10.1016/j.lwt.2010.01.007

KAYACIER, A; KARAMAN, S (2008) Rheological and some physicochemical characteristics of selected Turkish honeys. *Journal Texture Studies* 39(1): 17-27. http://dx.doi.org/10.1111/j.1745-4603.2007.00127.x

KERKVLIET, J D; MEIJER, H A J (2000) Adulteration of honey: Relation between microscopic analysis and $\delta^{13}C$ measurements. *Apidologie* 31: 717-726.

KELLY, J F D; DOWNEY, G; FOURATIER, V (2004) Initial study of honey adulteration by sugar solutions using midinfrared (MIR) spectroscopy and chemometrics. *Journal of Agricultural and Food Chemistry* 52(1): 33-39. http://dx.doi.org/10.1021/jf034985q

KELLY, J D; PETISCO, C; DOWNEY, G (2006a) Application of Fourier transform midinfrared spectroscopy to the discrimination between Irish artisanal honey and such honey adulterated with various sugar syrups. *Journal of Agricultural and Food Chemistry* 54(17): 6166-6171. http://dx.doi.org/10.1021/jf0613785

KELLY, J D; PETISCO, C; DOWNEY, G (2006b) Potential of near infrared transflectance spectroscopy to detect adulteration of Irish honey by beet invert syrup and high fructose corn syrup. *Journal of Near Infrared Spectroscopy* 14(1): 139-146. http://dx.doi.org/10.1255/jnirs.599

KENJERIĆ, F Č; SAVERIO, M; BENNEDETTI, S; PRIMORAC, L; KENJERIĆ, D Č (2009) Honey botanical origin determination by electronic nose. *Journal of Apicultural Research and Bee World* 48(2): 99-103. http://dx.doi.org/10.3896/IBRA.1.48.2.03

KHALIL, M I; MONIRUZZAMAN, M; BOUKRAÂ, L; BENHANIFIA, M; ISLAM, M A; ISLAM, M N; SULAIMAN, S A; GAN, S H (2012) Physicochemical and antioxidant properties of Algerian honey. *Molecules* 17(9): 11199-11215. http://dx.doi.org/doi:10.3390/molecules170911199

KISHORE, K; HALIM, A S; SYAZANA, M S N; SIRAJUDEEN, K N S (2011) Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. *Nutrition Research* 31(4): 322-325. http://dx.doi.org/10.1016/j.nutres.2011.03.001

KITZES, G; SCHUETTE, H A; ELVEHJEM, C A (1943) The B vitamins in honey. *The Journal of Nutrition* 26: 241-250.

KLOFUTAR, C; RUDAN-TASIC, D; KOTAR, B; MARINKO, R (1990) Determination of the total acidity of honey by the potentiometric titration technique. *Farmacevtski Vestnik* 41(1): 25-32.

KOLAYLI, S; KONGUR, N; GÜNDOĞDU, A; KEMER, B; DURAN, C; ALIYAZICIOĞLU, R; KÜÇÜK, M (2008) Mineral composition of selected honeys from Turkey. *Asian Journal of Chemistry* 20(3): 2421-2424.

KOLAYLI, S; YILDIZ, O; SAHIN, H; ALIYAZICIOGLU, R (2014) Biochemistry and physicochemical properties of honey. In *Boudraâ, L (Ed). Honey in Traditional and Modern Medicine.* CRC Press Taylor & Francis Group; Boca Ratón, Florida, USA. pp. 21-35.

KOWALSKI, S (2013) Changes of antioxidant activity and formation of 5-hydroxymethylfurfural in honey during thermal and microwave processing. *Food Chemistry* 141(2): 1378-1382. http://dx.doi.org/10.1016/j.foodchem.2013.04.025

KROPF, U; KOROŠEC, M; BERTONCELJ, J; OGRINC, N; NEČEMER, M; KUMP, P; GOLOB, T (2010) Determination of the geographical origin of Slovenian black locust, lime and chestnut honey. *Food Chemistry* 121(3): 839-846. http://dx.doi.org/10.1016/j.foodchem.2009.12.094

KÜCÜK, M; KOLAYLI, S; KARAOĞLU, S; ULUSOY, E; BALTACI, C; CANDAN, F (2007) Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chemistry* 100(2): 526-534. http://dx.doi.org/10.1016/j.foodchem.2005.10.010

KUMP, P; NECEMER, M; SNAJDER, J (1996) Determination of trace elements in bee honey, pollen and tissue by total reflection and radioisotope X-ray fluorescence spectrometry. *Spectrochimica Acta Part B* 51(5): 499-507. http://dx.doi.org/10.1016/0584-8547(95)01435-7

KUŚ, P M; CONGIU, F; TEPER, D; SROKA, Z; JERKOVIĆ, I; TUBEROSO, C I G (2014a) Antioxidant activity, color characteristics, total phenol content and general HPLC fingerprints of six Polish unifloral honey types. *LWT-Food Science and Technology* 55(1): 124-130. http://dx.doi.org/10.1016/j.lwt.2013.09.016

KUŚ, P M; JERKOVIĆ, I; TUBEROSO, C I G; MARIJANOVIĆ, Z; CONGIU, F (2014b) Cornflower (*Centaurea cyanus* L.) honey quality parameters: Chromatographic fingerprints, chemical biomarkers, antioxidant capacity and others. *Food Chemistry* 142: 12-18. http://dx.doi.org/10.1016/j.foodchem.2013.07.050

LACHMAN, J; ORSÁK, M; HEJTMÁNKOVÁ, A; KOVÁŘOVÁ, E (2010) Evaluation of antioxidant activity and total phenolics of selected Czech honeys. *LWT-Food Science and Technology* 43(1): 52-58. http://dx.doi.org/10.1016/j.lwt.2009.06.008

LEÓN-RUIZ, V; VERA, S; GONZÁLEZ-PORTO, A V; SAN ANDRÉS, M P (2011) Vitamin C and sugar levels as simple markers for discriminating Spanish honey sources. *Journal of Food Science* 76(3): C356-C361. http://dx.doi.org/10.1111/j.1750-3841.2011.02041.x

LEÓN-RUIZ, V; GONZÁLEZ-PORTO, A V; AL-HABSI, N; VERA, S; SAN ANDRÉS, M P; JAUREGI, P (2013a) Antioxidant, antibacterial and ACE-inhibitory activity of four monofloral honeys in relation to their chemical composition. *Food & Function* 4(11): 1617-1624. http://dx.doi.org/10.1039/c3fo60221d

LEÓN-RUIZ, V; VERA, S; GONZÁLEZ-PORTO, A V; SAN ANDRÉS, M P (2013b) Analysis of water-soluble vitamins in honey by isocratic RP-HPLC. *Food Analytical Methods* 6(2): 488-496. http://dx.doi.org/10.1007/s12161-012-9477-4

LI, Y; WAHDAT, F; NEEB, R (1995) Digestion-free determination of heavy metals (Pb, Cd, Cu) in honey using anodic stripping differential pulse voltammetry and potentiometric stripping analysis. *Fresenius' Journal of Analytical Chemistry* 351(7): 678-682. http://dx.doi.org/10.1007/BF00323346

LIANG, Y; CAO, W; CHEN, W J; XIAO; X H; ZHENG, J B (2009) Simultaneous determination of four phenolic components in citrus honey by high performance liquid chromatography using electrochemical detection. *Food Chemistry* 114(4): 1537-1541. http://dx.doi.org/doi:10.1016/j.foodchem.2008.11.024

LICHTENBERG-KRAAG, B; HEDTKE, C; BIENEFELD, K (2002) Infrared spectroscopy in routine quality analysis of honey. *Apidologie* 33(3): 327-337. http://dx.doi.org/10.1051/apido:2002010

LIN, S M; MOLAN, P C; CURSONS, R T (2009) The in vitro susceptibility of *Campylobacter* spp. to the antibacterial effect of manuka honey. *European Journal of Clinical Microbiology and Infectious Diseases* 28(4): 339-344. http://dx.doi.org/10.1007/s10096-008-0630-3

LIU, J R; YE, Y L; LIN, T Y; WANG, Y W; PENG, C C (2013) Effect of floral sources on the antioxidant, antimicrobial, and anti-inflammatory activities of honeys in Taiwan. *Food Chemistry* 139(1-4): 938-943.http://dx.doi.org/10.1016/j.foodchem.2013.02.015

LOLLI, M; BERTELLI, D; PLESSI, M; SABATINI, A G; RESTANI, C (2008) Classification of Italian honeys by 2D HR-NMR. *Journal of Agricultural and Food Chemistry* 56(4): 1298-1304. http://dx.doi.org/10.1021/jf072763c

LÓPEZ-MALO VIGIL, A; PALOU, E; PARISH, M E; DAVIDSON, P M (2005) Methods for activity assay and evaluation of results. In *Davidson, P M, Sofos, J N, Branen, A L (Eds). Antimicrobials in Food (3rd edition)*. Taylor & Francis; Boca Raton, Florida, USA. pp. 659-680.

LOW, N H; VONG, K V; SPORNS, P (1986) A new enzyme, β-glucosidase in honey. *Journal of Apicultural Research* 25(3): 178-181. http://dx.doi.org/10.1080/00218839.1986.11100713

MAJOR, N; MARKOVIĆ, K; KRPAN, M; ŠARIĆ, G; HRUŠKAR, M; VAHČIĆ, N (2011) Rapid honey characterization and botanical classification by an electronic tongue. *Talanta* 85(1): 569-574. http://dx.doi.org/10.1016/j.talanta.2011.04.025

MANUEL SUISSE DES DENRÉES ALIMENTAIRES (1974) Office Central Fédéral des Imprimés et du Matériel 23. Berna.

MATEO-CASTRO, R; JIMÉNEZ-ESCAMILLA, M; BOSCH-REIG, F (1992) Evaluation of the color of some Spanish unifloral honey types as a characterization parameter. *Journal of the AOAC International* 75(3): 537-542.

MATO, I; HUIDOBRO, J F; SÁNCHEZ, M P; MUNIATEGUI, S; FERNÁNDEZ-MUIÑO, M A; SANCHO, M T (1997) Enzymatic determination of total D-gluconic acid in honey. *Journal of Agricultural and Food Chemistry* 45(9): 3550-3553. http://dx.doi.org/10.1021/jf970012c

MATO, I; HUIDOBRO, J F; CENDÓN, V; MUNIATEGUI, S; FERNÁNDEZ-MUIÑO, M A; SANCHO, M T (1998a) Enzymatic determination of citric acid in honey by using polyvinylpolypyrrolidone clarification. *Journal of Agricultural and Food Chemistry* 46(1): 141-144. http://dx.doi.org/10.1021/jf970418p

MATO, I; HUIDOBRO, J F; SÁNCHEZ, M P; MUNIATEGUI, S; FERNÁNDEZ-MUIÑO, M A; SANCHO, M T (1998b) Enzymatic determination of L-malic acid in honey. *Food Chemistry* 62(4): 503-508. http://dx.doi.org/10.1016/S0308-8146(97)00166-0

MATO, I; HUIDOBRO, J F; SIMAL-LOZANO, J; SANCHO, M T (2003) Significance of nonaromatic organic acids in honey. *Journal of Food Protection* 66(12): 2371-2376.

MATO, I; HUIDOBRO, J F; SIMAL-LOZANO, J; SANCHO, M T (2006a) Rapid determination of nonaromatic organic acids in honey by capillary zone electrophoresis with direct ultraviolet detection. *Journal of Agricultural and Food Chemistry* 54(5): 1541-1550. http://dx.doi.org/10.1021/jf051757i

MATO, I; HUIDOBRO, J F; SIMAL-LOZANO, J; SANCHO, M T (2006b) Analytical methods for the determination of organic acids in honey. *Critical Reviews in Analytical Chemistry* 36(1): 3-11. http://dx.doi.org/10.1080/10408340500451957

MCCARTHY, J (1995) The antibacterial effects of honey. *American Bee Journal* May: 171-172.

MEDA, A; LAMIEN, C E; ROMITO, M; MILLOGO, J; NACOULMA, O G (2005) Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry* 91(3): 571-577. http://dx.doi.org/10.1016/j.foodchem.2004.10.006

MELLIOU, E; CHINOU, I (2011) Chemical constituents of selected unifloral Greek bee-honeys with antimicrobial activity. *Food Chemistry* 129(2): 284-290. http://dx.doi.org/10.1016/j.foodchem.2011.04.047

MENDES, T M F F; NIVALDO-BACCAN, S; CADORE, S (2006) Sample treatment procedures for the determination of mineral constituents in honey by inductively coupled plasma optical emission spectrometry. *Journal of the Brazilian Chemical Society* 17(1): 168-176. http://dx.doi.org/10.1590/S0103-50532006000100024

MENG, J; LI, F; LUO, L; WANG, X; XIAO, M (2014) Determination of zinc in acacia honey by square wave stripping voltammetry with a bismuth-film-modified montmorillonite doped carbon paste electrode. *Monatshefte für Chemie* 145(1): 161-166. http://dx.doi.org/10.1007/s00706-013-0976-9

MICHALKIEWICZ, A; BIESAGA, M; PYRZYNSKA, K (2008) Solid-phase extraction procedure for determination of phenolic acids and some flavonols in honey. *Journal of Chromatography A* 1187(1-2): 18-24. http://dx.doi.org/10.1016/j.chroma.2008.02.001

MILLER, D M; RUTZKE, M A (2003) Las espectroscopías de absorción y de emisión atómica. In *Nielsen, S (Ed). Análisis de los alimentos*. Acribia, S. A.; Zaragoza, Spain. pp 470-492.

MONIRUZZAMAN, M; KHALIL, M I; SULAIMAN, S A; GAN, S H (2013) Physicochemical and antioxidant properties Malaysian honeys produced by *Apis cerana, Apis dorsata* and *Apis mellifera*. *BMC Complementary and Alternative Medicine* 13: 43-55. http://dx.doi.org/10.1186/1472-6882-13-43

MONIRUZZAMAN, M; RODRÍGUEZ, I; RAMIL, M; CELA, R; SULAIMAN, S A; GAN, S H (2014) Assessment of gas chromatography time-of-flight accurate mass spectrometry for identification of volatile and semi-volatile compounds in honey. *Talanta* 129: 505-515. http://dx.doi.org/10.1016/j.talanta.2014.06.019

MOREIRA, R F A; DE MARIA, C A B (2005) Investigation of the aroma compounds from headspace and aqueous solution from the cambará (*Gochnatia velutina*) honey. *Flavour and Fragrance Journal* 20(1): 13-17. http://dx.doi.org/10.1002/ffj.1396

MOSSEL, B; BHANDARI, B; D'ARCY, B; AND CAFFIN, N (2000) Use of Arrhenius model to predict rheological behaviour in some Australian honeys. *Lebensmittel-Wissenschaft und-Technologie* 33(8): 545-552. http://dx.doi.org/10.1006/fstl.2000.0714

MOUAZEN, A M; AL-WALAAN, N (2014) Glucose adulteration in Saudi honey with visible and near infrared spectroscopy. *International Journal of Food Properties* 17(10): 2263-2274. http://dx.doi.org/10.1080/10942912.2013.791837

MUÑOZ, E; PALMERO, S (2006) Determination of heavy metals in honey by potentiometric stripping analysis and using a continuous flow methodology. *Food Chemistry* 94(3): 478-483. http://dx.doi.org/10.1016/j.foodchem.2005.01.022

MUTINELLI, F; BAGGIO, A; CAPOLONGO, F; PIRO, R; PRANDIN, L; BIASION, L (1997) A scientific note on oxalic acid by topical application for the control of varroasis. *Apidologie* 28: 461-462.

NAGAI, T; SAKAI, M; INOUE, R; INOUE, H; SUZUKI, N (2001) Antioxidative activities of some commercially honeys, royal jelly, and propolis. *Food Chemistry* 75(2): 237-240. http://dx.doi.org/10.1016/S0308-8146(01)00193-5

NANDA, V; SARKAR, B C; SHARMA, H K; BAWA, A S (2003) Physico-chemical properties and estimation of mineral content in honey produced from different plants in Northern India. *Journal of Food Composition and Analysis* 16(5): 613-619. http://dx.doi.org/10.1016/S0889-1575(03)00062-0

NANOS, C G; KARAYANNIS, M I (1991) Assay of reducing sugars in beverages, wines, honey and marmalades using potentiometric stripping analysis (PSA). *Fresenius' Journal of Analytical Chemistry* 340(4): 253-257. http://dx.doi.org/10.1007/BF00321779

NASUTI, C; GABBIANELLI, R; FLACIONI, G; CANTALAMESSA, F (2006) Antioxidative and gastroprotective activities of anti-inflammatory formulations derived from chestnut honey in rats. *Nutrition Research* 26(3): 130-137. http://dx.doi.org/10.1016/j.nutres.2006.02.007

NAVARRETE, M; CASADO, S; MINELLI, M; SEGURA, A; BONETTI, A; DINELLI, G; FERNÁNDEZ, A (2005) Direct determination of aliphatic acids in honey by coelectroosmotic capillary zone electrophoresis. *Journal of Apicultural Research* 44(2): 65-70. http://dx.doi.org/10.1080/00218839.2005.11101151

NCCLS (2002) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. *National Committee for Clinical Laboratory Standards (Sixth edition)*;Wayne, PA, USA.

NECEMER, M; KOSIR, I J; KUMP, P; KROPF, U; JAMNIK, M; BERTONCELJ, J; OGRINC, N; GOLOB, T (2009) Application of total reflection X-ray spectrometry in combination with chemometric methods for determination of the botanical origin of Slovenian honey. *Journal of Agricultural and Food Chemistry* 57(10): 4409-4414. http://dx.doi.org/10.1021/jf900930b

NEGUERUELA, A I; PÉREZ-ARQUILLUÉ, C (2000) Color measurement of rosemary honey in the solid state by reflectance spectroscopy with black background. *Journal of the AOAC Internationa*l 83(3): 669-674.

NISBET, C; GULER, A; CIFTCI, G; YARIM, G F (2009) The investigation of protein prophile of different botanical origin honey and density saccharose-adultered honey by SDS-Page Method. *Kafkas Univ Vet Fak Derg* 15(3):443-446.

NOOR, N; SARFRAZ, R A; ALI, S; SHAHID, M (2014) Antitumour and antioxidant potential of some selected Pakistani honeys. *Food Chemistry* 143: 362-366. http://dx.doi.org/10.1016/j.foodchem.2013.07.084

NORUMA, T; KIKUCHI, M; KAWAKAMI, Y (1997) Proton-donative antioxidant activity of fucoxanthin with 1,1-diphenyl-2-picrylhydrazyl (DPPH). *Biochemistry and molecular biology international* 42(2): 361-370. http://dx.doi.org/10.1080/15216549700202761

NOZAL, M J; BERNAL, J L; DIEGO, J C; GÓMEZ, L A; HIGES, M (2003a) HPLC determination of low molecular weight organic acids in honey with series-coupled ion-exclusion columns. *Journal of Liquid Chromatography and Related Technologies* 26(8): 1231-1253. http://dx.doi.org/10.1081/JLC-120020107

NOZAL, M J; BERNAL, J L; GÓMEZ, L A; HIGES, M; MEANA, A (2003b) Determination of oxalic acid and other organic acids in honey and in some anatomic structures of bees. *Apidologie* 34(2): 181-188. http://dx.doi.org/10.1051/apido:2003001

NOZAL-NALDA, M J; BERNAL-YAGÜE, J L; DIEGO-CALVA, J C; MARTÍN-GÓMEZ, M T M (2005) Classifying honeys from the Soria Province of Spain via multivariate analysis. *Analytical and Bioanalytical Chemistry* 382(2): 311-319. http://dx.doi.org/10.1007/s00216-005-3161-0

OELSCHLÄGEL, S; ESCHE, R; SPEER, K (2011) Organische Säuren im Honig: Ihre Bedeutung und Analytik. *Deutsche Lebensmittel-Rundschau* 107: 66-70.

OJEC-OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES (2002) Council Directive 2001/110/EC of 20 December 2001 relating to honey.

OROIAN, M; AMARIEI, S; ESCRICHE, I; LEAHU, A; DAMIAN, C; GUTT, G (2014) Chemical composition and temperature influence on the rheological behaviour of honeys. *International Journal of Food Properties* 17(10): 2228-2240. http://dx.doi.org/10.1080/10942912.2013.791835

ORTIZ-VALBUENA, A; FERNÁNDEZ-MAESO, M C; SUBRÁ MUÑOZ DE LA TORRE, E (1996) Principales características de la miel de La Alcarria. *Consejería de Agricultura y medio ambiente de la junta de comunidades de Castilla-La Mancha*; Toledo, España. pp. 26-35.

OSMAN, K A; AL-DOGHAIRI, M A; AL-REHIAYANI, S; HELAL, M I D (2007) Mineral contents and physicochemical properties of natural honey produced in Al-Qassim region, Saudi Arabia. *Journal of Food, Agriculture and Environment* 5(3-4): 142-146.

OUCHEMOUKH, S; SCHWEITZER, P; BACHIR BEY, M; DJOUDAD-KADJI, H; LOUAILECHE, H (2010) HPLC sugar profiles of Algerian honeys. *Food Chemistry* 121(2): 561-568. http://dx.doi.org/10.1016/j.foodchem.2009.12.047

ÖZBALZI, B; HAKKI BOYACI, I; TOPCU, A; KADILAR, C; TAMER, U (2013) Rapid analysis of sugars in honey by processing Raman spectrum using chemometric methods and artificial neural networks. *Food Chemistry* 136(3-4): 1444-1452. http://dx.doi.org/10.1016/j.foodchem.2012.09.064

ÖZCAN, M M; ÖLMEZ, Ç; ARSLAN, D; DURSUN, N (2012) Mineral and heavy metal contents of different honeys produced in Turkey. *Journal of Apicultural Research* 51(4): 353-358. http://dx.doi.org/10.3896/IBRA.1.51.4.10

PAPASTATHOPOULOS, D S; NIKOLELIS, D P; HADJIIOANNOU, T P (1977) Determination of reducing sugars in honey, marmalades and fruit juices using a copper ion-selective electrode. *Analyst* 102(1220): 852-857. http://dx.doi.org/10.1039/an9770200852

PASINI, F; GARDINI, S; MARCAZZAN, G L; CABONI, M F (2013) Buckwheat honeys: screening of composition and properties. *Food Chemistry* 141(3): 2802-2811. http://dx.doi.org/10.1016/j.foodchem.2013.05.102

PASQUINI, B; GOODARZI, M; ORLANDINI, S; BERETTA, G; FURLANETTO, S; DEJAEGHER, B (2014) Geographical characterisation of honeys according to their mineral content and antioxidant activity using a chemometric approach. *International Journal of Food Science and Technology* 49(5): 1351-1359. http://dx.doi.org/10.1111/ijfs.12436

PATACA, L C M; NETO, W B; MARCUCCI, M C; POPPI, R J (2007) Determination of apparent reducing sugars, moisture and acidity in honey by attenuated total reflectance-Fourier transform infrared spectrometry. *Talanta* 71(5): 1926-1931. http://dx.doi.org/10.1016/j.talanta.2006.08.028

PAWLOWSKA, M; ARMSTRONG, D W (1994) Evaluation of enantiomeric purity of selected amino acids in honey. *Chirality* 6(4): 270-276. http://dx.doi.org/10.1002/chir.530060409

PEKAL, A; PYRZYNSKA, K (2014) Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Analytical Methods* 7(9): 1776-1782. http://dx.doi.org/10.1007/s12161-014-9814-x

PELLERANO, R G; UNATES, M A; CANTARELLI, M A; CAMINA J M; MARCHEVSKY, E J (2012) Analysis of trace elements in multifloral Argentine honeys and their classification according to provenance. *Food Chemistry* 134(1): 578-582. http://dx.doi.org/10.1016/j.foodchem.2012.02.125

PÉREZ, C; HERRERA, A (1987) Análisis de aminoacidos proteinicos en mieles de los Monegros. *Alimentaria* 24: 167-172.

PÉREZ, E; RODRÍGUEZ-MALAVER, A J; VIT, P (2006) Antioxidant capacity of Venezuelan honey in Wistar rat homogenates. *Journal of Medicinal Food* 9(4): 510-516. http://dx.doi.org/10.1089/jmf.2006.9.510

PÉREZ-CERRADA, M; HERRERO-VILLEN, M A; MAQUIEIRA, A (1989) Sugar-rich food: determination of inorganic anions by ionic chromatography. *Food Chemistry* 34(4): 285-294. http://dx.doi.org/10.1016/0308-8146(89)90105-2

PÉREZ-MARTÍN, R A; VELA-HORTIGÜELA, L; LORENZO-LOZANO, P; ROJO-CORTINA, M D; DE LORENZO-CARRETERO, C (2008) In vitro antioxidant and antimicrobial activities of Spanish honeys. *International Journal of Food Properties* 11(4): 727-737. http://dx.doi.org/10.1080/10942910701586257

PERNA, A; INTAGLIETTA, I; SIMONETTI, A; GAMBACORTA, E (2013) A comparative study on phenolic profile, vitamin C content and antioxidant activity of Italian honeys of different botanical origin. *International Journal of Food Science and Technology* 48(9): 1899-1908. http://dx.doi.org/10.1111/ijfs.12169

PERSANO-ODDO, L; BALDI, E; ACCORTI, M (1990) Diastatic activity in some unifloral honeys. *Apidologie* 21(1): 17-24. http://dx.doi.org/10.1051/apido:19900103

PERSANO-ODDO, L; PIAZZA, M G; ZELLINI, G (1995) Caratteristiche cromatiche dei mieli uniflorali. *Apicoltura* 10: 109-120.

PERSANO-ODDO, L; PIAZZA, M G; PULCINI, P (1999) Invertase activity in honey. *Apidologie* 30(1): 57-65. http://dx.doi.org/10.1051/apido:19990107

PERSANO-ODDO, L; PULCINI, P (1999) A scientific note on the Phadebas method for honeys with low enzyme content. *Apidologie* 30(4): 347-348. http://dx.doi.org/10.1051/apido:19990411

PERSANO-ODDO, L; PIRO, R (2004) Main European unifloral honeys: descriptive sheets. *Apidologie* 35(Suppl. 1): S38-S81. http://dx.doi.org/10.1051/apido:2004049

PERSANO-ODDO, L; HEARD, T A; RODRÍQUEZ-MALAVER, A; PÉREZ, R A; FERNÁNDEZ-MUIÑO, M A; SANCHO, M T; SESTA, G; LUSCO, L; VIT, P (2008) Composition and antioxidant activity of *Trigona carbonaria* honey from Australia. *Journal of Medicinal Food* 11(4): 789-794. http://dx.doi.org/10.1089/jmf.2007.0724

PETROV, V (1974) Quantitative determination of amino acids in some Australian honeys. *Journal of Apicultural Research* 13(1): 61-66. http://dx.doi.org/10.1080/00218839.1974.11099760

PETRUS, K; SCHWARTZ, H; SONTAG, G (2011) Analysis of flavonoids in honey by HPLC coupled with coulometric electrode array detection and electrospray ionization mass spectrometry. *Analytical and Bioanalytical Chemistry* 400(8): 2555-2563. http://dx.doi.org/10.1007/s00216-010-4614-7

PIASENZOTTO, L; GRACCO, L; CONTE, L (2003) Solid phase microextraction (SPME) applied to honey quality control. *Journal of the Science of Food and Agriculture* 83(10): 1037-1044. http://dx.doi.org/10.1002/jsfa.1502

PIAZZA, M G; ACCORTI, M; PERSANO ODDO, L (1991) Electrical conductivity, ash, colour and specific rotatory power in Italian unifloral honeys. *Apicoltura* 7: 51-63.

PIDDOCK, L J (1990) Techniques used for determination of antimicrobial resistance and sensitivity in bacteria. *Journal of Applied Bacteriology* 68(4): 307-318. http://dx.doi.org/10.1111/j.1365-2672.1990.tb02880.x

PILZ-GÜTHER, D; SPEER, K (2004) Development of a GC method for simultaneous determination of organic acids in honey. *Deutsche Lebensmittel-Rundschau* 100(3): 84-87.

PIRES, J; ESTEVINHO, M L; FEÁS, X; CANTALAPIEDRA, J; IGLESIAS, A (2009) Pollen spectrum and physico-chemical attributes of heather (*Erica* sp.) honeys of north Portugal. *Journal of the Science of Food and Agriculture* 89(11): 1862-1870. http://dx.doi.org/10.1002/jsfa.3663

PIRINI, A; CONTE, L; FRANCIOSO, O; LERCKER, G (1992) Capillary gas chromatographic determination of free amino acids in honey as a means of discrimination between botanical sources. *Journal of High Resolution Chromatography* 15(3): 165-170. http://dx.doi.org/10.1002/jhrc.1240150306

PISANI, A; PROTANO, G; RICCOBONO, F (2008) Minor and trace elements in different honey types produced in Siena County (Italy). *Food Chemistry* 107(4): 1553-1560. http://dx.doi.org/10.1016/j.foodchem.2007.09.029

POHL, P (2009) Determination of metal content in honey by atomic absorption and emission spectrometries. *Trends in Analytical Chemistry* 28(1): 117-128. http://dx.doi.org/10.1016/j.trac.2008.09.015

POHL, P; STECKA, H; SERGIEL, I; JAMROZ, P (2012) Different aspects of the elemental analysis of honey by flame atomic absorption and emission spectrometry: a review. *Food Analytical Methods* 5(4): 737-751. http://dx.doi.org/10.1007/s12161-011-9309-y

POIANA, M; FUDA, S; MANZIU, E; POSTORINO, S; MINCIONE, B (1996) Research on commercial honeys in Italy-mineral fraction. *Industrie Alimentari* 35(348): 522-530.

PONTIS, J A; ALVES DA COSTA, L A M; DA SILVA, S J R; FLACH, A (2014) Color, phenolic and flavonid content, and antioxidant activity of honey from Roraima, Brazil. *Food Science and Technology Campinas* 34(1): 69-73. http://dx.doi.org/10.1590/S0101-20612014005000015

PRIOR, R L; WU, X; SCHAICH, K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry* 53(10): 4290-4302. http://dx.doi.org/10.1021/jf0502698

PROSEN, H.; KOKALJ, M.; DAMJAN, J.; SAMO K. (2010). Comparison of isolation methods for the determination of buckwheat volatile compounds. *Food Chemistry* 121(1): 298-306. http://dx.doi.org/10.1016/j.foodchem.2009.12.014

PRZYBYLOWSKI, P; WILCZYŃSKA, A (2001) Honey as an environmental marker. *Food Chemistry* 74(3): 289-291. http://dx.doi.org/10.1016/S0308-8146(01)00153-4

PULCINI, P; PIAZZA, M G; ALLEGRINI, F (2004) Total gluconic acid (AGT) content in Italian unifloral honeys. *Industrie Alimentari* 43: 263-268.

PYRZYNSKA, K; BIESAGA, M (2009) Analysis of phenolic acids and flavonoids in honey. *Trends in Analytical Chemistry* 28(7): 893-902. http://dx.doi.org/10.1016/j.trac.2009.03.015

QIU, P Y; DING, H B; TANG, Y K; XU, R J (1999) Determination of chemical composition of commercial honey by near-infrared spectroscopy. *Journal of Agricultural and Food Chemistry* 47(7): 2760-2765. http://dx.doi.org/10.1021/jf9811368

RADOVIC B., GOODACRE R., ANKLAM E. (2001a) Contribution of pyrolysis-mass spectrometry (Py-MS) to authenticity testing of honey. *Journal of Analytical and Applied Pyrolysis* 60(1): 79-87. http://dx.doi.org/10.1016/S0165-2370(00)00163-7

RADOVIC, B S; WHITE, R; PARKER, I; DENNIS, M J; SHARMAN, M; GEISS, H; ANKLAM, E (2001b) Contribution of high temperature gas chromatographic analysis of oligosaccharides and ion chromatographic analysis of various cations and anions to authenticity testing of honey. *Deutsche Lebensmittel-Rundschau* 97(10): 380-384.

RIBEIRO, R O R; MÁRSICO, E T; CARNEIRO, C S; MONTEIRO, M L G; CONTE-JÚNIOR, C A; MANO, S; DE JESÚS, E F O (2014) Classification of Brazilian honeys by physical and chemical analytical methods and low field nuclear magnetic resonance (LF ^{1}H NMR). *LWT - Food Science and Technology* 55(1): 90-95. http://dx.doi.org/10.1016/j.lwt.2013.08.004

RIZELIO, V M; GONZAGA, L V; BORGES, G S C; MALTEZ, H F; COSTA, A C O; FETT, R (2012a) Fast determination of cations in honey by capillary electrophoresis: A possible method for geographic origin discrimination. *Talanta* 99: 450-456. http://dx.doi.org/10.1016/j.talanta.2012.06.009

RIZELIO, V M; GONZAGA, L V; DA SILVA CAMPELO BORGES, G; MICKE, G A; FETT, R; COSTA, A C O (2012b) Development of a fast MECK method for determination of 5-HMF in honey samples. *Food Chemistry* 133(4): 1640-1645. http://dx.doi.org/10.1016/j.foodchem.2011.11.058

RODRÍGUEZ, B A; MENDOZA, S; ITURRIGA, M H; CASTAÑO-TOSTADO, E (2012). Quality parameters and antioxidant and antibacterial properties of some Mexican honeys. *Journal of Food Science* 71(1): C121-C127. http://dx.doi.org/10.1111/j.1750-3841.2011.02487.x

RODRÍGUEZ-FLORES, M S; ESCUREDO-PÉREZ, O; SEIJO-COELLO, M (2014) Characterization of *Eucalyptus Globulus* honeys produced in the Eurosiberian Area of the Iberian Peninsula. *International Journal of Food Properties* 17(10): 2177-2191. http://dx.doi.org/10.1080/10942912.2013.790050

RODRÍGUEZ-FLORES, M S; ESCUREDO, O; SEIJO, M (2015) Assessment of physicochemical and antioxidant characteristics of *Quercus pyrenaica* honeydew honeys. *Food Chemistry* 166: 101-106. http://dx.doi.org/10.1016/j.foodchem.2014.06.005

RODRÍGUEZ-OTERO, J L; PASEIRO, P; SIMAL, J; TERRADILLOS, L; CEPEDA, A (1992) Determination of Na, K, Ca, Mg, Cu, Fe, Mn and total cationic milliequivalents in Spanish commercial honeys. *Journal of Apicultural Research* 31(2): 65-69. http://dx.doi.org/10.1080/00218839.1992.11101264

ROGERS, K M; SIM, M; STEWART, S; PHILLIPS, A; COOPER, J; DOUANCE, C; PYNE, R; ROGERS, P (2014) Investigating C-4 sugar contamination of manuka honey and other New Zealand honey varieties using carbon isotopes. *Journal of Agriculture and Food Chemistry* 62(12): 2605-2614. http://dx.doi.org/10.1021/jf404766f

ROSA, A; TUBEROSO, C I G; ATZERI, A; MELIS, M P; BIFULCO, E; DESSÌ, M A (2011) Antioxidant profile of strawberry tree honey and its marker homogentisic acid in several models of oxidative stress. *Food Chemistry* 129(3): 1045-1053. http://dx.doi.org/10.1016/j.foodchem.2011.05.072

ROWLAND, C Y; BLACKMAN, A J; D´ARCY, B R; RINTOUL, G B (1995) Comparison of organic extractives found in leatherwood (*Eucryphia lucida*) honey and leatherwood flowers and leaves. *Journal of Agricultural Food Chemistry* 43(3): 753-763. http://dx.doi.org/10.1021/jf00051a036

RUIZ-MATUTE, A I; BROKL, M; SORIA, A C; SANZ, M L; MARTÍNEZ-CASTRO, I (2010) Gas chromatographic-mass spectrometric characterisation of tri- and tetrasaccharides in honey. *Food Chemistry* 120(2): 637-642. http://dx.doi.org/10.1016/j.foodchem.2009.10.050

RUOFF, K; IGLESIAS, M T; LUGINBÜHL, W; BOSSET, J O; BOGDANOV, S; AMADÒ, R (2006a) Quantitative analysis of physical and chemical measurands in honey by mid-infrared spectrometry. *European Food Research and Technology* 223(1): 22-29. http://dx.doi.org/10.1007/s00217-005-0085-z

RUOFF, K; LUGINBÜHL, W; BOGDANOV, S; BOSSET, J O; ESTERMANN, B; ZIOLKO, T; AMADÒ, R (2006b) Authentication of the botanical origin of honey by near-infrared spectroscopy. *Journal of Agricultural and Food Chemistry* 54(18): 6867-6872. http://dx.doi.org/10.1021/jf060770f

RUOFF, K; LUGINBÜHL, W; KUNZLI, R; IGLESIAS, M T; BOGDANOV, S; BOSSET, J O; VON DER OHE, K; VON DER OHE, W; AMADÒ, R (2006c) Authentication of the botanical and geographical origin of honey by mid-infrared spectroscopy. *Journal of Agricultural and Food Chemistry* 54(18): 6873-6880. http://dx.doi.org/10.1021/jf060838r

RUOFF, K; LUGINBÜHL, W; BOGDANOV, S; BOSSET, J O; ESTERMANN, B; ZIOLKO, T; KHERADMANDAN, S; AMADÒ, R (2007) Quantitative determination of physical and chemical measurands in honey by near-infrared spectrometry. *European Food Research and Technology* 225(3-4): 415-423. http://dx.doi.org/10.1007/s00217-006-0432-8

SABATINI, A G; MARCAZZAN, G L; COLOMBO, R; GARAGNANI, M (1994) Applicazione di un metodo enzimatico per la determinazione dell'acido formico e dell'acido lattico presenti nel miele. *Apicoltura* 9: 135-145.

SAKAČ, N; SAK-BOSNAR, M (2012) A rapid method for the determination of honey diastase activity. *Talanta* 93: 135-138. http://dx.doi.org/10.1016/j.talanta.2012.01.063

SAK-BOSNAR, M; SAKAČ, N (2012) Direct potentiometric determination of diastase activity in honey. *Food Chemistry* 135(2): 827-831. http://dx.doi.org/10.1016/j.foodchem.2012.05.006

SALASHINSKI, N A; BAZHENOVA, L S (1979) Determination of the activity of glucose oxidase of natural honeys. *Izvestiya Vysshikh Uchebnykh Zavedenii Pishchevaya Tekhnologiya* 6: 106-107.

SALINAS, F; ESPINOSA-MANSILLA, A; BERZAS-NEVADO, J J (1991) Flow-injection determination of HMF in honey by the Winkler method. *Fresenius' Journal of Analytical Chemistry* 340(4): 250-252. http://dx.doi.org/10.1007/BF00321778

SÁNCHEZ, M P; HUIDOBRO, J F; MUNIATEGUI, S; SANCHO, M T (2005) Evolution of acid phosphatase activity during the storage of honey. *Deutsche Lebensmittel-Rundschau* 101: 9-15.

SANCHO, M T; MUNIATEGUI, S; HUIDOBRO, J F; SIMAL, J (1991a) Correlation between the electrical conductivity of honey in humid and in dry matter. *Apidologie* 22(3): 221-227. http://dx.doi.org/10.1051/apido:19910306

SANCHO, M T; MUNIATEGUI, S; HUIDOBRO, J F; SIMAL, J (1991b) Mieles del País Vasco. II: Indices de formol y prolina. *Anales de Bromatología* XLIII-1: 87-99.

SANCHO, M T; MUNIATEGUI, S; SÁNCHEZ, M P; HUIDOBRO, J F; SIMAL, J (1991c) Relationships between electrical conductivity and total and sulphated ash contents in Basque honeys. *Apidologie* 22(5): 487-494. http://dx.doi.org/10.1051/apido:19910501

SANCHO, M T; MUNIATEGUI, S; SÁNCHEZ, M P; HUIDOBRO, J F; SIMAL-LOZANO, J (1992) Evaluating soluble and insoluble ash, alkalinity of soluble and insoluble ash and total alkalinity of ash in honey using electrical conductivity measurements at 20 ^{o}C. *Apidologie* 23(4): 291-297. http://dx.doi.org/10.1051/apido:19920403

SANCHO, M T; MATO, I; HUIDOBRO, J F; FERNANDEZ-MUIÑO, M A; PASCUAL-MATÉ, A (2013) Non-aromatic organic acids of honeys. In *Vit, P; Pedro, S R M; Roubik, D (Eds). Pot honey*: A *legacy of stingless bees*. Springer; Berlín, Germany. pp. 447-458.

SANCHO, M T; PASCUAL-MATÉ, A; RODRÍGUEZ-MORALES, E G; OSÉS, S M; ESCRICHE, I; PERICHE, A; FERNÁNDEZ-MUIÑO, M A (2016) Critical assessment of antioxidant-related parameters of honey. *International Journal of Food Science and Technology* 51(1): 30-36. http://dx.doi.org/10.1111/ijfs.12988

SANNA, G; PILO, M I; PIU, P C; TAPPARO, A; SEEBER, R (2000) Determination of heavy metals in honey by anodic stripping voltammetry at microelectrodes. *Analytica Chimica Acta* 415(1): 165-173. http://dx.doi.org/10.1016/S0003-2670(00)00864-3

SANT'ANA, L D; SOUSA, J P L M; SALGUEIRO, F B; LORENZON, M C A; CASTRO, R N (2012) Characterization of monofloral honeys with multivariate analysis of their chemical profile and antioxidant activity. *Journal of Food Science* 77(1): C135-C140. http://dx.doi.org/10.1111/j.1750-3841.2011.02490.x

SANT'ANA, L D; BUARQUE FERREIRA, A B; LORENZON, M C A; BERBARA, R L L; CASTRO, R N (2014) Correlation of total phenolic and flavonoid contents of Brazilian honeys with colour and antioxidant capacity. *International Journal of Food Properties* 17(1): 65-76. http://dx.doi.org/10.1080/10942912.2011.614368

SANZ, M L; GONZÁLEZ, M; DE LORENZO, C; SANZ, J; MARTÍNEZ-CASTRO, I (2004) Carbohydrate composition and physico chemical properties of artisanal honeys from Madrid (Spain): occurrence of *Echium* sp. honey. *Journal of the Science of Food and Agriculture* 84(12): 1577-1584. http://dx.doi.org/10.1002/jsfa.1823

SANZ, M L; GONZÁLEZ, M; DE LORENZO, C; SANZ, J; MARTÍNEZ-CASTRO, I (2005) A contribution to the differentiation between nectar honey and honeydew honey. *Food Chemistry* 91(2): 313-317. http://dx.doi.org/10.1016/j.foodchem.2004.06.013

SARKER, S D; NAHAR, L (2014) Modern methods of analysis applied to honey. In *Boudraâ, L (Ed)*. *Honey in Traditional and Modern Medicine.* CRC Press Taylor & Francis Group; Boca Ratón, Florida, USA. pp. 333-358.

SAXENA, S; GAUTAM, S; SHARMA, A (2010) Physical, biochemical and antioxidant properties of some Indian honeys. *Food Chemistry* 118(2): 391-397. http://dx.doi.org/10.1016/j.foodchem.2009.05.001

SCHADE, J E; MARSH, G L; ECKERT, J E (1958) Diastase activity and hydroxymethylfurfural in honey and their usefulness in detecting and heat adulteration. *Food Research* 23(5): 446-463. http://dx.doi.org/10.1111/j.1365-2621.1958.tb17592.x

SCHEPARTZ, A I; SUBERS, M H (1964) The glucose oxidase of honey. I. Purification and some general properties of the enzyme. *Biochimica et Biophysica Acta* 85(2): 228-337. http://dx.doi.org/10.1016/0926-6569(64)90243-3

SCHEPARTZ, A I; SUBERS, M H (1966) Catalase in honey. *Journal of Apicultural Research* 5(1): 37-43. http://dx.doi.org/10.1080/00218839.1966.11100130

SCHIEVANO, E; PEGGION, E; MAMMI, S (2010) H-1 Nuclear magnetic resonance spectra of chloroform extracts of honey for chemometric determination of its botanical origin. *Journal of Agricultural and Food Chemistry* 58(1): 57-65. http://dx.doi.org/10.1021/jf9022977

SCHIEVANO, E; STOCCHERO, M; MORELATO, E; FACCHIN, C; MAMMI, S (2012) An NMR-based metabolomic approach to identify the botanical origin of honey. *Metabolomics* 8(4) 679-690. http://dx.doi.org/10.1007/s11306-011-0362-8

SCHRAMM, D D; KARIM, M; SCHRADER, H R; HOLT, R R; CARDETTI, M; KEEN, C L (2003) Honey with high levels of antioxidants can provide protection to healthy human subjects. *Journal of Agricultural and Food Chemistry* 51(6): 1732-1735. http://dx.doi.org/10.1021/jf025928k

SEIF, A R M; ELFADIL, E B (2010) Identification of the floral origin of honey by amino acids composition. *Australian Journal Basic Applied Sciences* 4(4): 552-556.

SENGUL, M; ERTUGAY, F M; SENGUL, M (2005) Rheological, physical and chemical characteristics of mulberry pekmez. *Food Control* 16(1): 73-76. http://dx.doi.org/10.1016/j.foodcont.2003.11.010

SENYUVA, H Z; GILBERT, J; SILICI, S; CHARLTON, A; DAL, C; GÜREL, N; CIMEN, D (2009) Profiling Turkish honeys to determine authenticity using physical and chemical characteristics. *Journal of Agricultural and Food Chemistry* 57(9): 3911-3919. http://dx.doi.org/10.1021/jf900039s

SEREM, J C; BESTER, M J (2012) Physicochemical properties, antioxidant activity and cellular protective effects of honeys from southern Africa. *Food Chemistry* 133(4): 1544-1550. http://dx.doi.org/10.1016/j.foodchem.2012.02.047

SERGIEL, I; POHL, P; BIESAGA, M (2014) Characterization of honeys according to their content of phenolic compounds using high performance liquid chromatography/tandem mass spectrometry. *Food Chemistry* 145: 404-408. http://dx.doi.org/10.1016/j.foodchem.2013.08.068

SERRA-BONVEHÍ, J; VENTURA-COLL, F (2003) Flavour index and aroma profiles of fresh and processed honeys. *Journal of the Science of Food and Agriculture* 83(4): 275-282. http://dx.doi.org/10.1002/jsfa.1308

SERRA-BONVEHÍ, J; BENTABOL-MANZANARES, A; SANTOS-VILAR, J M (2004) Quality evaluation of broom honey (*Spartocytisus supranubius* L) produced in Tenerife (The Canary Islands). *Journal of the Science of Food and Agriculture* 84(10): 1097-1104. http://dx.doi.org/10.1002/jsfa.1792

SERRANO, S; RODRÍGUEZ, I; RINCÓN, F (2012) Optimization of polarimetric method for specific rotation determination in honey. Book of Abstracts of the II International Symposium on Bee Products: 41. http://ipb.pt/ihc2012/imagens/itf292.pdf

SGARIGLIA, M A; VATTUONE, M A; SAMPIETRO-VATTUONE, M M; SOBERÓN, J R; SAMPIETRO, D A (2010) Properties of honey from *Tetragonisca angustula fiebrigi* and *Plebeia wittmanni* of Argentina. *Apidologie* 41(6): 667-675. http://dx.doi.org/10.1051/apido/2010028

SHAO, Y N; HE, Y; BAO, Y D (2008) Application of visible/near infrared spectroscopy to discriminating honey brands based on independent component analysis and BP neural network. *Guang Pu Xue Yu Guang Pu Fen Xi/Spectroscopy and Spectral Analysis* 28: 602-605.

SHERLOCK, O; DOLAN, A; ATHMAN, R POWWER, A; GETHIN, G; COWMAN, S; HUMPHREYS, H (2010) Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. *BMC Complementary and Alternative Medicine* 10: 47. http://dx.doi.org/10.1186/1472-6882-10-47

SHI, M; GAO, Q; FENG, J; LU, Y (2012) Analysis of inorganic cations in honey by capillary zone electrophoresis with indirect UV detection. *Journal of Chromatographic Science* 50(6): 547-552. http://dx.doi.org/10.1093/chromsci/bms032

SIEGENTHALER, U (1975) Bestimmung der α-Amylase im Bienenhonig mit einem Handelsublichen, Farbmarkierten Substrat. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene* 66(4): 393-399.

SIEGENTHALER, U (1977) Eine einfache und rasche Methode zur Bestimmung der β-Glucosidase (Sacharase) in Honig. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene* 68: 251-258.

SILICI, S; SAGDIC, O; EKICI, L (2010) Total phenolic content, antiradical, antioxidant and antimicrobial activities of Rhododendron honeys. *Food Chemistry* 121(1): 238-243. http://dx.doi.org/10.1016/j.foodchem.2009.11.078

SILICI, S; SARIOGLU, K; KARAMAN, K (2013) Determination of polyphenols of some Turkish honeydew and nectar honeys using HPLC-DAD. *Journal of Liquid Chromatography & Related Technologies* 36(16): 2330-2341. http://dx.doi.org/10.1080/10826076.2012.720332

SILICI, S; SARIOGLU, K; DOGAN, M; KARAMAN, K (2014) HPLC-DAD analysis to identify the phenolic profile of rhododendron honeys collected from different regions in Turkey. *International Journal of Food Properties* 17(5): 1126-1135. http://dx.doi.org/10.1080/10942912.2012.698441

SILVA, L R; VIDEIRA, R; MONTEIRO, A P; VALENTÃO, P; ANDRADE, P B (2009) Honey from Luso región (Portugal): Physicochemical characteristics and mineral contents. *Microchemical Journal* 93(1): 73-77. http://dx.doi.org/10.1016/j.microc.2009.05.005

SILVA, I A A D; SILVA, T M S D; CAMARA, C A; QUEIROZ, N; MAGNANI, N; NOVAIS, J S D; SOLEDADE, L E B; LIMA, E D O; SOUZA, A L D; SOUZA, A G D (2013a) Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas, Northern Brazil. *Food Chemistry* 141(4): 3552-3558. http://dx.doi.org/10.1016/j.foodchem.2013.06.072

SILVA, T M S; DOS SANTOS, F P; EVANGELISTA-RODRIGUES, A; SILVA, E M S; SILVA, G S; SANTOS DE NOVAIS, J; DOS SANTOS; F A R; CAMARA, C A (2013b) Phenolic compounds, melissopalynological, physicochemical analysis and antioxidant activity of jandaíra (*Melipona subnitida*) honey. *Journal of Food Composition and Analysis* 29(1): 10-18. http://dx.doi.org/10.1016/j.jfca.2012.08.010

SILVANO, M F; VARELA, M S; PALACIO, M A; RUFFINENGO, S; YAMUL, D K (2014) Physicochemical parameters and sensory properties of honeys from Buenos Aires region. *Food Chemistry* 152: 500-507. http://dx.doi.org/10.1016/j.foodchem.2013.12.011

SIMAL, J; HUIDOBRO, J F (1984) Parámetros de calidad de la miel: Acidez (pH, libre, lactónica y total) e índice de formol. *Offarm* 3: 523-532.

SIMOVA, S; ATANASSOV, A; SHISHINIOVA, M; BANKOVA, V (2012) A rapid differentiation between oak honeydew honey and nectar and other honeydew honeys by NMR spectroscopy. *Food Chemistry* 134(3): 1706-1710. http://dx.doi.org/10.1016/j.foodchem.2012.03.071

SINGLETON, V L; ORTHOFER, R; LAMUELA-RAVENTOS, R M (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Methods in Enzymology* 299: 152-178. http://dx.doi.org/10.1016/S0076-6879(99)99017-1

SIVAKESAVA, S; IRUDAYARAJ, J (2001a) A rapid spectroscopic technique for determining honey adulteration with corn syrup. *Journal of Food Science* 66(6): 787-792. http://dx.doi.org/10.1111/j.1365-2621.2001.tb15173.x

SIVAKESAVA, S; IRUDAYARAJ, J (2001b) Prediction of inverted cane sugar adulteration of honey by Fourier transform infrared spectroscopy. *Journal of Food Science* 66(7): 972-978. http://dx.doi.org/10.1111/j.1365-2621.2001.tb08221.x

SIVAKESAVA, S; IRUDAYARAJ, J (2002) Classification of simple and complex sugar adulterants in honey by mid-infrared spectroscopy. *International Journal of Food Science and Technology* 37(4): 351-360. http://dx.doi.org/10.1046/j.1365-2621.2002.00573.x

SOCHA, R; JUSZCZAK, L; PIETRZYK, S; FORTUNA, T (2009) Antioxidant activity and phenolic composition of herbhoneys. *Food Chemistry* 113(2): 568-574. http://dx.doi.org/10.1016/j.foodchem.2008.08.029

SORIA, A C; MARTÍNEZ-CASTRO, I; SANZ, J (2008) Some aspects of dynamic headspace analysis of volatile components in honey. *Food Research International* 41: 838-848. http://dx.doi.org/10.1016/j.foodres.2008.07.010

SOUSA, M E B C; DIAS, L G; VELOSO, A C A; ESTEVINHO, L; PERES, A M; MACHADO, A A S C (2014) Practical procedure for discriminating monofloral honey with abroad pollen profile variability using an electronic tongue. *Talanta* 128: 284-292. http://dx.doi.org/10.1016/j.talanta.2014.05.004

SPANO, N; CIULU, M; FLORIS, I; PANZANELLI, A; PILO, M I; PIU, P C; SALIS, S; SANNA, G (2009) A direct RP-HPLC method for the determination of furanic aldehydes and acids in honey. *Talanta* 78(1): 310-314. http://dx.doi.org/10.1016/j.talanta.2008.11.015

SPEER, K; MONTAG, A (1986) Verteilung freier Aminosäuren in Honigen. *Deutsche Lebensmittel-Rundschau* 82: 248-253.

SPILIOTI, E; JAAKKOLA, M; TOLONEN, T; LIPPONEN, M; VIRTANEN, V; CHINOU, I; KASSI, E; KARAVOURNIOTI, S; MOUTSATSOU, P (2014) Phenolic acid composition, antiatherogenic and anticancer potential of honeys derived from various regions in Greece. *PLoS ONE* 9(4): e94860. http://dx.doi.org/10.1371/journal.pone.0094860

ŠTAJNER, D; POPOVIĆ, B M; ČANADANOVIČ-BRUNET, J; ĐILAS, S; ĆETKOVIĆ, G (2014) Nutritive composition and free radical scavenger activity of honey enriched with of *Rosa spp*. *LWT-Food Science and Technology* 55(1): 408-413. http://dx.doi.org/10.1016/j.lwt.2013.08.025

STANIMIROVA, I; ÜSTÜN, B; CAJKA, T; RIDDELOVA, K; HAJSLOVA, J; BUYDENS, L M C; WALCZAK, B (2010) Tracing the geographical origin of honeys based on volatile compounds profiles assessment using pattern recognition techniques. *Food Chemistry* 118(1): 171-176. http://dx.doi.org/10.1016/j.foodchem.2009.04.079

STINSON, E E; SUBERS, M H; PETTY, J; WHITE, J W JR (1960) The composition of honey. V. Separation and identification of the organic acids. *Archives of Biochemistry and Biophysics* 89(1): 6-12. http://dx.doi.org/10.1016/0003-9861(60)90003-5

STOYA, W; WACHENDOERFER, G; KARY, I; SIEBENTRITT, P; KAISER, E (1986) Formic acid as a therapeutic against varroatose and its effect on honey. *Deutsche Lebensmittel-Rundschau* 82: 217-221.

STOYA, W; WACHENDOERFER, G; KARY, I; SIEBENTRITT, P; KAISER, E (1987) Milchsäure als Therapeutikum gegen Varroatose und ihre Auswirkung auf den Honig. *Deutsche Lebensmittel-Rundschau* 83: 283-286.

SUÁREZ-LUQUE, S; MATO, I; HUIDOBRO, J F; SIMAL-LOZANO, J (2002a) Solid-phase extraction procedure to remove organic acids from honey. *Journal of Chromatography B* 770(1): 77-82. http://dx.doi.org/10.1016/S1570-0232(01)00583-9

SUÁREZ-LUQUE, S; MATO, I; HUIDOBRO, J F; SIMAL-LOZANO, J; SANCHO, M T (2002b) Rapid determination of minority organic acids in honey by high-performance liquid chromatography. *Journal of Chromatography A* 955(2): 207-214. http://dx.doi.org/10.1016/S0021-9673(02)00248-0

SUÁREZ-LUQUE, S; MATO, I; HUIDOBRO, J F; SIMAL-LOZANO, J (2005) Capillary zone electrophoresis method for the simultaneous determination of cations in honey. *Journal of Chormatography A* 1083(1-2): 193-198. http://dx.doi.org/10.1016/j.chroma.2005.06.011

SUÁREZ-LUQUE, S; MATO, I; HUIDOBRO, J F; SIMAL-LOZANO, J; SANCHO, M T (2006) Capillary zone electrophoresis method for the determination of inorganic anions and formic acid in honey. *Journal of Agricultural and Food Chemistry* 54(25): 9292-9296. http://dx.doi.org/10.1021/jf061536s

SULTANBAWA, Y; COZZOLINO, D; FULLER, S; CUSACK, A; CURRIE, M; SMYTH, H (2015) Infrared spectroscopy as a rapid tool to detect methylglyoxal and antibacterial activity in Australian honeys. *Food Chemistry* 172: 207-212. http://dx.doi.org/10.1016/j.foodchem.2014.09.067

SZCZESNA, T; RYBAK-CHMIELEWSKA, H (2004) The temperature correction factor for electrical conductivity of honey. *Journal of Apicultural Science* 48(2): 97-102. http://www.jas.org.pl/pdf/62?filename=jas_48_2_2004_11.pdf

TABART, J; KEVERS, C; PINCEMAIL, J; DEFRAIGNE, J O; DOMMES, J (2009) Comparative antioxidant capacities of phenolic compounds measured by various tests. *Food Chemistry* 113(4): 1226-1233. http://dx.doi.org/10.1016/j.foodchem.2008.08.013

TALPAY, B (1988) Inhaltsstoffe des Honigs-Citronensäure (Citrat). *Deutsche Lebensmittel-Rundschau* 84: 41-44.

TALPAY, B (1989) Inhaltsstoffe des Honigs-Ameisensäure (Formiat). *Deutsche Lebensmittel-Rundschau* 85: 143-147.

TANANAKI, C; THRASYVOULOU, A; GIRAUDEL, J L; MONTURY, M (2007) Determination of volatile characteristics of Greek and Turkish pine honey samples and their classification by using Kohonen self-organising maps. *Food Chemistry* 101(4): 1687-1693. http://dx.doi.org/10.1016/j.foodchem.2006.04.042

TEIXIDÓ, E; SANTOS, F J; PUIGNOU, L; GALCERAN, M T (2006) Analysis of 5-hydroxymethylfurfural in foods by gas chromatography-mass spectrometry. *Journal of Chromatography A* 1135(1): 85-90. http://dx.doi.org/10.1016/j.chroma.2006.09.023

TEIXIDÓ, E; NÚÑEZ, O; SANTOS, F J; GALCERAN, M T (2011) 5-Hydroxymethylfurfural content in foodstuffs determined by micellar electrokinetic chromatography. *Food Chemistry* 126(4): 1902-1908. http://dx.doi.org/10.1016/j.foodchem.2010.12.016

TEROL, A; PRATS, S; MAESTRE, S; TOLODÍ, J L (2012) Determination of maltose in food samples by high-temperature liquid chromatography coupled by ICP-AES. In *Preedy, V R (Ed). Dietary sugars: chemistry, analysis, function and effects*. The Royal Society of Chemistry; Cambridge, UK. pp. 425-442.

TERRAB, A; VEGA-PÉREZ, J M; DÍEZ, M J; HEREDIA, F J (2002) Characterization of northwest Moroccan honeys by gas chromatographic-mass spectrometric analysis of their sugar components. *Journal of the Science of Food and Agriculture* 82(2): 179-185. http://dx.doi.org/10.1002/JSFA.1011

TERRAB, A; GONÁLEZ-MIRET, L; HEREDIA, F J (2004) Colour characterisation of thyme and avocado honeys by diffuse reflectance spectrophotometry and spectroradiometry. *European Food Research and Technology* 218(5): 488-492. http://dx.doi.org/10.1007/s00217-004-0890-9

TERRAB, A; HEREDIA, F J (2004) Characterization of avocado (*Persea Americana* Mill) honeys by their physicochemical characteristics. *Journal of the Science of Food and Agriculture* 84(13): 1801-1805. http://dx.doi.org/10.1002/jsfa.1888

TEWARI, J; IRUDAYARAJ, J (2004) Quantification of saccharides in multiple floral honeys using Fourier transform infrared microattenuated total reflectance spectroscopy. *Journal of Agricultural and Food Chemistry* 52(11): 3237-3243. http://dx.doi.org/10.1021/jf035176

TEWARI, J; IRUDAYARAJ, J (2005) Floral classification of honey using mid-infrared spectroscopy and surface acoustic wave based z-Nose sensor. *Journal of Agricultural and Food Chemistry* 53(18): 6955-6966. http://dx.doi.org/10.1021/jf050139z

TEZCAN, F; KOLAYLI, S; SAHIN, H; ULUSOY, E; ERIM, B F (2011) Evaluation of organic acid, saccharide composition and antioxidant properties of some authentic Turkish honeys. *Journal of Food Nutrition Research* 50(1): 33-40.

TIWARI, K; TUDU, B; BANDYOPADHYAY, R; CHATTERJEE, A (2013) Identification of monofloral honey using voltammetric electronic tongue. *Journal of Food Engineering* 117(2): 205-210. http://dx.doi.org/10.1016/j.jfoodeng.2013.02.023

TOMÁS-BARBERÁN, F A; MARTOS, I; FERRERES, F; RADOVIC, B S; ANKLAM, E (2001) HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *Journal of the Science of Food and Agriculture* 81(5): 485-496. http://dx.doi.org/10.1002/jsfa.836

TOURN, M L; LOMBARD, A; BELLIARDO, F; BUFFA, M (1980) Quantitative analysis of carbohydrates and organic acids in honeydew, honey and royal jelly by enzymatic methods. *Journal of Apicultural Research* 19(2): 144-146. http://dx.doi.org/10.1080/00218839.1980.11100013

TRAUTVETTER, S; KOELLING-SPEER, I; SPEER, K (2009) Confirmation of phenolic acids and flavonoids in honeys by UPLC-MS. *Apidologie* 40(2): 140-150. http://dx.doi.org/10.1051/apido/2008072

TRÁVNÍČEK, P; VITEZ, T; PRIDAL, A (2012) Rheological properties of honey. *Scientia Agriculturae Bohemica* 43(4): 160-165. http://dx.doi.org/10.7160/sab.2012.430406

TRUZZI, C; ANNIBALDI, A; ILLUMINATI, S; FINALE, C; ROSSETTI, M; SCARPONI, G (2012) Determination of Very Low Levels of 5-(Hydroxymethyl)-2-furaldehyde (HMF) in Natural Honey: Comparison Between the HPLC Technique and the Spectrophotometric White Method. *Journal of Food Science* 77(7): C784-C790. http://dx.doi.org/10.1111/j.1750-3841.2012.02782.x

TRUZZI, C; ANNIBALDI, A; ILLUMINATI, S; FINALE, C; SCARPONI, G (2014) Determination of proline in honey: Comparison between official methods, optimization and validation of the analytical methodology. *Food Chemistry* 150: 477-481. http://dx.doi.org/10.1016/j.foodchem.2013.11.003

TU, Z H; JI, B P; MENG, C Y; ZHU, D Z; WANG, L G; QUING, Z S (2009) Possibilities of near-infrared spectroscopy for the assessment of principle components in honey. *Guang Pu Xue Yu Guang Pu Fen Xi/Spectroscopy and Spectral Analysis* 29(12): 3291-3294. http://dx.doi.org/10.3964/j.issn.1000-0593(2009)12-3291-04

TU, Z; ZHU, D; JI, B; CHEN, H; QUING, Z (2011) Adulteration detection of honey based on near-infrared spectroscopy. *Nongye Gongcheng Xuebao/Transactions of the Chinese Society of Agricultural Engineering* 27(11): 382-387. http://dx.doi.org/10.3969/j.issn.1002-6819.2011.11.071

TUBEROSO, C I G; JERKOVIC, I; BIFULCO, E; MARIJANOVIC, Z; CONGIU, F; BUBALO, D (2012) Riboflavin and lumichrome in Dalmatian sage honey and other unifloral honeys determined by LC-DAD technique. *Food Chemistry* 135(3): 1985-1990. http://dx.doi.org/10.1016/j.foodchem.2012.06.096

TUBEROSO, C I G; BOBAN, M; BIFULCO, E; BUDIMIR, D; PIRISI, F M (2013) Antioxidant capacity and vasodilatory properties of Mediterranean food: the case of Cannonau wine, myrtle berries liqueur and strawberry-tree honey. *Food Chemistry* 140(4): 686-691. http://dx.doi.org/10.1016/j.foodchem.2012.09.071

TUZEN, M; SILICI, C; MENDIL, D; SOYLAK, M (2007) Trace element levels in honeys from different regions of Turkey. *Food Chemistry* 103(2): 325-330. http://dx.doi.org/10.1016/j.foodchem.2006.07.053

ULUSOY, E; KOLAYLI, S; SARIKAYA, A O (2010) Antioxidant and antimicrobial activity of different floral origin honeys from Turkiye. *Journal of Food Biochemistry*, 34(Suppl. 1): 321-335. http://dx.doi.org/10.1111/j.1745-4514.2009.00332.x

VERZERA, A; CONDURSO, C (2012) Sampling Techniques for the Determination of the Volatile Fraction of Honey. In *Pawliszyn, J (Ed). Comprehensive sampling and sample preparation: analytical techniques for scientists. Vol. 4: Extraction techniques and applications: food and beverage. Food constituents: volatiles.* Elsevier; Amsterdam, Netherlands. pp. 87-117.

VIÑAS, P; BALSALOBRE, N; LÓPEZ-ERROZ, C; HERNÁNDEZ-CÓRDOBA, M (2004a) Liquid chromatographic analysis of riboflavin vitamers in foods using fluorescence detection. *Journal of Agricultural and Food Chemistry* 52(7): 1789-1794. http://dx.doi.org/10.1021/jf030756s

VIÑAS, P; BALSALOBRE, N; LÓPEZ-ERROZ, C; HERNÁNDEZ-CÓRDOBA, M (2004b) Determination of vitamin B6 compounds in foods using liquid chromatography with post-column derivatization fluorescence detection. *Chromatographia* 59(5-6): 381-386. http://dx.doi.org/10.1365/s10337-003-0173-9

VIÑAS, P; LÓPEZ-GARCÍA, I; BRAVO-BRAVO, M; BRICEÑO, M; HERNÁDEZ-CÓRDOBA, M (2012) Dispersive liquid-liquid microextraction coupled to liquid chromatography for thiamine determination in foods. *Analytical and Bioanalytical Chemistry* 403(4): 1059-1066. http://dx.doi.org/10.1007/s00216-012-5804-2

VIT, P; RODRÍGUEZ-MALAVER, A; ROUBIK, D W; MORENO, E; ALMEIDA, S B; SANCHO, M T; FERNÁNDEZ-MUIÑO, M A; ALMEIDA-ANACLETO, D; MARCHINI, L C; GIL, F; GONZÁLEZ, C; AGUILERA, G; NIEVES, B (2009) Expanded parameters to assess the quality of honey from Venezuelan *Apis mellifera*. *Journal of ApiProduct and ApiMedical Science* 1(3): 72-81. http://dx.doi.org/10.3896/IBRA.4.01.3.03

VOIDAROU, C; ALEXOPOULOS, A; PLESSAS, S; KARAPANOU, A; MANTZOURANI, I; STAVROPOULOU, E; FOTOU, K; TZORA, A; SKOUFOS, I; BEZIRTZOGLOU, E (2011) Antibacterial activity of different honeys against pathogenic bacteria. *Anaerobe* 17(6): 375-379. http://dx.doi.org/10.1016/j.anaerobe.2011.03.012

VON DER OHE W., DUSTMANN J.H., VON DER OHE K. (1991) Prolin als Kriterium der Reife des Honigs. *Deutsche Lebensmittel-Rundschau* 87: 383-386.

VON DER OHE, W; VON DER OHE, K; RAUDE-ROBERG, L; DUSTMANN, J H (1999) Vergleich der Methoden zur Bestimmung der Saccharase-Aktivität im Honig. *Apidologie* 30(5): 412-413. http://dx.doi.org/10.1051/apido:19990505

VORWOHL, G (1964a): Die Messung der elektrischen Leitfähigkeit des Honigs und die Verwendung der Meßwerte zur Sortendiagnose und zum Nachweis von Verfälschungen mit Zuckerfütterungs-Honig. *Zeitschrift Bienenforschung* 7: 37-47.

VORWOHL, G (1964b): Die Bezlehungen zwischen der elektrischen Leitfähigkeit der Honige und ihrer trachtmäßigen Herkunft. *Annales de l'Abeille* 7: 37-47.

WANG, J; KLIKS, M M; JUN, S; JACKSON, M; LI, Q X (2010) Rapid analysis of glucose, fructose, sucrose, and maltose in honeys from different geographic regions using Fourier transform infrared spectroscopy and multivariate analysis. *Journal of Food Science* 75(2): C208-C214. http://dx.doi.org/10.1111/j.1750-3841.2009.01504.x

WANG, X H; GHELDOF, N; ENGESETH, N J (2004) Effect of processing and storage on antioxidant capacity of honey. *Food Chemistry and Toxicology* 69(2): 96-101. http://dx.doi.org/10.1111/j.1365-2621.2004.tb15509.x

WEI, Z; WANG, J; LIAO, W (2009) Technique potential for classification of honey by electronic tongue. *Journal of Food Engineering* 94(3-4): 260-266. http://dx.doi.org/10.1016/j.jfoodeng.2009.03.016

WEI, Z; WANG, J; WANG, Y (2010) Classification of monofloral honeys from different floral origins and geographical origins based on rheometer. *Journal of Food Engineering* 96(3): 469-479. http://dx.doi.org/10.1016/j.jfoodeng.2009.08.028

WEI, Z; WANG, J (2011) Classification of monofloral honeys by voltammetric electronic tongue with chemometrics method. *Electrochimica Acta* 56(13): 4907-4915. http://dx.doi.org/10.1016/j.electacta.2011.02.065

WEI, Z; WANG, J (2014) Tracing floral and geographical origins of honeys by potentiometric and voltammetric electronic tongue. *Computers and Electronics in Agriculture* 108: 112-122. http://dx.doi.org/10.1016/j.compag.2014.07.014

WENHAN, L; LUQUING, L (1993) Determination of glucose oxidase activity with catalytic kinetic analysis-spectrophotometry. *Fenxi Huaxue (Chinese Journal of analytical chemistry)* 21(1): 66-69.

WHITE, J W JR; PAIRENT, F W (1959) Report on the analysis of honey. *Journal Association of Official Agricultural Chemists* 42: 341-348.

WHITE, J W JR; SUBERS, M H (1963) Studies on honey inhibine. 2. A chemical assay. *Journal of Apicultural Research* 2(2): 93-100. http://dx.doi.org/10.1080/00218839.1963.11100066

WHITE, J W JR; KUSHNIR, I; SUBERS, M H (1964) Effect of storage and processing temperatures on honey quality. *Food Technology* 18(4): 153-156.

WHITE, J W JR (1979a) Composition of honey. In *Crane, E (Ed). Honey. A comprehensive Survey.* Heinemann; London, UK. pp. 157-206.

WHITE, J W JR (1979b) Spectrophotometric Method for Hydroxymethylfurfural in Honey. *Journal of the AOAC International* 62(3): 509.

WILCZYŃSKA, A (2014) Effect of filtration on colour, antioxidant activity and total phenolics of honey. *LWT - Food Science and Technology* 57(2): 767-774. http://dx.doi.org/10.1016/j.lwt.2014.01.034

WILKINS, A L; LU, Y; TAN, S T (1995) Extractives from New Zealand honeys. 5. Aliphatic dicarboxylic acids in New Zealand Rewerewa (*Knightea excelsa*) honey. *Journal of Agricultural and Food Chemistry* 43(12): 3021-3025. http://dx.doi.org/10.1021/jf00060a006

WINKLER, O (1955) Beitrag zum Nachweis und zur Bestimmung von Oxymethylfurfural in Honig und Kunsthonig. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* 102(3): 160-167. http://dx.doi.org/10.1007/BF01683776

WITCZAK, M; JUSZCZAK, L; GALKOWSKA, D (2011) Non-Newtonian behaviour of heather honey. *Journal of Food Engineering* 104(4): 532-537. http://dx.doi.org/10.1016/j.jfoodeng.2011.01.013

ONG, Y F; MAKAHLEH, A; AL AZZAM, K M; YAHAYA, N; SAAD, B; AMRAH SULAIMAN, S A (2012) Micellar electrokinetic chromatography method for the simultaneous determination of furanic compounds in honey and vegetable oils. *Talanta* 97: 23-31. http://dx.doi.org/10.1016/j.talanta.2012.03.056

WOODCOCK, T; DOWNEY, G; KELLY, D; O'DONNELL, C P (2007) Geographical classification of honey samples by near-infrared spectroscopy: a feasibility study. *Journal of Agricultural and Food Chemistry* 55(22): 9128-9134. http://dx.doi.org/10.1021/jf072010q

WOODCOCK, T; DOWNEY, G; O'DONNELL, C P (2009) Near infrared spectral fingerprinting for confirmation of claimed PDO provenance of honey. *Food Chemistry* 114(2): 742-746. http://dx.doi.org/10.1016/j.foodchem.2008.10.034

YANNIOTIS, S; SKALTSI, S; KARABURNIOTI, S (2006) Effect of moisture content on the viscosity of honey at different temperatures. *Journal of Food Engineering* 72(4): 4372-4377. http://dx.doi.org/10.1016/j.jfoodeng.2004.12.017

ZAKARIA, A; SHAKAFF, A Y M; MASNAN, M J; AHMAD, M N; ADOM, A H; JAAFAR, M N; GHANI, S A; ABDULLAH, A H; AZIZ, A H A; KAMARUDIN, L M; SUBARI, N; FIKRI, N A (2011) A biomimetic sensor for the classification of honeys of different floral origin and the detection of adulteration. *Sensors* 11(8): 7799-7822. http://dx.doi.org/10.3390/s110807799

ZHAO, X; HE, Y; BAO, Y (2011) Non-destructive identification of the botanical origin of Chinese honey using visible/short wave-near infrared spectroscopy. *Sensor Letters* 9(3): 1055-1061. http://dx.doi.org/10.1016/j.foodres.2014.01.014

ZHOU, J; QI, Y; RITHO, J; DUAN, L; WU, L; DIAO, Q; LI, Y; ZHAO, J (2014a) Analysis of maltooligosaccharides in honey samples by ultra-performance liquid chromatography coupled with evaporative light scattering detection. *Food Research International* 56: 260-265. http://dx.doi.org/10.1016/j.foodres.2014.01.014

ZHOU, J; YAO, L; LI, Y; CHEN, L; WUA, L; ZHAO, J (2014b) Floral classification of honey using liquid chromatography-diode array detection-tandem mass spectrometry and chemometric analysis. *Food Chemistry* 145: 941-949. http://dx.doi.org/10.1016/j.foodchem.2013.08.117

ZHU, X; YE, F; YANG, J; XIAO, X; WIEN, H; LIU, R (2010) Determination of organic acids in honey by solid phase extraction-high performance liquid chromatography. *Chinese Journal of Chromatography (Se Pu)* 28(10): 945-949. http://dx.doi.org/10.3724/SP.J.1123.2010.00945

OBJECTIVES

OBJECTIVES

The main purpose of this work has been to characterize the honeys harvested in Castilla y León (Spain), on the basis of their quality control parameters, as well as on their melissopalynology, sensory, antioxidant, and antimicrobial data.

The specific aims have been:

- To thoroughly review the literature references about composition and analysis of honeys.
- To determine the botanical origins of honeys from Castilla y León.
- To check how honeys from Castilla y León fulfil the current European legal regulations.
- To compare sugars' and phenolic compounds' profiles of the analyzed honeys according to their botanical origins.
- To deeply study the assay for honeys' total flavonoids carried out in neutral media.
- To set up a reliable honey's antioxidant activity assessment by trolox equivalent antioxidant capacity (TEAC) method.
- To study antioxidant-related features of honeys from different botanical origins.
- To propose a suitable procedure for the determination of honeys' antibacterial activity against *Staphylococcus aureus*.
- To research possible differences among the antibacterial activities against *Staphylococcus aureus* of honeys from different botanical origins.

OBJETIVOS

El principal objetivo de este trabajo ha sido la caracterización de mieles producidas en Castilla y León (España) en función de sus parámetros de control de calidad, así como de sus análisis melisopalinológicos, sensoriales, antioxidantes y antimicrobianos.

Los objetivos específicos han sido:

- Realizar una revisión bibliográfica en profundidad sobre la composición de la miel y sus métodos de análisis más importantes.
- Determinar los orígenes botánicos de las mieles de Castilla y León.
- Comprobar que las mieles de Castilla y León cumplan con las actuales regulaciones legales Europeas.
- Comparar los perfiles de azúcares y de compuestos fenólicos de las mieles analizadas según sus orígenes botánicos.
- Estudiar en profundidad el ensayo de flavonoides totales llevado a cabo en medio neutro.
- Poner a punto el método de la capacidad antioxidante equivalente de trolox (TEAC) para analizar la actividad antioxidante en mieles.
- Estudiar los parámetros relacionados con la actividad antioxidante de mieles de diferentes orígenes botánicos.
- Proponer un procedimiento adecuado para la determinación de la actividad antibacteriana de las mieles frente *Staphylococcus aureus.*
- Buscar posibles diferencias entre las actividades antibacterianas frente *Staphylococcus aureus* de mieles de diferentes orígenes botánicos.

EXPERIMENTAL WORK

CHAPTER 4

CHARACTERIZATION OF HONEYS FROM CASTILLA Y LEÓN (SPAIN) ON THE BASIS OF THEIR SUGAR PROFILE AND OTHER PHYSICOCHEMICAL PARAMETERS

CHARACTERIZATION OF HONEYS FROM CASTILLA Y LEÓN (SPAIN) ON THE BASIS OF THEIR SUGAR PROFILE AND OTHER PHYSICOCHEMICAL PARAMETERS

ABSTRACT

As honey characterization may increase the commercial value of artisanal honeys, the purpose of this study was to characterize fifty-four representative artisanal honeys from Castilla y León region (North-Central Spain), on the basis of melissopalynology, sensory and physicochemical analyses. Hydroxymethylfurfural (HMF), diastase, moisture, conductivity, pH, free acidity, lactones, proline, specific rotation, sugar profile, crystallization indexes and other carbohydrate ratios were researched. All honeys fulfilled the European legal requirements. Fourteen carbohydrates were quantified: two monosaccharides, five disaccharides, six trisaccharides and one tetrasaccharide. Several carbohydrates were found to be characteristic of the most important honey types, although their concentrations in honey do not allow the classification of the main unifloral sources. Significant correlations were stablished among the studied parameters. Principal Component Analysis showed that sugars' profiles and other physicochemical parameters analysed proved not to be useful for botanical characterization.

1. Introduction

According to the Codex Alimentarius Standard for Honey (2001), this food is the natural sweet substance produced by honeybees from the nectar of flowers (blossom honeys), and from plants or trees exudates or excretions of plant-sucking insects (honeydew honeys). Honey is mainly composed by water (15-20%) and carbohydrates (70-80%), being the most important of which fructose (38%) and glucose (31%). It also contains a complex mixture of other di-, tri-, oligo- and polysaccharides (Bogdanov *et al.*, 2008). Moreover, about 200 minor but important constituents such as amino acids, proteins, organic acids, vitamins, minerals, Maillard reaction products, volatile compounds, enzymes and other phytochemical bioactive compounds (such as phenolic acids and flavonoids) have been found in small quantities in honey (Gheldof and Engeseth, 2002; Blasa *et al.*, 2006; Alvarez-Suarez *et al.*, 2010).

Castilla y León (Castile and Leon) is a region formed by nine provinces, located in the middle of the northern half of the Iberian Peninsula. It is the largest region of Spain and the third of the European Union, with an extension of 94,200 km^2. Almost the whole area has a continental-Mediterranean climate, being the main trees *Quercus* sp. The northern zone has an oceanic climate, with abundant vegetation and always green. It is constituted by meadows, deciduous forest (*Fagus* sp., *Quercus* sp. and *Castanea sativa* trees) and moors. The most

representative nectar-producing plants are Compositae, Cruciferae, Ericaceae, Fagaceae, Labiatae, Leguminosae and Rosaceae.

Climatic and floral diversity of Castilla y León give the bees (*Apis mellifera*) the opportunity to produce a wide variety of both floral and honeydew honeys. The main Castilla y León unifloral honeys are heather (*Erica* sp., *Calluna vulgaris*), honeydew (*Quercus* sp.) and chestnut (*Castanea sativa*). Other important honeys are rubus (*Rubus* sp.), lavender (*Lavandula* sp.), sunflower (Compositae type *Helianthus annuus*), clover (Leguminosae Type *Trifolium* sp.) and thyme (*Thymus* sp.). Such is the importance of beekeeping in Castilla y León that in 2014 it was the area with the largest number of the bee farms in Spain (16.5% of the total), being in 2013 the forth regarding annual honey production, with 3,983 tonnes (13%) (Subdirección General de Productos Ganaderos, 2015).

Floral source and geographical origin, as well as climatic conditions, bee species and postharvest beekeeping practices, influence the chemical composition, physical properties, sensory characteristics and biological activities of honeys (Anklam, 1998; Khalil *et al.*, 2011). Therefore, the complex botanical and geographical characterization and quality control of honeys, include the results of melissopalynology, physicochemical and sensory analysis, all of them linked to chemometric techniques, because these combined procedures have proved to be useful to guarantee honey authenticity (Lachman *et al.*, 2007; Kropf *et al.*, 2010).

The traditional approach for honey characterization is melissopalinology, based on the identification of pollen by microscopic examination (Louveaux *et al.*, 1978). However, this technique is tedious and depends on the qualification of the analyst (Anklam, 1998), and it does not always enable reliable identification. In general, a honey is considered unifloral (Maurizio, 1975), if it is produced mainly from one plant, being the pollen frequency of the plant higher than 45% of the total pollen (P) content (as *Erica* sp.). Unfortunately, this percentage is not valid in all cases, because of the great variability in nectar contribution of particular flowers compared to the amount of their pollen content (Tan *et al.*, 1989). This is the case of some unifloral honeys from Castilla y León, produced from under-represented (*Calluna vulgaris, Lavandula* sp.) or over-represented (*Castanea sativa*) pollen plants (Von der Ohe *et al.*, 2004). Moreover, although a ratio of HDE (honeydew elements)/P higher than 3 is generally required to establish a honey sample as honeydew honey (Louveaux *et al.*, 1978), many authors observed that honeydew honeys from *Quercus* sp. failed to fulfil this requirement (Serra-Bonvehí *et al.*, 1987; Escuredo *et al.*, 2012), so many researchers proposed alternative methods to complement the pollen analysis for the evaluation of honey's botanical origin.

Sensory analysis provides information about honey quality (Piana *et al.*, 2004). Some researchers showed that descriptive sensory analysis together with physicochemical data may help differentiate floral origins (Anupama *et al.*, 2003; Silvano *et al.*, 2014). Descriptive

sensory analysis is a useful technique that provides quantitative descriptions of products taking into account all sensations that are perceived.

The physicochemical quality criteria specified by the Directive 2001/110/EC (OJEC, 2002), are based on the analysis of fructose, glucose, sucrose, moisture, water insoluble content, electrical conductivity, free acid, diastase activity and hydroxymethylfurfural. Nevertheless, there are other parameters not included in the legislations that contribute to honey authenticity, among them colour, viscosity, specific rotation, water activity, minor compounds, and biological activities.

Honey composition has been widely used for its characterization, mainly by means of two different tools. On the one hand, the identification and quantification of potential chemical markers, such as the flavonoid hesperetin (Ferreres *et al.*, 1993) or the volatile compound methyl anthranilate (Serra-Bonvehí, 1988) in citrus honey. On the other hand, the description of chemical compounds' profiles that represent a "fingerprint" of a specific honey origin, as for example the profile of carbohydrates (Bentabol-Manzanares *et al.*, 2011; De la Fuente *et al.*, 2011; Consonni *et al.*, 2013), flavonoids and phenolic acids (Tomás-Barberán *et al.*, 2001; Escriche *et al.*, 2014), minerals (Chua *et al.*, 2012; Yücel and Sultanoğlu, 2013; Zhou *et al.*, 2013; Chen *et al.*, 2014; Rodríguez-Flores *et al.*, 2014), aminoacids (Hermosín *et al.*, 2003; Silici and Karaman, 2014), organic acids (Cherchi *et al.*, 1994; Suárez-Luque *et al.*, 2002; Mato *et al.*, 2006), vitamins (Viñas *et al.*, 2004; Ciulu *et al.*, 2011; León-Ruiz *et al.*, 2013) and volatile compounds (Escriche *et al.*, 2011; Yang *et al.*, 2012; Seisonen *et al.*, 2015).

Several researchers characterized honeys from different Spanish regions by physicochemical analysis. Illustrative examples are studies on honeys from Andalusia (Serrano *et al.*, 2004), Galicia (Seijo *et al.*, 1997; Escuredo *et al.*, 2013), Madrid (Soria *et al.*, 2004), Castilla la Mancha (León-Ruiz *et al.*, 2011, 2013), La Rioja and Basque Country (Sancho *et al.*, 1991c; Sanz *et al.*, 1995), Aragón (Perez-Arquillué *et al.*, 1994) and Tenerife (Canary Islands) (Bentabol-Manzanares *et al.*, 2014). Also chemical composition and physical analysis of honeys from some of the provinces of Castilla y León have been investigated: Burgos (Cavia *et al.*, 2002), Soria (Nozal *et al.*, 2005; Nozal-Nalda *et al.*, 2005), Salamanca and Zamora (Gómez-Bárez *et al.*, 2000; González-Paramás *et al.*, 2007).

Characterization of unifloral commercial honeys is a hard task that was initiated in response to consumer demands (Mateo and Bosch-Reig, 1998). Consumers show an increasing interest in quality foods and appreciate honeys from specific botanical/geographical origins. Thus, it is important to protect them from mislabelling, adulterations and frauds (Karabagias *et al.*, 2014). High quality honeys achieve high prices on the market. However, in big stores and supermarkets, those honeys are being replaced by run-of-the-mill imported honeys with lower prices. For this reason, to guarantee product quality, assessing the authenticity of local honeys

carrying out extensive honey compositional analyses, is a growing necessity for revaluing the products of each region (Estevinho *et al.*, 2012).

Within a research project aimed to characterize artisanal honeys produced in Castilla y León, in this study melissopalinology, sensory assessment, sugar profile and other interesting physicochemical parameters were determined.

2. Material and methods

2.1. Honey samples

This study was carried out on 54 artisanal honey samples declared as unifloral by the beekeepers. Samples were collected at apiaries located in Castilla y León region in 2011 (Figure 1). They were stored at 4ºC until analysis.

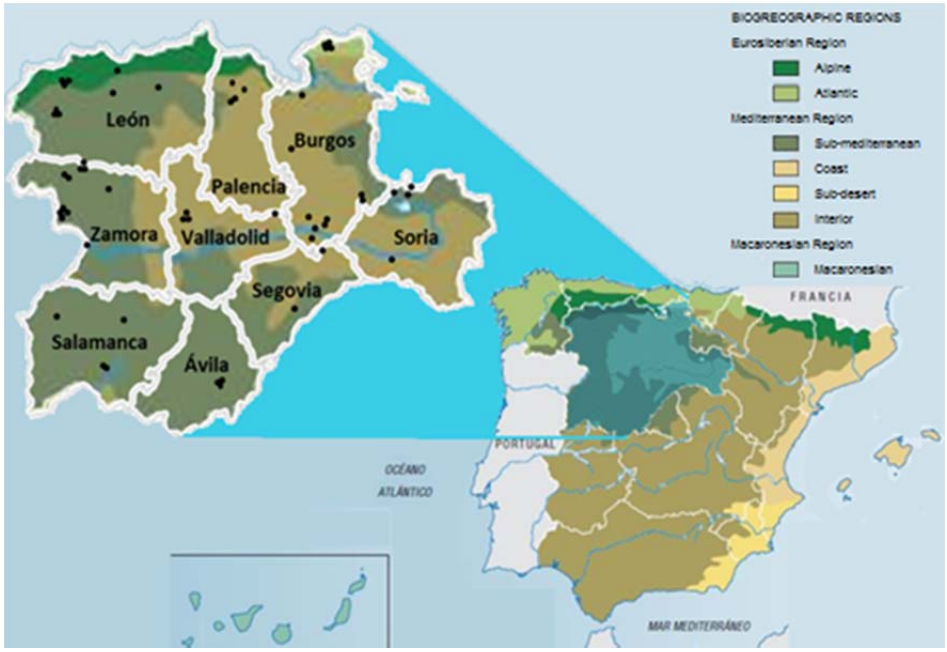

Figure 1. Map of Spain and Castilla y León region, showing the distribution of the raw honey samples studied (n = 54) harvested in 2011.

2.2. Melissopalynology

Botanical origin of the samples was monitored by pollen analysis. Honeys' sediments were treated and dyed according to the procedure described by Terradillos *et al.* (1994), and then subjected to qualitative melissopalinological analysis. Determination of frequency classes was performed at 1000X (Louveaux *et al.*, 1978), and calculation, reporting and interpretation of the results were accomplished following the recommendations of the current harmonized methods (Von der Ohe *et al.*, 2004), and those of several researchers regarding particular honeys: chestnut honeys (Seijo *et al.*, 1997; OJEU, 2007; Escuredo *et al.*, 2012) and oak honeydew honeys (Mateo and Bosch-Reig, 1998; Rodríguez-Flores *et al.*, 2015). Pollen grains (P) and such honeydew elements (HDE) as fungal spores, hyphae and microscopic

algae were counted. Then, the ratio HDE/P was calculated. According to the literature (Louveaux *et al.*, 1978; Von der Ohe *et al.*, 2004), a given honey is considered to be predominately honeydew if HDE/P exceeds 3 (Von der Ohe *et al.*, 2004). For unifloral honeys from under-represented or over-represented pollen plants, as well as honeydew honeys from *Quercus* sp. that did not meet the proposed requirement HDE/P>3, some physicochemical parameters and sensory assessment were necessary for proper botanical classification.

2.3. Sensory analysis

Descriptive sensory analysis was carried out by a panel of trained assessors, in order to help classify the samples by their botanical origins and establish the most important terms used for the distinction of monofloral honeys.

The evaluation form based on the odour and aroma wheel for honey of the International Honey Commision (Piana *et al.*, 2004) was used.

2.4. Physicochemical parameters

Most physicochemical properties were analysed according to the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 2012), the Harmonised Methods of the International Honey Commision (Bogdanov, 2009) and the Spanish legislation (BOE, 1986), with some modifications. The determined parameters were: Hydroxymethylfurfural (mg/kg), diastase activity (Schade degrees), moisture (%), electrical conductivity (mS/cm), pH, free, lactone and total acidity (meq/kg), proline (%), optical rotation (º), sugar profile (%), crystallization indexes and other sugars' ratios.

2.4.1. Hydroxymethylfurfural

HMF was determined according to White (1979a) method. Five grams of honey was dissolved and made up to 50 ml with distilled water after clarification with Carrez reagents (I and II). The solution was filtered and then, the HMF content (mg/kg) was calculated by subtracting the background absorbance of the filtered honey solution measured at 336 nm from the absorbance at 284 nm (test solution) against an aliquot of the same solution treated with sodium bisulphite, using a Varian/Agilent Cary 400 UV/VIS Bio double-beam Lab Spectrophotometer.

HMF was determined following the equation (Eq. (1)), where D is the dilution factor and W the sample weight (g).

$$(1)\ \text{HMF (mg/kg)} = (Abs_{284}-Abs_{336}) \times 149.7 \times 5 \times D/W$$

2.4.2. Diastase activity

Diastase activity was measured spectrophotometrically using a buffered solution of honey and soluble starch, incubated in a thermostatic bath at 40ºC. One millilitre of this solution was removed at periodic intervals and added rapidly to an iodine solution (developing a blue colour that decreases with the time), reading the absorbance at 660 nm of less than 0.235 (endpoint of the reaction). Diastase number was calculated using the time taken for the absorbance to reach de endpoint, and the results were expressed in Schade (or Goethe) units.

2.4.3. Moisture

Water content of a previous liquefied honey was determined by measuring the refractive index at 20ºC (Abbe refractometer Officine Galileo). Chataway conversion table revised by Wedmore was used to calculate the moisture content (%).

2.4.4. Electrical conductivity

Electrical conductivity of a 20% (w/v dry matter basis) honey solution in CO_2-free deionized water was measured at 20ºC (Conductivity meter Crison-Micro CM 2202). The results were expressed as mS/cm.

2.4.5. pH, free acidity, lactone acidity and total acidity

These parameters were determined using the potentiometric titrator 751 GPD Titrino METROHM. pH was measured in a honey solution prepared with 10.00 g of honey and 75 mL CO_2-free distilled water. Thereafter, this solution was titrated with 0.05 M NaOH at a rate of 5.0 ml/min up to pH 8.50 for free acidity determination (meq/kg). Immediately, 10 mL 0.05 M NaOH was added, and without delay, the solution was titrated with 0.05 M HCl up to pH 8.30 for lactone acidity determination (meq/kg). Total acidity is the sum of both free acid and lactones.

2.4.6. Proline

Proline content (mg/100 g) was determined spectrophotometrically. Test solution contained the honey solution and ninhydrin in acidic medium (with formic acid) was placed in a boiling water bath for 15 min. After cooling and adding isopropanol solution, absorbance was measured at 520 nm against a blank containing distilled water instead of honey solution. Colour correction was made substituting ninhydrin in acidic medium for distilled water against a blank of water and calibration curve was made using proline standard solution, both subjected to the same treatment as test solution. The absorbance value of colour correction was subtracted from the test solution value before calculating, and calibration curve was used for proline determination.

2.4.7. Specific rotation

Angular rotation was determined in a solution of 12.00 g of honey diluted to 100 ml with distilled water, after clarification with Carrez reagents (I and II). Specific optical rotation $[\alpha]_D^{20}$ was read at 20ºC the following day after filtration, using a digital polarimeter Cecchinato MOD D-400, equipped with a sodium lamp. Results were read in angular degrees on a 200 mm basis polarimeter tube.

2.4.8. Sugar profile

Sugars were determined by using a GC-FID method according to Pourtallier´s derivatisation procedure. A solution of 3.00 g of honey was filled up to 500 ml with distiller water after addition of mannitol used as internal standard. A volume of 100 µl of the solution was transferred to a conical bottomed vial and dried under nitrogen at 50ºC. Thereafter, sugars were derivatized to their oximes by adding 200 µl of oxime reagent (12 mg/ml hydroxylamine hydrochloride in pyridine). After heating at 75ºC during 30 min and cooling at room temperature, the sugars' oximes were silylated by adding 100 µl of hexamethyldisilazane and 10 µl of trifluoroacetic acid. After centrifugation, the trimethylsilyl derivatives were separated and quantified by gas chromatography in a Carlo Erba 4160 Chromatograph equipped with a cold on-column injector, a Flame Ionization Detector and a Mega 5 (25 m×0.25 mm×0.15 µm) capillary column. 0.6 µl of each sample was analysed under the following GC conditions: injector temperature 70°C, oven temperature programmed at 49°C/min from 70 to 140°C and then from 140 to 300°C at 6°C/min and carrier gas helium. Data acquisition and analysis of the chromatographic peak areas were carried out using Borwin Integrator Software. For qualitative analysis, relative retention times to mannitol both for standards and sample peaks were used. Sugars' concentrations were calculated by the internal standard method on the basis of the response factors for sugars' standards eluted under the same conditions as samples. For compounds with two anomeric forms, total area was calculated as the sum of areas for both anomers. Results were expressed in g of sugars per 100 g of honey.

2.4.9. Statistical analysis

One way analysis of the variance (ANOVA) was applied when the assumptions of normality of data, homogeneity of variances and independence of variables were met. Sometimes different transformations were carried out for data normalization, such as x^2, 1/x, (x+1)/2, log x and $x^{0.5}$, among others. The Student-Newman-Keels test was used to perform a multiple comparison of means, where group differences were considered statistically significant at the 95.0% confidence level. When homoscedasticity was met but normality of the data was not possible, the nonparametric procedure Kruskal-Wallis ANOVA to compare the medians instead of the means was done. To determine which medians were significantly different, the median notch in the Box-and-Whisker Plot was used. The ANOVA analysis was not determined when data did not meet homoscedasticity condition. The unequal sample sizes

lead to the heterogeneity of variances. Correlations among variables were reached and multivariate statistical Principal Component Analysis (PCA) was also carried out. The statistical package software Statgraphics Centurion XVI.II (2010) was employed.

3. Results and discussion

3.1. Botanical origin identification

Although samples were considered monofloral honeys by beekeepers, pollen analysis was carried out to confirm the identity of the honey source. The results are summarised in Table 1. Seventy-one different pollen types, belonging to thirty-six botanical families were identified. The number of pollen types identified in each honey sample range from 8 to 27, with an average of 18 per sample. The average number of pollen was similar from those reported by other authors (Thrasyvoulou and Manikis, 1995). They constitute the common palynological spectra of Castilla y León honeys (Serra-Bonvehí and Ventura-Coll, 1993; Valencia-Barrera *et al.*, 2000; Herrero *et al.*, 2002). All samples contained the pollen types *Trifolium* sp., *Genista* sp. and *Rubus* sp., and 80% of the samples contained *Castanea sativa* and Ericaceae pollens (*Calluna vulgaris* and *Erica vagans* types). Twenty seven pollen types were common to more than a quarter of samples. Melissopalinology showed that not all samples were monofloral, being 15 out of the 54 honey samples multifloral honeys rich in broom, clover, chestnut and/or sunflower. Honeydew honey was the most important botanical origin of the studied samples (33.3%), followed by monofloral honeys from heather (18.5%), chestnut (7.4%), lavender (7.4%) and clover (5.6%).

Table 1. Botanical origins of the samples studied. Predominant pollen (P), Secondary pollen (S), Important minor pollen (I).

Sample	Geographycal origin	Botanical denomination	*P*	*S*	*I*
1	León	Chesnut	*Castanea sativa*	-	*Erica* sp.; Leguminosae Type *Genista* sp.; Leguminosae Type *Trifolium* sp.
2	León	Chesnut	*Castanea sativa*	-	Leguminosae Type *Genista* sp.; Leguminosae Type *Trifolium* sp.; *Calluna vulgaris; Erica* sp.
3	León	Chesnut	*Castanea sativa*	-	Leguminosae Type *Trifolium* sp.; Leguminosae Type *Genista* sp.; *Erica* sp.; *Calluna vulgaris*
4	Salamanca	Chesnut	*Castanea sativa*	-	*Rubus* sp.; Leguminosae Type *Genista* sp.; *Erica* sp.
5	Ávila	Clover	Leguminosae Type *Trifolium* sp.	Leguminosae Type *Genista* sp.	*Thymus* sp.; *Rubus* sp.
6	Ávila	Clover	Leguminosae Type *Trifolium* sp.	Leguminosae Type *Genista* sp.	*Rubus* sp.; *Thymus* sp.
7	Ávila	Clover	Leguminosae Type *Trifolium* sp.	Leguminosae Type *Genista* sp.	*Rubus* sp.; *Castanea sativa;* Labiatae Type *Mentha* sp.
8	Burgos	Heather	*Ericaceae (Erica* sp., *Calluna vulgaris)*	Leguminosae Type *Genista* sp.	*Rubus* sp.; Leguminosae Type *Trifolium* sp.
9	Burgos	Heather	*Ericaceae (Erica* sp., *Calluna vulgaris)*	Leguminosae Type *Trifolium* sp.	*Rubus* sp.; Leguminosae Type *Genista* sp.; Compostiae Type *Centaurea jacea*
10	Burgos	Heather (*Erica* sp.)	*Erica* sp.	-	Leguminosae Type *Genista* sp.; Leguminosae Type *Trifolium* sp.; *Calluna vulgaris; Rubus* sp.

Sample	Geographycal origin	Botanical denomination	*P*	*S*	*I*
11	Burgos	Heather (*Erica* sp. and *Calluna vulgaris*)	*Erica* sp.	-	Leguminosae Type *Genista* sp.*; Calluna vulgaris;* Leguminosae Type *Trifolium* sp.*; Rubus* sp.
12	Burgos	Heather (*Erica* sp. and *Calluna vulgaris*)	*Erica* sp.	-	*Rubus* sp.*; Calluna vulgaris;* Leguminosae Type *Trifolium* sp.*;* Leguminosae Type *Genista* sp.
13	Burgos	Heather (*Erica* sp. and *Calluna vulgaris*)	*Erica* sp.	*Calluna vulgaris*	Leguminosae Type *Genista* sp.*; Rubus* sp.*;* Leguminosae Type *Trifolium* sp.
14	Soria	Heather (Ling)	-	*Calluna vulgaris*, Leguminosae Type *Trifolium* sp.	Leguminosae Type *Genista* sp.*; Erica* sp.*; Rubus* sp.
15	Soria	Heather (Ling)	-	*Calluna vulgaris;* Leguminosae Type *Genista* sp.*;* Leguminosae Type *Trifolium* sp.	*Rubus* sp.*; Erica* sp.*; Thymus* sp.
16	León	Heather (Ling)	-	*Castanea sativa*	*Erica* sp.*;* Leguminosae Type *Genista* sp.*; Calluna vulgaris;* Leguminosae Type *Trifolium* sp.
17	Soria	Heather (Ling)	-	*Erica* sp.	*Calluna vulgaris;* Leguminosae Type *Trifolium* sp.*;* Leguminosae Type *Genista* sp.*; Rubus* sp.*;* Leguminosae Type *Onobrychis sp;* Cruciferae Type *Diplotaxis* sp.
18	Valladolid	Honeydew	-	-	Umbelliferae Type *Daucus carota;* Leguminosae Type *Trifolium* sp.*; Castanea sativa;* Compostiae Type *Centaurea jacea;* Leguminosae Type *Genista* sp.*; Rubus* sp.*;* Leguminosae Type *Onobrychis sp;* Scrophulariaceae *Type Scrophularia* sp.*;* Compositae Type *Helianthus annuus*
19	Salamanca	Honeydew	-	-	Leguminosae Type *Trifolium* sp.*;* Leguminosae Type *Genista* sp.*; Erica* sp.*; Echium* sp.*;* Scrophulariaceae Type *Veronica* sp.*; Castanea sativa; Rubus* sp.*; Thymus* sp.*;* Leguminosae Type *Lotus sp*
20	Zamora	Honeydew	-	-	Leguminosae Type *Genista* sp.*; Castanea sativa; Erica* sp.*;* Leguminosae Type *Trifolium* sp.*; Calluna vulgaris; Rubus* sp.
21	Segovia	Honeydew	-	-	Leguminosae Type *Trifolium* sp.*; Rubus* sp.*; Erica* sp.*; Echium* sp.*;* Leguminosae Type *Genista* sp.*; Calluna vulgaris*
22	Palencia	Honeydew	-		*Erica* sp.*;* Leguminosae Type *Genista* sp.*;* Leguminosae Type *Trifolium* sp.*;* Leguminosae Type *Onobrychis* sp.*; Rubus* sp.
23	Palencia	Honeydew	-	-	*Erica* sp.*;* Leguminosae Type *Genista* sp.*;* Leguminosae Type *Onobrychis* sp.*; Calluna vulgaris;* Leguminosae Type *Trifolium* sp.*;* Compositae Type *Helianthus annuus; Rubus* sp.
24	Palencia	Honeydew	-	-	Compositae Type *Helianthus annuus; Rubus* sp.*; Erica* sp.*;* Leguminosae Type *Onobrychis* sp.*;* Leguminosae Type *Genista* sp.
25	León	Honeydew	-	*Castanea sativa*	Leguminosae Type *Trifolium* sp.*; Rubus* sp.*;* Leguminosae Type *Genista* sp.*; Calluna vulgaris; Erica* sp.
26	Zamora	Honeydew	-	*Castanea sativa*	*Calluna vulgaris;* Leguminosae Type *Genista* sp.*; Erica* sp.
27	Zamora	Honeydew	-	*Castanea sativa*	*Erica* sp.*;* Leguminosae Type *Trifolium* sp.*;* Leguminosae Type *Genista* sp.*; Rubus* sp.*;* Rosaceae Type *Crataegus monogyna; Calluna vulgaris*
28	Zamora	Honeydew	-	*Castanea sativa*	Leguminosae Type *Genista* sp.*;* Leguminosae Type *Trifolium* sp.*; Rubus* sp.*; Erica* sp.
29	Zamora	Honeydew	-	*Castanea sativa;* Leguminosae Type *Genista* sp.	Leguminosae Type *Trifolium* sp.*; Rubus* sp.*; Thymus* sp.
30	Zamora	Honeydew	-	*Castanea sativa;* Leguminosae Type *Genista* sp.	Leguminosae Type *Trifolium* sp.*; Rubus* sp.

Sample	Geographycal origin	Botanical denomination	*P*	*S*	*I*
31	Burgos	Honeydew	-	*Erica* sp.	Leguminosae Type *Trifolium* sp.; *Rubus* sp.; Leguminosae Type *Genista* sp.; *Calluna vulgaris*
32	León	Honeydew	-	Leguminosae Type *Genista* sp.	*Castanea sativa; Rubus* sp.; Leguminosae Type *Trifolium* sp.; *Erica* sp.; *Calluna vulgaris*
33	Zamora	Honeydew	*Castanea sativa*	-	Leguminosae Type *Genista* sp.; *Rubus* sp.; Leguminosae Type *Trifolium* sp.; *Calluna vulgaris; Erica* sp.
34	Zamora	Honeydew	*Castanea sativa*	-	Leguminosae Type *Genista* sp.; Leguminosae Type *Trifolium* sp.; *Erica* sp.; *Rubus* sp., *Calluna vulgaris*
35	Salamanca	Honeydew	*Castanea sativa*	-	Leguminosae Type *Genista* sp.; *Erica* sp.; *Rubus* sp.; Leguminosae Type *Trifolium* sp.
36	Valladolid	Lavender	-	Compositae Type *Helianthus annuus*	Leguminosae Type *Trifolium* sp.; Cruciferae Type *Diplotaxis* sp.; Leguminosae Type *Genista* sp.; *Rubus* sp.; *Lavandula* sp.
37	Valladolid	Lavender	-	Leguminosae Type *Genista* sp.	*Echium* sp.; Leguminosae Type *Lotus* sp.; *Rubus* sp.; Leguminosae Type *Onobrychis* sp.; Leguminosae Type *Trifolium* sp.; Compostiae Type *Centaurea jacea; Lavandula* sp.
38	Soria	Lavender	-	Leguminosae Type *Genista* sp.; *Castanea sativa;* Leguminosae Type *Trifolium* sp.	*Rubus* sp.; *Lavandula* sp.; Cruciferae Type *Diplotaxis sp*
39	Segovia	Lavender	-	*Reseda* sp.; Leguminosae Type *Genista* sp.	*Lavandula* sp.; Leguminosae Type *Trifolium* sp.; *Rubus* sp.; Compositae Type *Helianthus annuus; Castanea sativa*
40	Palencia	Multifloral	-	-	*Erica* sp.; Leguminosae Type *Onobrychis* sp.; *Castanea sativa;* Scrophulariaceae Type *Veronica* sp.; Cruciferae Type *Diplotaxis* sp.; *Compositae type C;* Leguminosae Type *Trifolium* sp.; Leguminosae Type *Genista* sp.; *Calluna vulgaris; Rubus* sp.
41	León	Multifloral	-	*Castanea sativa;* Leguminosae Type *Genista* sp.	Leguminosae Type *Trifolium* sp.; *Rubus* sp.
42	Zamora	Multifloral	-	*Castanea sativa;* Leguminosae Type *Genista* sp.; Leguminosae Type *Trifolium* sp.	*Rubus* sp.; *Thymus* sp.; Scrophulariaceae Type *Veronica* sp.
43	Burgos	Multifloral	-	Compositae Type *Helianthus annuus*	Leguminosae Type *Genista* sp.; *Reseda* sp.; *Rubus* sp.; Leguminosae Type *Vicia* sp.; Compostiae Type *Centaurea jacea; Trifolium* sp.; *Salix* sp.
44	Valladolid	Multifloral	-	Compositae Type *Helianthus annuus*	Umbelliferae Type *Daucus carota;* Leguminosae Type *Trifolium* sp.; Leguminosae Type *Genista* sp.; Compostiae Type *Centaurea jacea; Rubus* sp.; *Onobrychis* sp.
45	Burgos	Multifloral	-	Compositae Type *Helianthus annuus*	Leguminosae Type *Trifolium* sp.; *Rubus* sp.; *Castanea sativa;* Cruciferae Type *Raphanus raphanistrum;* Leguminosae Type *Genista* sp.; Leguminosae Type *Onobrychis* sp.
46	Burgos	Multifloral	-	Leguminosae Type *Genista* sp.	*Rubus* sp.; Compostiae Type *Helianthus annuus;* Leguminosae Type *Trifolium* sp.; Leguminosae Type *Onobrychis* sp.; *Reseda* sp.; Umbelliferae Type *Daucus carota*
47	Burgos	Multifloral	-	Leguminosae Type *Genista* sp.; Compositae Type *Helianthus annuus*	Leguminosae Type *Trifolium* sp.; *Rubus* sp.; Leguminosae Type *Onobrychis* sp.; Scrophulariaceae Type *Veronica* sp.; *Lavandula* sp.
48	Burgos	Multifloral	-	Leguminosae Type *Genista* sp.; Leguminosae Type *Trifolium* sp.	*Rubus* sp.; *Reseda* sp.; Compostiae Type *Helianthus annuus; Sedum* sp.; *Hypecoum* sp.
49	Zamora	Multifloral	-	Leguminosae Type *Genista* sp.; *Leguminosae* Type *Trifolium* sp.	*Rubus* sp.; Scrophulariaceae Type *Veronica* sp.; *Thymus* sp.

Sample	Geographycal origin	Botanical denomination	*P*	*S*	*I*
50	Zamora	Multifloral	-	Leguminosae Type *Trifolium* sp.; *Castanea sativa;* Leguminosae Type *Genista* sp.	*Echium* sp.; *Rubus* sp.; Leguminosae Type *Lotus* sp.
51	Salamanca	Multifloral	-	Leguminosae Type *Trifolium* sp.; Leguminosae Type *Genista* sp.	*Rubus* sp.; *Thymus* sp.
52	Burgos	Multifloral	-	*Reseda* sp.; Leguminosae Type *Genista* sp.	Cruciferae Type *Raphanus raphanistrum; Rubus* sp.; Scrophulariaceae Type *Veronica* sp.; Leguminosae Type *Trifolium* sp.; Leguminosae Type *Lotus* sp.; Labiatae Type *Mentha* sp.
53	León	Multifloral	*Castanea sativa*	Leguminosae Type *Genista* sp.	Leguminosae Type *Trifolium* sp.; *Rubus* sp.
54	León	Multifloral	*Castanea sativa*	Leguminosae Type *Genista* sp.	Leguminosae Type *Trifolium* sp.; *Rubus* sp.; *Erica* sp.

3.1.1. Honeydew honeys

In this work, the samples classified as honeydew honeys from *Quercus* sp. (mainly *Quercus ilex*, *Quercus robur* and *Quercus pyrenaica*) showed a HDE/PG ratio between 0.34 and 3.4, being 0.83 the medium ratio. The majority of the samples did not meet the proposed requirement of HDE/PG higher than 3 ("honeydew index"), since the number of microscopic honeydew elements was extremely low (Louveaux *et al.*, 1978). Other researchers also reported low HDE/pollen ratios in honeydew honeys from Spain. Bentabol-Manzanares *et al.* (2011) classified samples from Tenerife (Canary Islands) as suspected honeydew honeys of unknown botanical origin with a medium ratio lower than 0.3. Serra-Bonvehí *et al.* (1987) described an average index of 0.06 for oak honeydew honeys from the Iberian Peninsula (denominating this kind of honey *"forest honey"* instead of honeydew honeys because of its low index), Mateo and Bosch-Reig (1998) reported values between 0.07 and 1.70, Escriche *et al.* (2012) values between 1.90 and 2.50, and in the north of Spain, Escuredo *et al.* (2012) and Rodríguez-Flores *et al.* (2015) classified samples as honeydew honeys with mean values for HDE/P of 0.16 and 0.03 respectively. These values are considered low or very-low according to the classification of Louveaux *et al.* (1978). Low content of honeydew elements could be due to residual contamination of the beehives by previous blooms (Serra-Bonvehí *et al.*, 1987), and the fact that *Quercus* sp. is a tree extremely poor in honeydew elements (Ricciardelli D'Albore, 1998). Maurizio (1975) reported that the appearance of honeydew elements in honey sediment could depend on climatic factors and it could be absent in honeydew honeys from dry areas. Honeydew honeys from *Quercus* species are collected mainly in summer, being it a very hot and dry season in Castilla y León region (Mateo and Bosch-Reig, 1998). Since honeydew index was not found as very useful to classify Spanish oak honeydew honeys, Mateo and Bosch-Reig (1997, 1998) suggested the use of sensory analysis and physicochemical criteria such as electrical conductivity and pH to complement the microscopic determination. Descriptive sensory analysis, conductivity values higher than 0.800 *m*S/cm and pH values higher than 4.3 were considered (Mateo and Bosch-Reig, 1997). Other physicochemical characteristics such as specific rotation index (in general nectar

honeys are laevorotatory and honeydew honeys dextrorotatory) (Bogdanov *et al.*, 2004; Bertoncelj *et al.*, 2011), darker colours, higher antioxidant capacity, polyphenol content and di- and trisaccharide sugar content than blossom honeys (specially melezitose trisaccharide), and lower content of reducing sugars (glucose and fructose), could be considered for honeydew honey classification (Mateo and Bosch-Reig, 1997; Bogdanov *et al.*, 2004; Iglesias *et al.*, 2004; Rodríguez-Flores *et al.*, 2015). Some authors quantified also *Quercus* sp. pollen grains as an additional indicator of the presence of honeydew honey (Iglesias *et al.*, 2004; Soria *et al.*, 2004), but Rodríguez-Flores *et al.* (2015) indicated that *Quercus* pollen is present at very low levels, because oaks flower in early spring. As other authors reported, high presence of pollens from anemophylous and nectarless plants is typical of honeydew honeys (Bentabol-Manzanares *et al.*, 2011).

Soria *et al.* (2005) developed the expression HD%=104.5EC-1.353*(F+G)%+65.47, based on the percentage of fructose (F) plus glucose (G) and on the electrical conductivity (EC) for the estimation of the percentage of honeydew. Honeys with values higher than 70% could be classified as honeydew honeys, those with values lower than 45% as nectar honeys and those with values from 45% to 75% as honey blends or forest honeys (Soria *et al.*, 2004). According to this expression, only 1 out of the 18 samples classified as honeydew honeys could be honeydew blends. But the 100% of honeys classified as chestnut honeys and ling honeys and the 75% of the samples classified as multifloral honeys rich in *Castanea sativa* pollen, had values of HD% higher than 70%, so, although this expression could be useful, it is necessary to be careful with its application. The sensory characteristics used for honeydew discrimination were very dark colour, medium sweetness and warm odour and aroma like caramel, toast, wood, dried fruit and cooked fruit (prunes, raisins).

3.1.2. Chestnut honeys

Chestnut is an over-represented pollen plant, so, according to the harmonized methods of the IHC, to be considered monofloral, its pollen frequency has to be higher than 86% (Von der Ohe *et al.*, 2004). Nevertheless, in Spain, such chestnut honeys as those with the Geographical Protected Indication “Mel de Galicia” (OJEU, 2007) are considered monofloral with pollen percentages above 70% (Seijo *et al.*, 1997; Escuredo *et al.*, 2012). Escuredo *et al.* (2014) classified as chestnut honeys those samples with chestnut pollen percentage above 64.1% and Anjos *et al.* (2015) with percentages between 60-65%. In our study, honeys classified as “chestnut samples” contained more than 68% chestnut pollen grains and their most important sensory descriptors were a very dark colour, light bitter taste, and animal and warm odour and aroma.

In the West area of Castilla y León (Zamora, Salamanca and León provinces), both oak and chestnut trees are common. Chestnut honeydew honeys could be produced (Iglesias *et al.*, 2004) since the weather conditions stimulate secretions of the living parts of *Castanea sativa*

trees that are picked up by bees. González-Paramás *et al.* (2007) claimed that sweet chestnut honeys from the mentioned three provinces could be naturally made with a mixture of nectar and honeydew. With regard to the samples analysed in this study, 3 out of the 18 suspected *Quercus* sp. honeydew honeys had high content in *Castanea sativa* pollen (above 45%), while two out of the four chestnut honeys had an important content in honeydew elements (average of 0.64). Botanical classification of our samples was carried out on the basis of both melissopalinology and sensory characteristics. Thus, in this case, chestnut samples were not considered honeydew honeys.

3.1.3. Heather honeys

Heather honeys from Castilla y León region include mainly Ericaceae species that flowering in summer such as *Erica cinerea* (bell heather), *Erica vagans* (Cornish heather) and *Calluna vulgaris* (ling heather). For monofloral heather honeys from *Erica* sp., the pollen frequency have to be more than 45% of the total pollen, but heather *Calluna vulgaris* honeys (Ling honeys) can be under-represented in some cases, so they are considered monofloral with a pollen percentage above 10% and typical sensory attributes for this kind of honey (Von der Ohe *et al.*, 2004). In this study, dark colour, medium bitter taste, and floral, warm-caramelized and woody odour and aroma descriptors were considered for ling identification. Four out of the ten heather samples were from *Calluna vulgaris*, three from *Erica* sp. and the other three were heather samples with melissopalinology and sensory characteristics of both *Erica* sp. and *Calluna vulgaris*.

3.1.4. Lavender honeys

Our lavender honeys came mainly from such *Lavandula* species as *Lavandula latifolia* (spike lavender) and *Lavandula stoechas* (French lavender). Two samples came from "Lavandin" (*Lavandula x intermedia* or *Lavandula angustifolia x latifolia*). Lavender pollen is under-represented in honey. Lavandin has been widely cultivated in recent years due to its flavouring properties (Castro-Vázquez *et al.*, 2014). As hybrid, lavandin is sterile, and the number of pollen grains is particularly low, being almost impossible to properly classify a Lavandin honey by melissopalinology (Guyot-Declerck *et al.*, 2002). In this study, identification of lavender honeys was carried out by both melissopalinology and sensory analysis. The most important sensory characteristics of our lavender honeys were light colour, medium-high sweetness, floral/fruity and vegetal odour and aroma (that was more aromatic and balsamic in honeys from spike lavender). For the classification of lavandin honeys, the beehives location within extensive lavandin cropping areas was also taken into account.

3.1.5. Clover honeys

In Castilla y Leon, clover honeys come from Leguminosae Type *Trifolium* species. All clover honeys of this study were from Ávila province.

3.2. Physicochemical parameters

Table 2 shows the averages, standard deviations, maximum and minimum values of HMF, diastase activity, moisture content, electrical conductivity, pH, free acidity, lactones, total acidity, proline content and specific optical rotation.

Table 2. Mean, standard deviation (SD), minimum and maximum values of the physicochemical parameters of honeys from Castilla y León.

	Chesnut (n=4)	Clover (n=3)	Heather (n=10)	Honeydew (n=18)	Lavender (n=4)	Multifloral (n=15)
	Mean±SD (min; max)	Mean±SD (min; max)	Mean±SD (min; max)	Mean±SD (min; max)	Mean±SD (min; max)	Mean±SD (min; max)
HMF (mg/kg)	0.58 ± 0.59^{A} (ND; 1.40)	$7.02\pm3.91^{A,B}$ (4.33; 11.50)	$3.84\pm5.81^{A,B}$ (ND; 16.60)	$2.61\pm4.00^{A,B}$ (ND; 12.40)	$6.10\pm7.56^{A,B}$ (ND; 17.14)	5.52 ± 6.69^{B} (0.05; 25.15)
Diastase (º Schade)	24.19±4.11 (20.98; 30.18)	24.33±5.73 (18.99; 30.39)	20.33±14.29 (8.58; 47.98)	24.02±5.79 (15.46; 37.40)	19.80±7.84 (10.98; 28.88)	23.73±8.32 (12.25; 43.06)
Moisture (%)	16.5 ± 0.4^{a} (16.0; 16.9)	16.7 ± 1.7^{a} (15.2; 18.6)	$16.2\pm1.1^{a,b}$ (14.5; 17.4)	$15.6\pm0.6^{a,b}$ (14.4; 16.7)	15.2 ± 0.5^{b} (14.7; 15.8)	$15.5\pm0.6^{a,b}$ (14.6; 16.7)
Conductivity (mS/cm)	1.092±0.108 (0.999; 1.247)	0.493±0.191 (0.337; 0.706)	0.838±0.154 (0.625; 1.126)	0.998±0.112 (0.801; 1.213)	0.372±0.235 (0.177; 0.701)	0.718±0.376 (0.326; 1.494)
pH	4.66±0.12 (4.57; 4.81)	3.98±0.33 (3.64; 4.29)	4.36±0.41 (4.00; 5.25)	4.65±0.22 (4.35; 4.97)	3.97±0.26 (3.75; 4.35)	4.39±0.35 (3.91; 4.98)
Free acidity (meq/kg)	37.4 ± 3.6^{a} (33.7; 41.5)	42.4 ± 6.4^{a} (38.3; 49.7)	37.3 ± 7.5^{a} (24.5; 47.2)	39.1 ± 5.2^{a} (30.3; 48.2)	25.3 ± 4.1^{b} (20.5; 29.7)	27.6 ± 4.9^{b} (20.2; 38.9)
Lactones (meq/kg)	1.8±1.3 (1.0; 3.8)	4.8±1.0 (4.1; 6.0)	5.6±2.5 (2.3; 8.3)	2.1±1.1 (0.6; 4.3)	2.3±1.9 (0.0; 4.6)	2.1±0.8 (0.7; 3.3)
Total acidity (meq/kg)	$39.3\pm4.8^{b,c}$ (34.8; 45.3)	47.2 ± 5.7^{c} (43.5; 53.8)	$42.9\pm8.8^{b,c}$ (27.0; 54.8)	$41.1\pm5.9^{b,c}$ (31.3; 52.5)	27.7 ± 5.7^{a} (20.5; 32.3)	$29.7\pm5.1^{a,b}$ (21.7; 41.8)
Lactones/free acidity	0.05±0.03 (0.03; 0.09)	0.12±0.04 (0.08; 0.16)	0.15±0.06 (0.05; 0.26)	0.05±0.20 (0.02; 0.09)	0.09±0.07 (0.00; 0.17)	0.08±0.03 (0.03; 0.15)
Proline (mg/100 g)	$71.58\pm10.37^{B,C}$ (58.83; 81.00)	73.61 ± 2.96^{C} (70.28; 75.95)	$66.69\pm19.85^{A,B}$ (52.29; 116.89)	$74.97\pm13.68^{B,C}$ (55.34; 112.92)	$59.56\pm24.27^{A,B,C}$ (29.89; 89.02)	53.93 ± 10.29^{A} (39.46; 77.36)
Specific rotation (º)	$-5.41\pm4.57^{a,b}$ (-11.38; -0.25)	$-9.96\pm2.00^{b,c}$ (-11.63; -7.75)	-13.45 ± 6.60^{c} (-21.00; -2.50)	-1.81 ± 3.72^{a} (-8.63; 4.38)	$-7.69\pm4.59^{a,b}$ (-11.75; -1.50)	$-7.37\pm4.03^{a,b}$ (-13.50; -1.38)

Mean values within the same row having different lower-case letter are significantly different by Student-Newman-Kreus multiple range test (p<0.05). Medians values within the same row having different capital letter are significantly different by the median nocht in the Box-and-Whisker Plot (p<0.05). No letters mean that ANOVA could not be determined. ND: Not Detected.

3.2.1. Hydroxymethylfurfural

HMF is one of the most important quality factors. It is an indicator of honey freshness, because in fresh honeys HMF is absent or only present in small amounts, increasing during its processing or aging (Bogdanov *et al.*, 1999; Ruoff and Bogdanov, 2004). As expected, HMF of all the analysed honeys was lower than the maximum value of 40 mg/kg established by the regulations (OJEC, 2002), because our samples were fresh raw honeys that did not suffer

overheat or storage before being analysed. Only 13% samples had HMF values higher than 10 mg/kg. Chestnut honeys showed the lowest HMF average (0.58 mg/kg) and clover the highest one (7.02 mg/kg). The maximum HMF content was found in a multifloral honey from Burgos province (25.15 mg/kg).

3.2.2. Diastase activity

Together with HMF, the activity of diastase (the most heat-resistant enzyme in honey), is an indicator of honey freshness. Diastase activity is not only susceptible to aging and overheating, but also varies in relation with the source of honey (Persano-Oddo *et al.*, 1990; Juan-Borrás *et al.*, 2014). According to Bogdanov *et al.* (2004), this parameter could only be used for the floral discrimination of fresh honeys. However, despite being fresh honeys, our samples showed similar diastase values, so that this parameter was not useful for its botanical characterization. Diastase averages ranged between 19.80º Schade (lavender) and 24.33º Schade (clover). Two *Calluna vulgaris* honeys showed the minimum (8.58º Schade) and maximum (47.98º Schade) values for diastase index. All samples complied with the minimum value of 8 Schade units (OJEC, 2002). In the literature, diastase index vary greatly among honeys (Annex 1/Table 1). Similar values of diastase activity were found by Marini *et al.* (2004) for Italian chestnut honeys (24.47º Schade), Kamboj *et al.* (2013) for Indian clover honeys (26.08º Schade), Andrade *et al.* (1999) for Portuguese heather honeys (23.61º Schade), Rodríguez-Flores *et al.* (2015) for Spanish honeydew honeys (23.90º Schade) and Serra-Bonvehí and Ventura-Coll (1993) for Spanish lavender honeys (20.90º Schade). Thrasyvoulou and Manikis (1995) reported a higher diastase index for honeydew, chestnut and heather Greek honeys, while Golob and Plestenjak (1999) reported a lower diastase number in honeydew honeys and chestnut honeys from Slovenia.

3.2.3. Moisture

Water content of honey is related to different factors such as botanical origin of nectar, climatic factors, harvesting season and degree of maturity, among others (Finola *et al.*, 2007). The moisture in honey is of great importance. Moisture contributes to honey's stability and tendency to granulation during storage (Singh and Bath, 1997). High water content leads to undesirable honey fermentation, flavour loss and spoilage, decreasing the shelf-life and the quality of the product (Costa *et al.*, 1999; Terrab *et al.*, 2003a). Current regulations (OJEC, 2002) establish a maximum moisture value of 20%, being 23% the maximum value for ling heather (*Calluna vulgaris*) honey as an exception. After ling heather, clover honey is a type of honey with high moisture content (Bogdanov *et al.*, 1999). In the first revision of the Codex Alimentarius Standard for Honey (1987), the maximum moisture content established for clover honey was the same as for ling honey, but in the last revision (Codex Alimentarius Standard for Honey, 2001), a maximum of 20% was considered appropriate for clover honey. In this study, the moisture percentage for samples varied from 14.4% found in a honeydew

honey and 18.6% found in a clover honey. Average moisture values ranged from 15.17% for lavender honeys and 16.73% for clover honeys, indicating optimum harvesting practices and a good degree of maturity (Downey *et al.*, 2005) of all the analysed honeys of this study. Clover and chestnut possessed significantly higher values than lavender samples. In the literature (Annex 1/Table 1), higher mean values were reported for heather honeys from different geographical origins and different Ericaceae species (Alves *et al.*, 2013; Moise *et al.*, 2013). Our research showed lower moisture averages for clover and lavender samples than those reported by other authors for these honeys (Malacalza *et al.*, 2005; Estevinho *et al.*, 2013). Nanda *et al.* (2003) reported the highest moisture percentages for *Trifolium* sp. in comparison with other honey types. With regard to chestnut and honeydew honeys, variable moisture values were reported in the literature (Mateo and Bosch-Reig, 1998; Golob and Plestenjak, 1999; Terrab *et al.*, 2003b; Küçük *et al.*, 2007).

3.2.4. Electrical conductivity

In respect of electrical conductivity (EC), this parameter depends on the mineral content, inorganic ions, organic acids, proteins and other components such as sugars and polyols, which can act as electrolytes (White, 1979b). In the current regulations, electrical conductivity has replaced the tedious determination of honey ash content in routine honey control (Codex Alimentarius Standard for Honey, 2001), because many researchers found high statistically significant linear correlations between both parameters (Sancho *et al.*, 1991a; Pires *et al.*, 2009; Silva *et al.*, 2009; Feás *et al.*, 2010a). EC is directly related to the floral origin, being important for botanical discrimination and characterisation. For this reason, according to the legislation, honey samples with values lower than 0.8 mS/cm are classified as floral honeys, having honeydew and chestnut honeys values higher than 0.8 mS/cm. There are some exceptions such as bell (*Erica cinerea*) and ling (*Calluna vulgaris*) heather honeys that can exceed this value, due to their high mineral content. In our samples, as expected, chestnut and honeydew honeys showed the highest electrical conductivity averages (1.092 mS/cm and 0.998 mS/cm respectively), with minimum values higher than 0.8 mS/cm, while lavender and clover showed the lowest averages (0.372 mS/cm and 0.493 mS/cm respectively). Heather honey showed an average of 0.838 mS/cm, being the average of 0.979 mS/cm for ling heather honeys and 0.745 mS/cm for the rest of heather samples (blends of *E. cinerea* and *E. vagans* or blends of both with *Calluna vulgaris*). The range of conductivity varied between 0.177 mS/cm from a lavender honey and 1.494 mS/cm from a multifloral honey rich in *Castanea sativa* pollen. Four multifloral honeys with high content in *Castanea sativa* pollen showed an electrical conductivity average of 1.159 mS/cm, higher than honeydew and chestnut averages. In general, lower mean values for clover (Vanhanen *et al.*, 2011), heather (Marini *et al.*, 2004) and lavender (Persano-Oddo *et al.*, 2004) honeys, higher values for chestnut honeys (Devillers *et al.*, 2004) and similar values for honeydew honeys from *Quercus* sp. (González-Lorente *et al.*, 2008) were found in the literature references (Annex

1/Table 1). Other honeydew honeys such as metcalfa (Persano-Oddo *et al.*, 1995) or fir (Karabagias *et al.*, 2014) showed higher conductivity averages than oak honeydew honeys.

3.2.5. pH

Due to the presence of organic acids, the honey pH generally varies from 3.5 to 5.5 (Bogdanov *et al.*, 2004). Blossom honeys have values between 3.5 and 4.5, being higher than 4.5 the pH for honeydew honeys because of its higher mineral content. Nectar chestnut honey is an exception, presenting honeydew-like pH (Bogdanov, 2011; Pasini *et al.*, 2013). Honeys with pH lower than 3.5 are considered as fragile for an adequate preservation because they are prone to ferment (Chefrour *et al.*, 2009). pH is important during honey extraction and storage, because it affects the texture and shelf life of honey, and can help increase its stability against microbial spoilage (Terrab *et al.*, 2002; Bogdanov *et al.*, 2004; Gomes *et al.*, 2010). Honey pH is not directly related to free acidity because the mineral salts act as a buffer (Abu-Tarboush *et al.*, 1993). In this study, lavender and clover honeys showed the lowest pH average, followed by heather and multifloral honeys, all of them with pH mean values lower than 4.5, as it was reported by other researchers for blossom honeys (Annex 1/Table 1). On the other hand, chestnut and honeydew honeys showed pH averages above 4.5. In general, higher values of pH were described in the literature for European chestnut honeys (mean values between 4.90 and 5.90). Our results for chestnut samples were in agreement with those found by León-Ruiz *et al.* (2011) for the same unifloral honeys from Castilla la Mancha (Central Spain). Similar pH values were reported for *Trifolium* sp. honeys (Kamboj *et al.*, 2013; Özcan and Ölmez, 2014), heather honeys (Thrasyvoulou and Manikis, 1995; Mateo and Bosch-Reig, 1998; Terrab *et al.*, 2003a), oak honeydew honeys (Vela *et al.*, 2007; González-Lorente *et al.*, 2008; Pérez-Martín *et al.*, 2008; León-Ruiz *et al.*, 2011) and lavender honeys (Persano-Oddo *et al.*,2004; Chakir *et al.*, 2011; Castro-Vázquez *et al.*, 2014). Within the group of heather honeys, ling samples showed higher pH values (mean of 4.73) than the other heather samples (*Erica* sp. honeys and *Erica-Calluna* honeys) (mean of 4.11), which could be attributed to their floral or geographical origins, since ling honeys came from the Sub-Mediterranean phytosociological area of Soria and León provinces, while the other heather honeys came from the Atlantic-European and the Interior Peninsular phytosociological areas in Burgos (Figure 2). Multifloral honeys rich in chestnut from León province showed the highest pH values (average of 4.92), being the pH of these samples even higher than the one of chestnut honeys from the same province (mean value of 4.65).

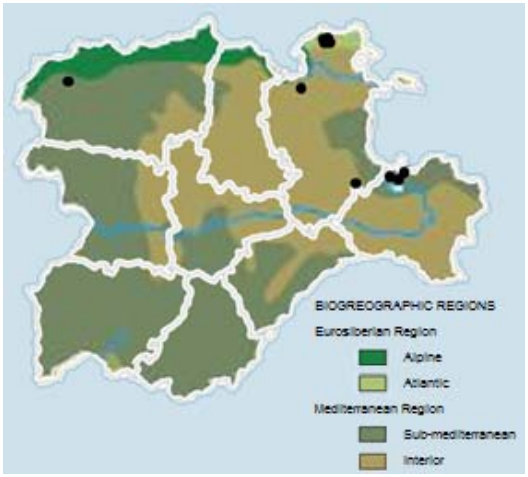

Figure 2. Heather honeys distribution in the phytosociological map of Castilla y León.

3.2.6. Free, lactone and total acidity

Acidity contributes to the honey flavour and aroma (Isla *et al.*, 2011). It is divided into free, lactone and total acidity. Organic acids are responsible for free acidity, being gluconic acid the most important. Free acids are in equilibrium with their corresponding lactones (or internal esters), and some inorganic ions such as phosphate, chloride or sulphate (Terrab *et al.*, 2004; Gomes *et al.*, 2010).

In this study, all samples showed free acidity values within the allowed European limits (below 50 meq/kg of honey) (Codex Alimentarius Standard for Honey, 2001; OJEC, 2002). High acidity values in honey can be indicative of sugars fermentation, being the resulting alcohol converted into organic acids by microbial action (Mato *et al.*, 2003; Cavia *et al.*, 2007). Lavender honeys, followed by multifloral samples, showed statistically significantly lower free acidity averages than the rest of the samples. Clover honeys presented the highest mean value. In general, both the Spanish and/or Portuguese chestnut honeys analysed by León-Ruiz *et al.* (2011), Anjos *et al.* (2015) and the chestnut honeys of this research, showed higher free acidity values than those reported by other authors for honeys from the rest of Europe (Devillers *et al.*, 2004; Fallico *et al.*, 2004; Kropf *et al.*, 2010). The literature references also described lower values of free acidity for clover honeys from other geographical origins than those obtained in our study (Nanda *et al.*, 2003; Malacalza *et al.*, 2005). In respect of the rest of unifloral honeys, our results agreed with the data reported for heather honeys harvested in Spain and Portugal (Nozal-Nalda *et al.*, 2005; Alves *et al.*, 2013), oak honeydew (Iglesias *et al.*, 2004; Pérez *et al.*, 2007) and lavender honeys (Pérez-Arquillué, 1995; Nozal-Nalda *et al.*, 2005; Anjos *et al.*, 2015), although other researchers described a great variability of data (Annex 1/Table 1). The main factors that have been described as responsible for the differences in honeys' free acidity values are related to their botanical and geographical origins, harvest season and storage conditions (Pérez-Arquillué *et al.*, 1995; Acquarone *et al.*, 2007; Kahraman *et al.*, 2010; Alves *et al.*, 2013).

Lactones are considered as a sort of acidity reserve when honey is exposed to alkaline conditions (Terrab *et al.*, 2002). Lactones vary irregularly, not providing useful information for honey floral discrimination (Bogdanov *et al.*, 2004). However, its determination is interesting because their hydrolysis increases free acid (Cavia *et al.*, 2007). In this study, the highest lactone acidity average was found in heather honeys followed by clover honeys, being lactones low in the rest of unifloral samples. In the literature, variable data for lactones were found in chestnut, heather, honeydew and lavender honeys, with averages lower than 13 meq/kg (Annex 1/Table 1). Lactones' values higher than 15 meq/kg were reported for clover honeys (Nanda *et al.*, 2003; Malacalza *et al.*, 2005).

Total acidity is the sum of free acidity and lactones. In our study, lavender honeys showed the lowest average and clover honeys the highest. Total acidity values ranged from 20.5 meq/kg in a lavender sample to 54.8 meq/kg in a heather sample.

Lactones/Free acidity (L/FA) might be related to honeys' origin. White *et al.* (1962) suggested a possible inverse relation between the L/FA ratio and pH, based on the equilibrium between organic acids and their corresponding lactones, depending on the pH of the medium. The lower the pH value is, the highest the lactone quantity and L/FA ratio will be. As expected, our unifloral honeys with the highest pH averages (chestnut and honeydew), showed the lowest lactones and L/FA ratio. But lavender samples with the lowest pH, had low lactones and medium L/FA ratio.

3.2.7. Proline

Proline is the most abundant free amino acid in honey (White, 1978). It mainly comes from honeybee salivate secretions, so that it would not be a suitable parameter for honey's botanical origin characterization (Von der Ohe *et al.*, 1991). Other authors claim that proline also comes from the pollen that bees consume in early life (González-Paramás *et al.*, 2006), and some researchers reported that honey's proline quantity depends on the time spent by the bee in processing the nectar. According to some literature references, proline is characteristic of the type of honey that in turn depends on the extent and duration of the flowering (Cotte *et al.*, 2004; Kečkeš *et al.*, 2013). Some researchers found the highest proline values in honeydew honeys (Dinkov, 2001; Terrab *et al.*, 2002; Baroni *et al.*, 2009), whereas other researchers reported the highest concentrations in chestnut honeys (Šarić *et al.*, 2008; Bertoncelj *et al.*, 2011), concluding that the main source of proline in honey would be the pollen, because chestnut honeys possess high pollen content (Hermosín *et al.*, 2003). The desirable proline content in honeys should be higher than 200 mg/kg (Bogdanov, 2011), being values lower than 180 mg/kg possible indicators of sugar adulteration or an insufficient honey ripeness (Bogdanov *et al.*, 1999).

In our study, all samples showed levels of proline above 180 mg/kg, indicating that honeys were ripened and not adulterated. Values ranged between 298.9 mg/kg corresponding to a

lavender honey to 1168.9 mg/kg corresponding to a heather honey. Multifloral and lavender honeys had the lowest proline averages and honeydew, clover and chestnut honeys the highest ones. Nevertheless, the high variability of proline content found in this research, which agrees with the literature (Annex 1/Table 1), seems to indicate that proline concentration is not very useful for honey botanical characterization (Sancho *et al.*, 1991b; Sánchez *et al.*, 2001; Serrano *et al.*, 2004; Iglesias *et al.*, 2006; Kivrak, 2015).

3.2.8. Specific rotation

Honey has the property of rotate the plane of polarized light. Some sugars rotate the polarized light angle to the left, presenting a negative optical rotation value (laevorotatory sugars such as fructose $[\alpha]_D^{20}$=-92.4º), while others rotate to the right, with positive optical activity (dextrorotatory, such as glucose $[\alpha]_D^{20}$=+52.7º). The overall value for the optical rotation depends on the concentration of the different honey sugars (Bogdanov *et al.*, 1999; Dinkov, 2003). Nectar honeys are laevorotatory, due to the normal preponderance of fructose over glucose, in contrast to honeydew honeys, which are usually dextrorotatory due to its lower fructose content and its higher oligosaccharide mass fraction, mainly melezitose ($[\alpha]_D^{20}$=+88.2°) and erlose ($[\alpha]_D^{20}$=+121.8°) (Persano-Oddo *et al.*, 1995; Bogdanov and Martin, 2002). Some countries such as Greece, Italy and UK have applied this parameter to distinguish between blossom and honeydew honeys, but this procedure has not been harmonized yet (Bogdanov *et al.*, 1999). Many authors have separated blossom honeys from honeydew honeys measuring optical rotation, finding all blossom honeys laevorotatory and honeydew honeys dextrorotatory (Persano-Oddo *et al.*, 1995; Bogdanov *et al.*, 2004; Bertoncelj *et al.*, 2011). In our case, all the samples had a negative optical rotation average, even honeydew honeys (although these samples owned the lowest fructose average content, and the highest melezitose and erlose average contents, as shown in section 3.2.9). In the case of honeydew honeys, this negative value were the lowest (-1.81º), having 6 out of the 18 honeydew honeys positive optical rotation values. Both the highest negative optical rotation average (-13.45º) and the highest negative value (-21.00º) corresponded to heather honeys. The minimum value of -2.50º in heather honeys was found in a ling heather sample, with a low percentage of *Calluna vulgaris* pollen and a few honeydew elements. For chestnut honeys, variable data were described in literature, but all of them were negative values (Annex 1/Table 1). Regarding clover and heather honeys, our values were similar than those reported by Nanda *et al.* (2003) for Indian *Trifolium alexandrinum* honeys and by Marini *et al.* (2004) and Persano-Oddo *et al.* (2004) for Italian *Erica* sp. honeys. In the literature positive optical rotation averages were reported for honeydew honeys. Can *et al.* (2015) reported very low positive optical rotation for oak honeys (0.74º), while higher positive specific rotation values were found in metcalfa (17.00º) or fir (12.60º) honeydew samples (Persano-Oddo *et al.*, 1995; Bertoncelj *et al.*, 2011). With regard to lavender honeys, Persano-Oddo *et al.* (2004) reported mean values similar to ours for European lavender samples.

Conversely, lower negative values for spike lavender (*Lavandula latifolia*) and French lavender (*Lavandula stoechas*) from Spain were found by Pérez-Arquillué *et al.* (1995).

3.2.9. Sugar profile

In this study, 14 sugars were identified and quantified, including two monosaccharides (fructose and glucose), five disaccharides (sucrose, trehalose, maltose, gentiobiose and isomaltose), six trisaccharides (raffinose, erlose, melezitose, maltotriose, panose and isomaltotriose) and one tetrasaccharide (maltotetrose). Figure 3 displays a typical GC sugar profile. Table 3 shows the mean values, standard deviations and concentrations ranges for each sugar.

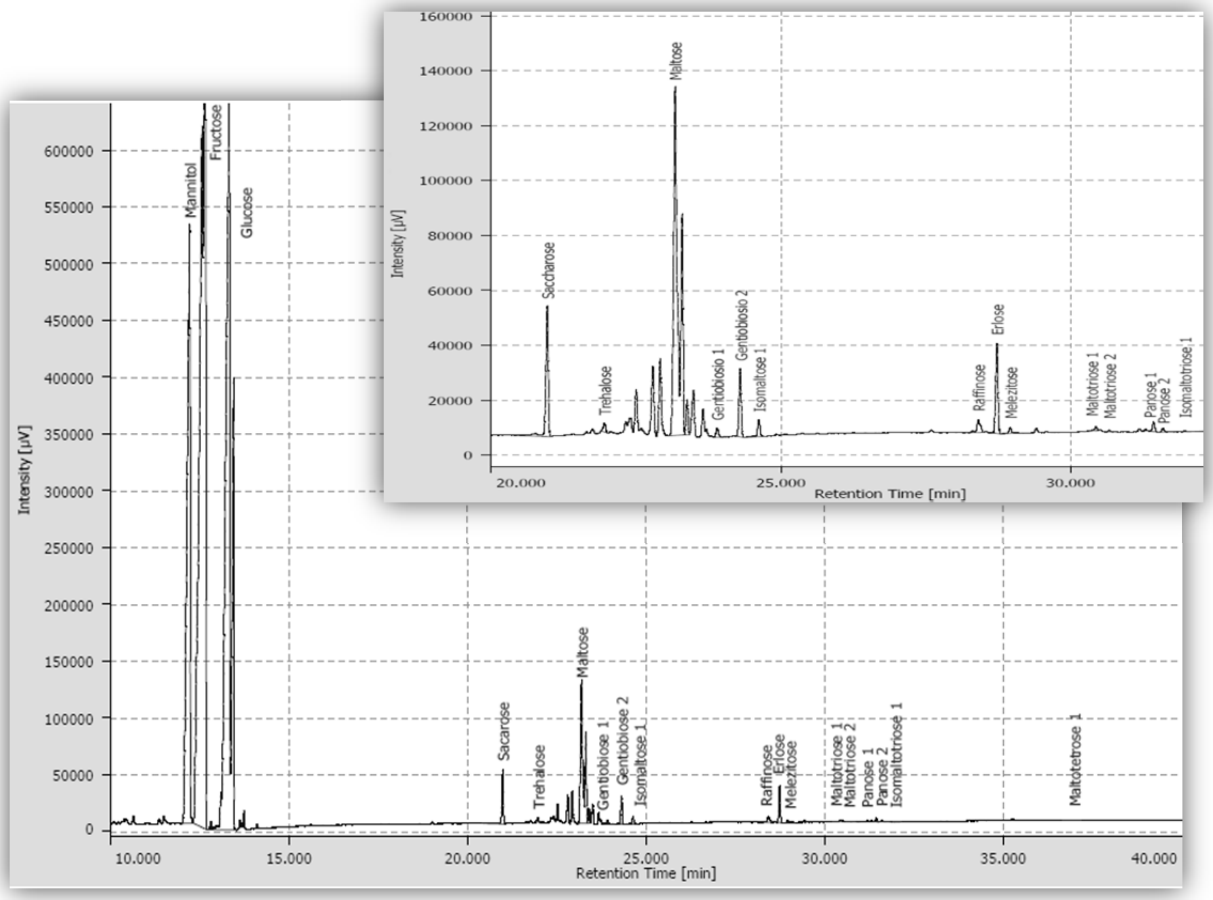

Figure 3. GC sugar profile of a honey sample from Castilla y León.

Table 3. Mean, standard deviation (SD), minimum and maximum values of the sugar profiles of honeys from Castilla y León.

	Chesnut (n=4)	Clover (n=3)	Heather (n=10)	Honeydew (n=18)	Lavender (n=4)	Multifloral (n=15)
	Mean±SD (min; max)	Mean±SD (min; max)	Mean±SD (min; max)	Mean±SD (min; max)	Mean±SD (min; max)	Mean±SD (min; max)
Fructose (%)	37.53±4.40 (32.69; 43.38)	38.08±1.03 (37.12; 39.17)	39.93±2.27 (35.55; 42.45)	36.24±1.24 (34.56; 38.93)	38.52±0.68 (37.59; 39.05)	39.07±1.76 (35.81; 42.60)
Glucose (%)	$27.30±2.84^{b}$ (24.74; 31.35)	$30.24±1.89^{a,b}$ (28.87; 32.40)	$31.03±3.02^{a}$ (26.26; 35.10)	$26.86±1.17^{b}$ (24.75; 28.83)	$31.38±1.38^{a}$ (29.39; 32.54)	$30.07±3.35^{a,b}$ (24.14; 35.31)
Sucrose (%)	0.10±0.07 (0.02; 0.18)	0.23±0.05 (0.18; 0.27)	0.26±0.24 (0.01; 0.73)	0.37±0.28 (0.07; 0.85)	1.50±2.47 (0.05; 5.18)	0.37±0.33 (0.05; 1.22)
Trehalose (%)	0.06±0.05* (0.03; 0.13)	0.07±0.01* (0.06; 0.07)	0.11±0.09* (0.04; 0.3)	0.08±0.04* (0.03; 0.16)	0.08±0.01* (0.07; 0.1)	0.11±0.06* (0.05; 0.27)
Maltose (%)	$4.26±1.15^{a,b}$ (2.54; 4.90)	$5.14±1.14^{a}$ (3.89; 6.11)	$3.34±1.03^{b}$ (2.32; 5.96)	$4.32±1.17^{a,b}$ (2.91; 6.56)	$4.04±0.89^{a,b}$ (3.19; 5.27)	$4.23±0.73^{a,b}$ (2.93; 5.54)
Gentiobiose (%)	0.19±0.02 (0.16; 0.20)	0.11±0.03 (0.08; 0.13)	0.17±0.11 (0.04; 0.35)	0.19±0.05 (0.10; 0.26)	0.11±0.04 (0.08; 0.17)	0.16±0.06 (0.09; 0.27)
Isomaltose (%)	1.61±0.19 (1.34; 1.74)	1.06±0.41 (0.6; 1.38)	1.06±0.85 (0.29; 3.12)	1.75±0.33 (1.17; 2.49)	0.88±0.29 (0.6; 1.16)	1.26±0.42 (0.65; 1.97)
Raffinose (%)	0.07±0.09* (0.01; 0.2)	0.06±0.04* (0.03; 0.10)	0.05±0.05* (ND; 0.12)	0.08±0.06* (0.03; 0.24)	0.03±0.02* (0.01; 0.05)	0.08±0.06* (0.01; 0.17)
Erlose (%)	$0.41±0.29^{A,B}$ (0.08; 0.72)	$0.76±0.08^{A}$ (0.67; 0.8)	$0.24±0.26^{B}$ (0.02; 0.93)	$0.84±0.34^{A}$ (0.29; 1.42)	$0.81±0.54^{A,B}$ (0.32; 1.4)	$0.69±0.41^{A,B}$ (0.28; 1.71)
Melezitose (%)	$0.15±0.14^{A,B}$ (0.05; 0.36)	$0.08±0.02^{B}$ (0.06; 0.10)	$0.16±0.23^{B}$ (ND; 0.68)	$0.50±0.49^{A}$ (0.10; 1.73)	$0.07±0.03^{B}$ (0.04; 0.10)	$0.25±0.29^{A,B}$ (0.05; 1.22)
Maltotriose (%)	0.05±0.02 (0.02; 0.07)	0.09±0.01 (0.09; 0.10)	0.07±0.08 (ND; 0.28)	0.09±0.02 (0.06; 0.16)	0.11±0.05 (0.05; 0.15)	0.09±0.05 (0.03; 0.20)
Panose (%)	$0.20±0.06^{A,B}$ (0.11; 0.24)	$0.25±0.12^{A,B,C,D}$ (0.16; 0.38)	$0.13±0.12^{D}$ (ND; 0.41)	$0.25±0.05^{A}$ (0.14; 0.33)	$0.15±0.02^{C,D}$ (0.13; 0.18)	$0.17±0.06^{B,C}$ (0.04; 0.25)
Isomaltotriose (%)	0.04±0.02 (0.01; 0.05)	0.01±0.00 (0.01; 0.01)	0.04±0.07 (ND; 0.22)	0.04±0.02 (ND; 0.08)	0.01±0.01 (ND; 0.02)	0.02±0.02 (ND; 0.06)
Maltotetraose (%)	0.00±0.01 (ND; 0.01)	ND	0.03±0.09 (ND; 0.28)	0.01±0.01 (ND; 0.03)	0.00±0.01 (ND; 0.01)	0.03±0.05 (ND; 0.14)
Total sugars (%)	$71.95±6.67^{a}$ (65.80; 81.40)	$76.20±1.31^{a}$ (74.80; 77.40)	$76.59±4.69^{a}$ (69.30; 82.50)	$71.62±2.63^{a}$ (67.10; 780)	$77.68±1.99^{a}$ (76.00; 80.10)	$76.59±4.14^{a}$ (69.80; 84.50)
Fructose+glucose (%)	64.83±7.22 (57.43; 74.73)	68.32±2.90 (65.99; 71.57)	70.96±5.00 (61.81; 77.55)	63.10±2.22 (59.59; 67.76)	69.89±2.04 (66.98; 71.59)	69.14±4.72 (62.60; 77.24)
Total disaccharides (DS) (%)	$6.22 ±1.12^{A}$ (4.59; 6.95)	$6.62±1.57^{A,B}$ (4.82; 7.75)	$4.93±1.32^{B}$ (3.63; 7.41)	$6.71±1.22^{A}$ (5.05; 9.29)	$6.60±3.12^{A,B}$ (4.58; 11.25)	$6.13±0.82^{A}$ (4.66; 7.69)
Total tri- and tetrasaccharides (TS) (%)	$0.92±0.53^{a,b}$ (0.28; 1.47)	$1.25±0.24^{a,b}$ (1.02; 1.49)	$0.69±0.56^{a}$ (0.13; 1.65)	$1.81±0.67^{b}$ (0.74; 3.45)	$1.17±0.56^{a,b}$ (0.58; 1.83)	$1.35±0.58^{a,b}$ (0.56; 2.51)
Total DS + TS (%)	7.14±1.14 (5.79; 8.40)	7.87±1.76 (5.84; 8.99)	5.63±1.74 (3.90; 9.06)	8.52±1.29 (6.29; 11.34)	7.77±3.61 (5.16; 13.08)	7.46±1.06 (5.88; 9.72)

Mean values within the same row having different lower-case letter are significantly different by Student-Newman-Kreus multiple range test (p<0.05). Medians values within the same row having different capital letter are significantly different by the median nocht in the Box-and-Whisker Plot (p<0.05).* There is not statistically significant differences between the means or medians at the 95.0% confidence level (p-value greater than or equal to 0.05). No letters or * mean that ANOVA could not be determined. ND: Not Detected.

Honey shows a great variability both in carbohydrate composition and carbohydrate content, which is strongly related to the floral origin, and to a lesser extent to the seasonal climatic conditions and the geographical origin (Anklam, 1998; Mateo and Bosch-Reig, 1998).

As expected, fructose followed by glucose, were the main carbohydrates in the studied honeys. The fructose content varied between 32.69% and 43.38%, both obtained in chestnut honeys. Heather honeys possessed the maximum mean value (39.93%) and honeydew honeys the lowest one (36.24%), followed by chestnut honeys (37.53%), agreeing with the research of Escuredo *et al.* (2013). In terms of glucose content, the minimum value was 24.14% corresponding to a multifloral honey rich in chestnut, and the maximum of 35.31% to other multifloral honey, in this case rich in leguminosae type *Trifolium* sp. Lavender honeys, with the highest average (31.38%) together with heather samples, presented significantly higher values than honeydew (average of 26.86%) and chestnut (average of 27.30%) honeys. Compared to the other multifloral honeys (mean glucose value of 31.34%), all multifloral samples rich in chestnut from León province had very low glucose values (mean value of 24.90%), similar to those found in chestnut samples. Heather honeys from *Calluna vulgaris* had lower glucose average (28.00%) than the other heather honeys (33.05%). These results were in agreement with those reported in the quoted bibliography, where low glucose and fructose values for chestnut and honeydew samples and high for heather and lavender ones were found (Annex 1/Table 1). These values did not differ much among those reported by Mateo and Bosch-Reig (1997) for Spanish heather and lavender honeys, Ouchemoukh *et al.* (2010) for Algerian clover honeys and Escuredo *et al.* (2013) for Spanish chestnut and honeydew honeys.

In respect of disaccharides content, all samples contained all disaccharides determined in this study. In general, maltose was the major disaccharide in honey, agreeing with literature (Serra-Bonvehí and Ventura-Coll, 1993; Bentabol-Manzanares *et al.*, 2014), and the third main sugar in abundance. Maltose represented 67% of the total disaccharides, within a range from 2.32% (a heather honey) to 6.56% (a honeydew honey), with the clover samples showing the highest average (5.24%), significantly different than the lowest one showed in heather honeys (3.34%). Lower values of maltose were reported for European chestnut honeys by Primorac *et al.* (2011) and Escuredo *et al.* (2013). Shin and Ustunol (2005) showed higher values for clover honeys (10.20%), in contrast to the lower values (1.45% and 1.65% in *Melilotus* sp. and *Trifolium* sp. respectively) obtained by Ouchemoukh *et al.* (2010). Other researchers reported similar averages than ours for Spanish heather (Bentabol-Manzanares *et al.*, 2014), honeydew (Mateo and Bosch-Reig, 1998; Bentabol-Manzanares *et al.*, 2011) and lavender honeys (Serra-Bonvehí and Ventura-Coll, 1993; Mateo and Bosch-Reig, 1997). High amount of maltose could indicate adulteration by sugar syrup or starch hydrolysate (Horvath and Molnar-Perl, 1997; Cotte *et al.*, 2003). Higher averages for sucrose than for maltose were reported for Citrus (Mateo and Bosch-Reig, 1997) and lavender (Cotte *et al.*, 2003) honeys.

Martins *et al.* (2008) showed higher mean values for isomaltose than for maltose in "Serra da Lousa" (Portugal) heather honeys in 1992 and 1993. But in samples harvested in 1991, maltose mean value was higher. Differences among the samples from different harvests demonstrate a strong influence of harvest year on the sugar composition. In our study, only a ling heather sample showed isomaltose as the major disaccharide, followed by maltose. For most samples it was the second disaccharide in abundance. A heather honey possessed the maximum isomaltose content (3.12%), although the maximum average was for honeydew honeys (1.75%), followed by chestnut honeys, and the minimum for lavender ones (0.88%). Values of isomaltose were in agreement with the results found in the literature for European chestnut (Persano-Oddo *et al.*, 1995; Cotte *et al.*, 2003), Spanish oak honeydew (Mateo and Bosch-Reig, 1998; De la Fuente *et al.*, 2007) and lavender (Serra-Bonvehí and Ventura-Coll, 1993; Mateo and Bosch-Reig, 1997) honeys. Some researchers reported higher isomaltose concentration in honeydew honeys than in blossom ones (Mateo and Bosch-Reig, 1997; Bentabol-Manzanares *et al.*, 2011). In the literature, the averages for Spanish heather honeys widely ranged, varying from 0.38% (Terrab *et al.*, 2003a) to 3.60% (Serra-Bonvehí and Granados-Tarrés, 1993).

Sucrose is an important sugar from a legislative point of view, with a general maximum of 5% (OJEC, 2002). As exceptions, some honeys can have a maximum of 10% (such as clover honeys belonging to *Medicago sativa* genus) or 15% (such as lavender honeys). None of our honeys reached these limits, showing low concentrations for this sugar. The maximum value was found in one lavender sample (5.18%). Chestnut honeys had the lowest sucrose average (0.10%) and lavender the highest (1.50%), in accordance with those reported by Cotte *et al.* (2003). Ling heather honeys had lower average than the rest of heather samples (0.04% versus 0.40%). These low sucrose values suggest an advanced stage of honeys' ripening, by the conversion of sucrose into glucose and fructose (Terrab *et al.*, 2001; Pasini *et al.*, 2013), as well as the absence of artificial feeding of bees with sucrose syrups (Escuredo *et al.*, 2013). With regard to sucrose content, in the literature higher mean values than those found in our study were reported for chestnut (Golob and Plestenjak, 1999; Šarić *et al.*, 2008) and clover honeys (Shin and Ustunol, 2005; Ouchemoukh *et al.*, 2010). Meanwhile, the distribution for oak honeydew, heather and lavender honeys was broad, ranging between ND (Not Detected)-3.36%, 0.02-4.12% and 0.24-8.01%, respectively (Terrab *et al.*, 2003a; Nozal *et al.*, 2005; Nozal-Nalda *et al.*, 2005; Moise *et al.*, 2013).

Gentiobiose and trehalose were the minor disaccharides found in our samples. Their quantities were lower than 0.35%, with mean values lower than 0.19% for gentiobiose and 0.11% for trehalose. The literature references showed very low concentrations for these sugars (Terrab *et al.*, 2003a; Nozal *et al.*, 2005; Martins *et al.*, 2008; De la Fuente *et al.*, 2011; Escuredo *et al.*, 2014; Can *et al.*, 2015), being the quantities of trehalose higher than those of gentiobiose with a maximum mean value of 2.70% versus 0.22%, respectively (Cotte *et al.*,

2003; Nozal *et al.*, 2005; Rybak-Chmielewska *et al.*, 2013). Bentabol-Manzanares *et al.* (2011) observed higher concentration of trehalose in honeydew honeys (average of 1.89%) than in blossom honeys (average of 1.67%), although in a latter study the same authors reported a similar mean concentration (1.81%) in heather honeys from *Erica arborea* (Bentabol-Manzanares *et al.*, 2014).

Among the oligosaccharides, erlose was the most abundant and the only trisaccharide detected in every sample analysed in this study. Melezitose, raffinose, maltotriose and panose were detected in all the samples except in one heather honey; and isomaltotriose was absent in five multifloral honeys, two honeys from heather, one from honeydew and another one from lavender.

Melezitose was also proposed to differentiate honeydew and blossom honeys (Persano-Oddo and Piro, 2004), being considered an indicator of the presence of honeydew (Ouchemoukh *et al.*, 2010), together with other trisaccharides such as erlose or raffinose (Weston and Brocklebank, 1999; Bogdanov, 2011). In our research, the maximum averages for melezitose, erlose and raffinose were found in honeydew honeys (0.50%, 0.84% and 0.08%, respectively). These sugars were also detected in samples from all the botanical origins. On the one hand, no statistically significantly differences were found for raffinose among the botanical origins. On the other hand, clover and honeydew possessed significantly higher values of erlose than heather honeys, and honeydew had significantly higher values of melezitiose than clover, heather and lavender samples. Erlose is an intermediate trisaccharide in the metabolism of nectar sugars by honeybees (White and Maher, 1953; Kolayli *et al.*, 2012). In general, the presence of melezitose in blossom honeys is considered to be a result of their mixture with honeydew (Da Costa Leite *et al.*, 2000), meanwhile the origin of raffinose in blossom honey is not clear, being suggested that it could be in the nectar composition or it could also come from honeydew contaminations (White *et al.*, 1986; Kaškonienė *et al.*, 2010). Our data were in agreement with the values reported by other researchers, where these three sugars were found in similar concentration as those in our study (Mateo and Bosch-Reig, 1997; Cotte *et al.*, 2003; Nozal *et al.*, 2005; De la Fuente *et al.*, 2007; De la Fuente *et al.*, 2011). Maximum averages in the literature ranged from 0.52% (lavender honeys) to 2.47% (oak honeydew honeys) for melezitose (Nozal *et al.*, 2005; Can *et al.*, 2015); 0.24% (chestnut honeys) to 1.96% (heather honeys) for erlose (Cotte *et al.*, 2003; Nozal *et al.*, 2005); and 0.22% (chestnut honeys) to 2.03% (heather honeys) for raffinose (Devillers *et al.*, 2004; Martins *et al.*, 2008). The melezitose average reported in Spanish oak honeydew honeys by Escuredo *et al.* (2014) was lower than the mean value found in our research (0.21%). Other authors found higher melezitose concentrations in Spanish (Mateo and Bosch-Reig, 1997; Terrab *et al.*, 2003b; Nozal *et al.*, 2005) and European (Can *et al.*, 2015) oak honeydew honeys, and proposed melezitose content as a marker of the floral origin. In European fir honeydew honeys melezitose averages were higher than in oak honeydew samples, so that

melezitose could be a marker of honeydew honeys from different botanical origins (Golob and Plestenjak, 1999; Devillers *et al.*, 2004; Rybak-Chmielewska *et al.*, 2013). Bentabol-Manzanares *et al.* (2011) reported lower melezitose average in suspected honeydew honeys than in blossom ones.

The rest of trisaccharides (maltotriose, panose and isomaltotriose) were found in very low concentrations (always below 0.41%), in agreement with the averages reported in the literature (less than 0.66%) by different authors (Cotte *et al.*, 2003; De la Fuente *et al.*, 2007; De la Fuente *et al.*, 2011). High concentration of trisaccharides such as maltotriose could indicate an adulteration with different syrups (Cotte *et al.*, 2003).

Finally, maltotetraose, the only tetrasaccharide analysed, was present at very low concentrations in 14 out of the 54 samples: six honeydew honeys, five multifloral samples, two heather honeys and one lavender sample. Both maximum value (0.28%) and maximum average (0.03%) belonged to heather honeys. No data were found in the literature for maltotetraose for unifloral honeys of the same botanical origins as those of our research.

In respect of current regulations (OJEC, 2002), all samples fulfilled the legal requirements for the sum of fructose and glucose (more than 45% for honeydew and honeydew blend honeys and over 60% for blossom honeys). One *Castanea sativa* sample had 57.43% of monosaccharides, being under the limit stablished for blossom honeys. Sensory characteristics and pollen analysis of this honey revealed that it was a mixture of chestnut and honeydew honey, thus fulfilling the legal limit of 45% for honeydew-blend (mixture of blossom and honeydew honeys).

As expected, honeydew honeys showed the minimum average of the sum of fructose and glucose, and the highest mean value for total disaccharides and oligosaccharides, being the opposite situation for heather honeys. Chestnut honeys had quite low average value for the sum of fructose and glucose, and also quite low mean values for total disaccharides and oligosaccharides. Heather honeys presented significantly higher disaccharide content than chestnut, honeydew and multifloral samples, and significantly higher oligosaccharide content than honeydew ones. De la Fuente *et al.* (2011), also found for Spanish heather honeys the lowest average for disaccharides and low concentrations of trisaccharides, although the values of these researchers were higher than those found in our study (10.38% and 1.23% versus 4.93% and 0.69%, respectively). The total sugars averages varied between 71.62% (honeydew samples) and 77.78% (lavender samples). These data agree with the research of Escuredo *et al.* (2013), who reported lower mean contents for oak honeydew and chestnut honeys than for heather samples from the North of Spain. Our data of total monosaccharides and total carbohydrates were within the wide intervals reported in the literature references for honeys from the same botanical origins than our samples (Annex 1/Table 1), where in general, heather honeys showed the highest values for both parameters (Persano-Oddo and Piro, 2004;

Feás *et al.*, 2010b; De la Fuente *et al.*, 2011; Waś *et al.*, 2011) and honeydew honeys the lowest values of total monosaccharides (Persano-Oddo *et al.*, 1995; Pérez *et al.*, 2007).

Sugar profiles were proposed to help differentiate floral and honeydew honeys. According to Bogdanov *et al.* (2008), blossom honeys contain more oligosaccharides such as sucrose, maltose, trehalose or panose, among others. In our research, honeydew honeys were characterized by lower values of monosaccharides and higher data of di- and oligosaccharides, mainly melezitose, erlose and raffinose, but differences with blossom honeys in relation to carbohydrates' profile were no significant. Our carbohydrate data for honeydew samples agreed with literature (Mateo and Bosch-Reig, 1998; Weston and Brocklebank, 1999; Bogdanov *et al.*, 2008). The numerous di- and trisaccharides in honeydew honeys could be produced by microbial activity and enzymatic reactions in the intestinal tract of the aphids and during honey ripening (Kolayli *et al.*, 2012). Furthermore, Serra-Bonvehí *et al.* (1987) and Mateo and Bosch-Reig (1997) reported that honeydew honeys had more maltose and isomaltose than blossom ones. Our honeydew honeys presented the highest isomaltose concentration, but even though the maltose average was high in honeydew honeys, it was not the highest one. Cotte *et al.* (2003) characterised chestnut honeys by low trisaccharides quantity. In our study, chestnut samples were also poor in oligosaccharides.

3.2.10. Crystallization indexes and other carbohydrate ratios

Honeys' granulation is a natural process of paramount importance because an improper crystallisation could lead to problems in handling and processing (Özbalci *et al.*, 2013). It also affects honeys' textural properties, making this foodstuff less appealing to the consumer (Cavia *et al.*, 2002).

Table 4. Mean, standard deviation (SD), minimum and maximum values of the sugars ratios of artisanal honeys from Castilla y León.

	Chesnut (n=4)	**Clover (n=3)**	**Heather (n=10)**	**Honeydew (n=18)**	**Lavender (n=4)**	**Multifloral (n=15)**
	Mean±SD (min; max)	Mean±SD (min; max)	Mean±SD (min; max)	Mean±SD (min; max)	Mean±SD (min; max)	Mean±SD (min; max)
fructose/glucose ratio	1.37±0.04 (1.32; 1.40)	1.26±0.05 (1.21; 1.29)	1.29±0.08 (1.19; 1.40)	1.34±0.05 (1.25; 1.43)	1.23±0.03 (1.20; 1.28)	1.31±0.13 (1.18; 1.59)
glucose/moisture ratio	1.66±0.15 (1.50; 1.86)	1.81±0.08 (1.74; 1.90)	1.92±0.17 (1.65; 2.14)	1.74±0.11 (1.56; 1.98)	2.07±0.09 (2.00; 2.18)	1.94±0.26 (1.52; 2.37)
(glucose-moisture) /fructose ratio	0.29±0.04 (0.25; 0.33)	0.35±0.01 (0.34; 0.37)	0.37±0.05 (0.28; 0.43)	0.31±0.03 (0.26; 0.36)	0.42±0.03 (0.39; 0.45)	0.37±0.08 (0.21; 0.49)
maltose/isomaltose ratio	2.68±0.86 (1.55; 3.63)	5.16±1.28 (3.93; 6.48)	5.17±3.67 (0.92; 11.45)	2.77±1.22 (1.42; 6.20)	5.23±2.57 (2.93; 8.11)	3.80±1.73 (2.14; 7.89)

No letters mean that ANOVA could not be determined.

The honey granulation tendency was predicted on the basis of different crystallization indexes (Table 4). This foodstuff generally crystallizes quicker with fructose/glucose ratio (F/G) lower than 1.11; and glucose content, glucose/water ratio (G/W), (glucose-moisture)/fructose ratio ((G-W)/F) and melezitose values higher than 35%, 2.16, 0.49 and 10% respectively. On the

contrary, honeys with F/G higher than 1.33, and glucose content, G/W and (G-W)/F lower than 28%, 1.70 and 0.30 respectively, generally remains liquid for longer periods (Serra-Bonvehí, 1989; Sancho *et al.*, 1991d; Lupano, 1997; Manikis and Thrasyvoulou, 2001; Cavia *et al.*, 2002; Smanalieva and Senge, 2009). Rapid granulation occurs in honeys in one month, medium in 1-12 months and slow crystallization in longer than a year.

Manikis and Thrasyvoulou (2001) observed that glucose was not very useful granulation index in samples that had medium crystallisation tendency. They also observed that melezitose was a poor index because Greek fir honeys had more than 10% of melezitose and possessed a slow crystallisation tendency. F/G and G/W ratios were also unsuitable to predict honey crystallization, because the former did not take into account water content, and the latter did not consider the inhibitory action of fructose (Sancho *et al.*, 1991d). Moreover, G/W was not satisfactory for honeys with low moisture content (Tabouret, 1979), although some researchers indicated that it was more reliable than F/G index for the prediction of honey crystallization (Manikis and Thrasyvoulou, 2001). (G-W)/F demonstrated to be a better crystallization index because fructose was included (Serra-Bonvehí, 1989; Sancho *et al.*, 1991d).

In the honeys studied, F/G values varied between 1.18 (multifloral honey rich in legumes) and 1.59 (multifloral honey rich in *Castanea sativa* pollen). G/W values varied between 1.50 (chestnut honey) and 2.37 (multifloral honey rich in sunflower). In the literature, F/G ratios considerably varied in honeys from the same botanical origins (Annex 1/Table 1). These broad ranges depended on the accompanying flora, being indicative of the wide variety of vegetation sources, from which the honey samples came from.

Depending on the crystallization index employed, the granulation tendency of each of the five unifloral groups of this study was different. Lavender was always the honey that possessed the fastest granulation tendency, while honeydew and chestnut honeys presented the slowest one. Honeydew and chestnut samples showed glucose averages lower than 28% and F/G mean values higher than 1.33. Moreover, chestnut samples had a G/W average lower than 1.70 and a (G-W)/F mean value lower than 0.30. According to the more reliable granulation index ((G-W)/F)), crystallization speed increased in the following order: chestnut < honeydew < clover < heather < lavender. Using F/G index, granulation in chestnut honeys was also the slowest; being the slowest in honeydew samples if glucose and melezitose contents were considered, as well.

The vast majority of the researchers reported that both chestnut and honeydew were the honeys that remained liquid for longer times (Mateo and Bosch-Reig, 1997; Smanalieva and Senge, 2009; Primorac *et al.*, 2011). Escuredo *et al.* (2014) reported higher F/G (more than 1.40) and lower G/W (less than 1.50) ratios for chestnut and honeydew samples than the values described in our study for these honeys. Moderate crystallization tendency rates were

found for heather honeys in comparison with other honey types (Smanalieva and Senge, 2009; Escuredo *et al.*, 2014), agreeing with our data. In comparison with data of the literature references, higher F/G values for chestnut samples and lower G/W results for chestnut and lavender honeys were found (Persano-Oddo *et al.*, 1995; Mateo and Bosch-Reig, 1998; Cotte *et al.*, 2003; Persano-Oddo and Piro, 2004; Can *et al.*, 2015), whereas for the rest of monofloral honeys, our averages were within the intervals proposed by other researchers (Annex 1/Table 1).

It is interesting to highlight that in addition to the lowest glucose average, multifloral honeys from León province rich in chestnut pollen had the highest F/G average (1.54), the lowest G/W (1.53) and the lowest (G-W)/F (0.22), being this values considerably different than the averages found for the rest of multifloral honeys (1.25, 2.5 and 0.41 respectively).

Maltose/isomaltose ratio (M/I) was proposed as a possible marker of honey adulteration with such cheap sweet products as invert syrup, corn syrup and high fructose corn syrup (Horvath and Molnar-Perl, 1997). These syrups possess high amounts of glucose or high quantities of different di- and oligosaccharides such as maltose, maltotriose or sucrose (Anklam, 1998). Horvath and Molnar-Perl (1997) suggested that high M/I values might indicate the adulteration with starch hydrolysate, and low ratios the use of high fructose syrup. These researchers described the ranges of M/I for authentic honeys between 0.50 and 21.80. However, further studies demonstrated that this ratio should only be used for guidance, because some genuine honeys with natural M/I high index could be erroneously rejected (Nozal *et al.*, 2005). In our research, the M/I ratios ranged between 0.92 and 11.45 (both values belonged to heather honeys), being all values within the interval proposed for authentic honeys (Horvath and Molnar-Perl, 1997). Chestnut honeys, followed by honeydew samples, showed the lowest averages (2.68 and 2.77 respectively), and lavender the highest (5.23). Bentabol-Manzanares *et al.* (2011), whose data also fulfilled the proposed interval (Horvath and Molnar-Perl, 1997), showed a higher M/I average for honeydew honeys (7.04) than for blossom ones (5.53). On the other hand, Nozal *et al.* (2005) reported averages considerably lower than ours for heather, lavender and forest honeys from our same region.

3.3. Statistical analysis

Correlations between all considered parameters by Pearson test were studied in order to define which of them were significant (Annex 1/Table 2). One hundred ninety-nine significant correlations ($p<0.05$) were found between the physicochemical parameters studied, the most important of which are cited below.

Electrical conductivity showed a significant correlation with pH ($r=0.8484$). This correlation could be explained by the fact that conductivity depends on the mineral content of honey, related with the buffering capacity (Bertoncelj *et al.*, 2011). Conductivity and pH were also correlated with glucose ($r=-0.7374$ and $r=-0.7235$, respectively) and different parameters

related with glucose, such as total sugars and the different sugar ratios. Moderate correlations were found among conductivity and pH with other sugars such as isomaltose and gentiobiose (r>0.6). Honeydew and chestnut honeys had the highest conductivity, gentiobiose and isomaltose content and lowest glucose concentration. pH was neither correlated with free acidity nor with total acidity, probably because of the buffer capacity of such honey components as mineral compounds (White, 1979a; Terrab *et al.*, 2002; Ojeda de Rodríguez *et al.*, 2004).

As expected, the rotatory power was correlated with total sugars (r=-0.6661), especially fructose (r=-0.7628), glucose (r=-0.7468) and isomaltose (r=0.7275). Lower but also significant correlations were found with panose (r=0.6777) and total oligosaccharide content (r=0.6023).

Fructose and glucose were strongly correlated (r=0.7971). Other correlations between sugars were found, being the most important of which, glucose and isomaltose (r=-0.8197), fructose and panose (r=-0.7200), gentiobiose and isomaltose (r=0.8154), panose and isomaltose (r=0.8357), and isomaltose and isomaltotriose (r=0.7386).

In addition to fructose (r=0.8730) and glucose (r=0.8973), the most important sugars correlated with the total sugars content were isomaltose (r=-0.7260), panose (r=-0.6384) and gentiobiose (r=-0.6153).

Principal Component Analysis was applied to data structure study in a reduced dimension while retaining the maximum amount of variability present in data. Four components were extracted, since they had eigenvalues greater than or equal to 1.0. Together they account for 82.07% of the variability in the original data. In this case the combination of the variables was not informative enough for honey discrimination according to their floral origin.

REFERENCES

ABU-TARBOUSH, H M; AL-KAHTANI, H A; EL-SARRAGE, M S (1993) Floral-type identification and quality evaluation of some honey types. *Food Chemistry* 46(1): 13-17. http://dx.doi.org/10.1016/0308-8146(93)90068-q

ACQUARONE, C; BUERA, P; ELIZALDE, B (2007) Pattern of pH and electrical conductivity upon honey dilution as a complementary tool for discriminating geographical origin of honeys. *Food Chemistry* 101(2): 695-703. http://dx.doi.org/10.1016/j.foodchem.2006.01.058

ALVAREZ-SUAREZ, J M; TULIPANI, S; DÍAZ, D; ESTEVEZ, Y; ROMANDINI, S; GIAMPIERI, F; DAMIANI, E; ASTOLFI, P; BOMPADRE, S; BATTINO, M (2010) Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. *Food and Chemical Toxicology* 48(8/9): 2490-2499. http://dx.doi.org/10.1016/j.fct.2010.06.021

ALVES, A; RAMOS, A; GONÇALVES, M M; BERNARDO, M; MENDES, B (2013) Antioxidant activity, quality parameters and mineral content of Portuguese monofloral honeys. *Journal of Food Composition and Analysis* 30(2): 130-138. http://dx.doi.org/10.1016/j.jfca.2013.02.009

ANDRADE, P B; AMARAL, M T; ISABEL, P; CARVALHO, J C M F; SCABRA, R M; PROENÇA DA CUNHA, A (1999) Physicochemical attributes and pollen spectrum of Portuguese heather honeys. *Food Chemistry* 66(4): 503-510. http://dx.doi.org/10.1016/S0308-8146(99)00100-4

ANJOS, O; IGLESIAS, C; PERES, F; MARTÍNEZ, J; GARCÍA, A; TABOADA, J (2015) Neural networks applied to discriminate botanical origin of honeys. *Food Chemistry* 175: 128-136. http://dx.doi.org/10.1016/j.foodchem.2014.11.121

ANKLAM, E (1998) A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry* 63(4): 549-562. http://dx.doi.org/10.1016/S0308-8146(98)00057-0

ANUPAMA, D; BHAT, K K; SAPNA, V K (2003) Sensory and physico-chemical properties of commercial samples of honey. *Food Research International* 36(2): 183-191. http://dx.doi.org/10.1016/S0963-9969(02)00135-7

AOAC-ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (2012) Official Methods of Analysis of AOAC International. *Latimer J W (Ed).* Gaithersburg, Maryland, USA.

BARONI, M V; ARRUA, C; NORES, M L; FAYE, P; DÍAZ, M P; CHIABRANDO, A; WUNDERLIN, D A (2009) Composition of honey from Córdoba (Argentina): Assessment of North/South provenance by chemometrics. *Food Chemistry* 114(2): 727-733. http://dx.doi.org/10.1016/j.foodchem.2008.10.018

BENTABOL-MANZANARES, A; HERNÁNDEZ-GARCÍA, Z; RODRÍGUEZ-GALDÓN, B; RODRÍGUEZ-RODRÍGUEZ, E; DÍAZ-ROMERO, C (2011) Differentiation of blossom and honeydew honeys using multivariate analysis on the physicochemical parameters and sugar composition. *Food Chemistry* 126(2): 664-672. http://dx.doi.org/10.1016/j.foodchem.2010.11.003

BENTABOL-MANZANARES, A; HERNÁNDEZ-GARCÍA, Z; RODRÍGUEZ-GALDÓN, B; RODRÍGUEZ-RODRÍGUEZ, E; DÍAZ ROMERO, C (2014) Physicochemical characteristics of minor monofloral honeys from Tenerife, Spain. *LWT - Food Science and Technology* 55(2): 572-578. http://dx.doi.org/10.1016/j.lwt.2013.09.024

BERTONCELJ, J; GOLOB, T; KROPF, U; KOROŠEC, M (2011) Characterisation of Slovenian honeys on the basis of sensory and physicochemical analysis with a chemometric approach. *International Journal of Food Science & Technology* 46(8): 1661-1671. http://dx.doi.org/10.1111/j.1365-2621.2011.02664.x

BLASA, M; CANDIRACCI, M; ACCORSI, A; PIACENTINI, M P; ALBERTINI, M C; PIATTI, E (2006) Raw millefiori honey is packed full of antioxidants. *Food Chemistry* 97(2): 217-222. http://dx.doi.org/10.1016/j.foodchem.2005.03.039

BOE-BOLETIN OFICIAL DEL ESTADO (1986) Orden de 12 de junio de 1986 por la que se aprueban los métodos oficiales de análisis para la miel (B.O.E nº 145 de 18 de junio de 1986). https://www.boe.es/boe/dias/1986/06/18/pdfs/A22195-22202.pdf

BOGDANOV, S (2009) Harmonised methods of the International Honey Commission. http://www.ihc-platform.net/ihcmethods2009.pdf (Accessed 12/12/2014)

BOGDANOV, S (2011) Honey Composition. In *Bogdanov, S (Ed). The Honey Book*. pp. 27-36. http://www.bee-hexagon.net/honey/ (Accessed 08/11/2014)

BOGDANOV, S; LÚLLMANN, C; MARTIN, P; VON DER OHE, W (1999) Honey quality and international regulatory standards: review by the International Honey Commission. *Bee World* 80(2): 61-69. http://dx.doi.org/10.1080/0005772X.1999.11099428

BOGDANOV, S; MARTIN, P (2002) Honey authenticity: a review. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene* 93: 232-254. Available from: http://www.bee-hexagon.net/files/file/fileE/Honey/AuthenticityRevue_Internet.pdf

BOGDANOV, S; RUOFF, K; PERSANO-ODDO, L (2004) Physico-chemical methods for the characterisation of unifloral honeys: a review. *Apidologie* 35(Suppl. 1): S4-S17. http://dx.doi.org/10.1051/apido:2004047

BOGDANOV, S; JURENDIC, T; SIEBER, R; GALLMANN, P (2008) Honey for nutrition and health: A review. *Journal of the American College of Nutrition* 27(6): 677-689. http://dx.doi.org/10.1080/07315724.2008.10719745

CAN, Z; YILDIZ, O; SAHIN, H; TURUMTAY, E A; SILICI, S; KOLAYLI, S (2015) An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles. *Food Chemistry* 180: 133-141. http://dx.doi.org/10.1016/j.foodchem.2015.02.024

CASTRO-VÁZQUEZ, L; LEON-RUIZ, V; ALAÑON, M E; PÉREZ-COELLO, M S; GONZÁLEZ-PORTO, A V (2014) Floral origin markers for authenticating Lavandin honey (*Lavandula angustifolia x latifolia*). Discrimination from Lavender honey (*Lavandula latifolia*). *Food Control* 37: 362-370. http://dx.doi.org/10.1016/j.foodcont.2013.09.003

CAVIA, M M; FERNÁNDEZ-MUIÑO, M A; GÖMEZ-ALONSO, E; MONTES-PÉREZ, M J; HUIDOBRO, J F; SANCHO, M T (2002) Evolution of fructose and glucose in honey over one year: influence of induced granulation. *Food Chemistry* 78(2): 157-161. http://dx.doi.org/10.1016/S0308-8146(01)00393-4

CAVIA, M M; FERNÁNDEZ-MUIÑO, M A; ALONSO-TORRE, S R; HUIDOBRO, J F; SANCHO, M T (2007) Evolution of acidity of honeys from continental climantes: Influence of induced granulation. *Food Chemistry* 100(4): 1728-1733. http://dx.doi.org/10.1016/j.foodchem.2005.10.019

CHAKIR, A; ROMANE, A; MARCAZZAN, G L; FERRAZZI, P (2011) Physicochemical properties of some honeys produced from different plants in Morocco. *Arabian Journal of Chemistry*. http://dx.doi.org/10.1016/j.arabjc.2011.10.013

CHEFROUR, A; DRAIAIA, R; TAHAR, A; AIT-KAKI, Y; BENNADJA, S; BATTESTI, M J (2009) Physicochemical characteristics and pollen spectrum of some north-east Algerian honeys. *African Journal of Food, Agriculture, Nutrition and Development* 9(5): 1276-1293. http://dx.doi.org/10.4314/ajfand.v9i5.45101

CHEN, H; FAN, C; CHANG, Q; PANG, G; HU, X; LU, M; WANG, W (2014) Chemometric determination of the botanical origin for Chinese honeys on the basis of mineral elements determined by ICP-MS. *Journal of Agricultural and Food Chemistry* 62(11): 2443-2448. http://dx.doi.org/10.1021/jf405045q

CHERCHI, A; SPANEDDA, L; TUBEROSO, C; CABRAS, P (1994) Solid-phase extraction and high-performance liquid chromatographic determination of organic acids in honey. *Journal of Chromatography A* 669(1/2): 59-64. http://dx.doi.org/10.1016/0021-9673(94)80336-6

CHUA, L S; ABDUL-RAHAMAN, N-L; SARMIDI, M R; AZIZ, R (2012) Multi-elemental composition and physical properties of honey samples from Malaysia. *Food Chemistry* 135(3): 880-887. http://dx.doi.org/10.1016/j.foodchem.2012.05.106

CIULU, M; SOLINAS, S; FLORIS, I; PANZANELLI, A; PILO, M I; PIU, P C; SPANO, N; SANNA, G (2011) RP-HPLC determination of water-soluble vitamins in honey. *Talanta* 83(3): 924-929. http://dx.doi.org/10.1016/j.talanta.2010.10.059

CODEX ALIMENTARIUS STANDARD FOR HONEY (2001) Codex standard for honey 12-1981. Revised Codex Standard for Honey. Standards and Standard Methods, Volume 11. http://www.Codexalimentarius.net (Accessed 12/12/2014)

CONSONNI, R; CAGLIANI, L R; COGLIATI, C (2013) Geographical discrimination of honeys by saccharides analysis. *Food Control* 32(2): 543-548. http://dx.doi.org/10.1016/j.foodcont.2013.01.038

COSTA, L S M; ALBUQUERQUE, M L S; TRUGO, L C; QUINTEIRO, L M C; BARTH, O M; RIBEIRO, M; DE MARÍA, C A B (1999) Determination of non-volatile compounds of different botanical origin Brazilian honeys. *Food Chemistry* 65(3): 347-352. http://dx.doi.org/10.1016/S0308-8146(98)00230-1

COTTE, J .; CASABIANCA, H; CHARDON, S; LHERITIER, J; GRENIER-LOUSTALOT, M (2003) Application of carbohydrate analysis to verify honey authenticity. *Journal of Chromatography A* 1021(1/2): 145-155. http://dx.doi.org/10.1016/j.chroma.2003.09.005

COTTE, J F; CASABIANCA, H; CHARDON, S; LHERITIER, J; GRENIER-LOUSTALOT, M F (2004) Chromatographic analysis of sugars applied to the characterization of monofloral honey. *Analytical and Bioanalytical Chemistry* 380(4): 698-705. http://dx.doi.org/ 10.1007/s00216-004-2764-1

DA COSTA-LEITE, J M; TRUGO, L C; COSTA, L S M; QUINTEIRO, L M C; BARTH, O M; DUTRA, V M L; DE MARIA, C A B (2000) Determination of oligosaccharides in Brazilian honeys of different botanical origin. *Food Chemistry* 70(1): 93-98. http://dx.doi.org/10.1016/S0956-7135(99)00115-2

DE LA FUENTE, E; VALENCIA-BARRERA, R M; MARTÍNEZ-CASTRO, I; SANZ, J (2007) Occurrence of 2-hydroxy-5-methyl-3-hexanone and 3-hydroxy-5-methyl-2-hexanone as indicators of botanic origin in eucalyptus honeys. *Food Chemistry* 103(4): 1176-1180. http://dx.doi.org/10.1016/j.foodchem.2006.10.020

DE LA FUENTE, E; RUIZ-MATUTE, A I; VALENCIA-BARRERA, R M; SANZ, J; MARTÍNEZ CASTRO, I (2011) Carbohydrate composition of Spanish unifloral honeys. *Food Chemistry* 129(4): 1483-1489. http://dx.doi.org/10.1016/j.foodchem.2011.05.121

DEVILLERS, J; MORLOT, M; PHAM-DELÈGUE, M H; DORÉ, J C (2004) Classification of monofloral honeys based on their quality control data. *Food Chemistry* 86(2): 305-312. http://dx.doi.org/10.1016/j.foodchem.2003.09.029

DINKOV, D (2001) A report on the Bulgarian bee honey ripeness according to amount of the aminoacid proline. Suplement 1: 71-74.

DINKOV, D (2003) A scientific note on the specific optical rotation of three honey types from Bulgaria. *Apidologie* 34(4): 319-320. http://dx.doi.org/10.1051/apido:2003017

DOWNEY, G; HUSSEY, K; KELLY, J D; WALSHE, T F; MARTIN, P G (2005) Preliminary contribution to the characterisation of artisanal honey produced on the island of Ireland by palynological and physico-chemical data. *Food Chemistry* 91(2): 347-354. http://dx.doi.org/10.1016/j.foodchem.2004.06.020

ESCRICHE, I; KADAR, M; JUAN-BORRÁS, M; DOMENECH, E (2011) Using flavonoids, phenolic compounds and headspace volatile profile for botanical authentication of lemon and orange honeys. *Food Research International* 44(5): 1504-1513. http://dx.doi.org/10.1016/j.foodres.2011.03.049

ESCRICHE, I; KADAR, M; DOMENECH, E; GIL-SÁNCHEZ, L (2012) A potentiometric electronic tongue for the discrimination of honey according to the botanical origin. Comparison with traditional methodologies: Physicochemical parameters and volatile profile. *Journal of Food Engineering* 109(3): 449-456. http://dx.doi.org/10.1016/j.jfoodeng.2011.10.036

ESCRICHE, I; KADAR, M; JUAN-BORRÁS, M; DOMENECH, E (2014) Suitability of antioxidant capacity, flavonoids and phenolic acids for floral authentication of honey. Impact of industrial thermal treatment. *Food Chemistry* 142: 135-143. http://dx.doi.org/10.1016/j.foodchem.2013.07.033

ESCUREDO, O; FERNÁNDEZ-GONZÁLEZ, M; SEIJO, M C (2012) Differentiation of blossom honey and honeydew honey from North-West Spain. *Agriculture* 2(1): 25-37. http://dx.doi.org/10.3390/agriculture2010025

ESCUREDO, O; MÍGUEZ, M; FERNÁNDEZ-GONZÁLEZ, M; SEIJO, M C (2013) Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. *Food Chemistry* 138(2/3): 851-856. http://dx.doi.org/10.1016/j.foodchem.2012.11.015

ESCUREDO, O; DOBRE, I; FERNÁNDEZ-GONZÁLEZ, M; SEIJO, M C (2014) Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. *Food Chemistry* 149: 84-90. http://dx.doi.org/10.1016/j.foodchem.2013.10.097

ESTEVINHO, L M; FEÁS, X; SEIJAS, J A; VÁZQUEZ-TATO, M P (2012) Organic honey from Trás-Os-Montes region (Portugal): Chemical, palynological, microbiological and bioactive compounds characterization. *Food and Chemical Toxicology* 50(2): 258-264. http://dx.doi.org/10.1016/j.fct.2011.10.034

ESTEVINHO, M L; VÁZQUEZ-TATO, M P; SEIJAS, J A; FEÁS, X (2013) Palynological, physicochemical, and microbiological attributes of organic lavender (*Lavandula stoechas*) honey from Portugal. *Acta Alimentaria* 42(1): 36-44. http://dx.doi.org/10.1556/AAlim.42.2013.1.4.

FALLICO, B; ZAPPALÀ, M; ARENA, E; VERZERA, A (2004) Effects of conditioning on HMF content in unifloral honeys. *Food Chemistry* 85(2): 305-313. http://dx.doi.org/10.1016/j.foodchem.2003.07.010

FEÁS, X; PIRES, J; ESTEVINHO, M L; IGLESIAS, A; DE ARAUJO, J P P (2010a) Palynological and physicochemical data characterisation of honeys produced in the Entre-Douro e Minho region of Portugal. *International Journal of Food Science & Technology* 45(6): 1255-1262. http://dx.doi.org/10.1111/j.1365-2621.2010.02268.x

FEÁS, X; PIRES, J; IGLESIAS, A; ESTEVINHO, M L (2010b) Characterization of artisanal honey produced on the Northwest of Portugal by melissopalynological and physico-chemical data. *Food and Chemical Toxicology* 48(12): 3462-3470. http://dx.doi.org/10.1016/j.fct.2010.09.024

FERRERES, F; GARCÍA-VIGUERA, C; TOMÁS-LORENTE, F; TOMÁS-BARBERÁN. F A (1993) Hesperetin: A marker of the floral origin of citrus honey. *Journal of the Science of Food and Agriculture* 61(1): 121-123. http://dx.doi.org/10.1002/jsfa.2740610119

FINOLA, M S; LASAGNO, M C; MARIOLI, J M (2007) Microbiological and chemical characterization of honeys from central Argentina. *Food Chemistry* 100(4): 1649-1653. http://dx.doi.org/10.1016/j.foodchem.2005.12.046

GHELDOF, N; ENGESETH, N J (2002) Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *Journal of Agricultural and Food Chemistry* 50(10): 3050-3055. http://dx.doi.org/10.1021/jf0114637

GOLOB, T; PLESTENJAK, A (1999) Quality of Slovene Honey. *Food Technology and Biotechnology* 37(3): 195-201.

GOMES, S; DIAS, L G; MOREIRA, L L; RODRIGUES, P; ESTEVINHO, L (2010) Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. *Food and Chemical Toxicology* 48(2): 544-548. http://dx.doi.org/10.1016/j.fct.2009.11.029

GÓMEZ-BÁREZ, J A; GARCIA-VILLANOVA, R J; ELVIRA-GARCIA, S; RIVAS-PALÁ, T; GONZALEZ-PARAMÁS, A M; SÁNCHEZ-SÁNCHEZ, J (2000) Geographical discrimination of honeys through the employment of sugar patterns and common chemical quality parameters. *European Food Research and Technology* 210(6): 437-444. http://dx.doi.org/10.1007/s002170050578

GONZÁLEZ-LORENTE, M; DE LORENZO-CARRETERO, C; PÉREZ-MARTÍN, R A (2008) Sensory attributes and antioxidant capacity of spanish honeys. *Journal of Sensory Studies* 23(3): 293-302.

GONZÁLEZ-PARAMÁS, A M; GÓMEZ-BÁREZ, J A; CORDÓN-MARCOS, C; GARCÍA-VILLANOVA, R J; SÁNCHEZ-SÁNCHEZ, J (2006) HPLC-fluorimetric method for analysis of amino acids in products of the hive (honey and bee-pollen). *Food Chemistry* 95(1): 148-156. http://dx.doi.org/10.1016/j.foodchem.2005.02.008

GONZÁLEZ-PARAMÁS, A M; GARCÍA-VILLANOVA, R J; GÓMEZ BÁREZ, J A; SÁNCHEZ SÁNCHEZ, J; ARDANUY ALBAJAR, R (2007) Botanical origin of monovarietal dark honeys (from heather, holm oak, pyrenean oak and sweet chestnut) based on their chromatic characters and amino acid profiles. *European Food Research and Technology* 226(1/2): 87-92. http://dx.doi.org/10.1007/s00217-006-0512-9

GUYOT-DECLERCK, C; RENSON, S; BOUSETA, A; COLLIN, S (2002) Floral quality and discrimination of *Lavandula stoechas, Lavandula angustifolia,* and *Lavandula angustifolia x latifolia* honeys. *Food Chemistry* 79(4): 453-459. http://dx.doi.org/10.1016/S0308-8146(02)00216-9

HERMOSÍN, I; CHICÓN, R M; CABEZUDO, D (2003) Free amino acid composition and botanical origin of honey. *Food Chemistry* 83(2): 263-268. http://dx.doi.org/10.1016/S0308-8146(03)00089-X

HERRERO, B; VALENCIA-BARRERA, R M; SAN MARTÍN, R; PANDO, V (2002) Characterization of honeys by melissopalynology and statistical analysis. *Canadian Journal of Plant Science* 82(1): 75-82. http://dx.doi.org/10.4141/P00-187

HORVÁTH, K; MOLNÁR-PERL, I (1997) Simultaneous quantitation of mono-, di- and trisaccharides by GC-MS of their TMS ether oxime derivatives: II: In Honey. *Chromatographia* 45(1): 328-335.

IGLESIAS, M T; DE LORENZO, C; POLO, M D C; MARTÍN-ÁLVAREZ, P J; PUEYO, E (2004) Usefulness of amino acid composition to discriminate between honeydew and floral honeys. Application to Honeys from a Small Geographic Area. *Journal of Agricultural and Food Chemistry* 52(1): 84-89. http://dx.doi.org/10.1021/jf030454q

IGLESIAS, M T; MARTÍN-ÁLVAREZ, P J; POLO, M C; DE LORENZO, C; GONZÁLEZ, M; PUEYO, E (2006) Changes in the free amino acid contents of honeys during storage at ambient temperature. *Journal of Agricultural and Food Chemistry* 54(24): 9099-9104. http://dx.doi.org/10.1021/jf061712x

ISLA, M I; CRAIG, A; ORDOÑEZ, R; ZAMPINI, C; SAYAGO, J; BEDASCARRASBURE, E; ÁLVAREZ, A; SALOMÓN, V; MALDONADO, L (2011) Physico chemical and bioactive properties of honeys from Northwestern Argentina. *LWT-Food Science and Technology* 44(9): 1922-1930. http://dx.doi.org/10.1016/j.lwt.2011.04.003

JUAN-BORRÁS, M; DOMENECH, E; HELLEBRANDOVA, M; ESCRICHE, I (2014) Effect of country origin on physicochemical, sugar and volatile composition of acacia, sunflower and tilia honeys. *Food Research International* 60: 86-94. http://dx.doi.org/10.1016/j.foodres.2013.11.045

KAHRAMAN, T; BUYUKUNAL, S K; VURAL, A; ALTUNATMAZ, S S (2010) Physico-chemical properties in honey from different regions of Turkey. *Food Chemistry* 123(1): 41-44. http://dx.doi.org/10.1016/j.foodchem.2010.03.123

KAMBOJ, R; BERA, M B; NANDA, V (2013) Chemometric classification of northern India unifloral honey. *Acta Alimentaria* 42(4): 540-551. http://dx.doi.org/10.1556/AAlim.42.2013.4.9

KARABAGIAS, I K; BADEKA, A V.; KONTAKOS, S; KARABOURNIOTI, S; KONTOMINAS, M G (2014) Botanical discrimination of Greek unifloral honeys with physico-chemical and chemometric analyses. *Food Chemistry* 165: 181-190. http://dx.doi.org/10.1016/j.foodchem.2014.05.033

KAŠKONIENĖ, V; VENSKUTONIS, P R; ČEKSTERYTĖ, V (2010) Carbohydrate composition and electrical conductivity of different origin honeys from Lithuania. *LWT - Food Science and Technology* 43(5): 801-807. http://dx.doi.org/10.1016/j.lwt.2010.01.007

KEČKEŠ, J; TRIFKOVIĆ, J; ANDRIĆ, F; JOVETIĆ, M; TEŠIĆ, Ž; MILOJKOVIĆ-OPSENICA, D (2013) Amino acids profile of Serbian unifloral honeys. *Journal of the Science of Food and Agriculture* 93(13): 3368-3376. http://dx.doi.org/10.1002/jsfa.6187

KHALIL, M I; ALAM, N; MONIRUZZAMAN, M; SULAIMAN, S A; GAN, S H (2011) Phenolic acid composition and antioxidant properties of Malaysian honeys. *Journal of Food Science* 76(6): C921-C928. http://dx.doi.org/10.1111/j.1750-3841.2011.02282.x

KIVRAK, İ (2015) Free amino acid profiles of 17 Turkish unifloral honeys. *Journal of Liquid Chromatography & Related Technologies* 38(8): 855-862. http://dx.doi.org/10.1080/10826076.2014.976712

KROPF, U; KOROŠEC, M; BERTONCELJ, J; OGRINC, N; NEČEMER, M; KUMP, P; GOLOB, T (2010) Determination of the geographical origin of Slovenian black locust, lime and chestnut honey. *Food Chemistry* 121(3): 839-846. http://dx.doi.org/10.1016/j.foodchem.2009.12.094

KOLAYLI, S; BOUKRAÂ, L; ŞAHIN, H; ABDELLAH, F (2012) Sugars in honey. In Preedy, V R (Ed). *Dietary Sugars: Chemistry, Analysis, Function and Effects*. Royal Society of Chemistry Publishing; Cambridge, UK. pp. 3-15. http://dx.doi.org/10.1039/9781849734929

KÜÇÜK, M; KOLAYLI, S; KARAOĞLU, Ş; ULUSOY, E; BALTACI, C; CANDAN, F (2007) Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chemistry* 100(2): 526-534. http://dx.doi.org/10.1016/j.foodchem.2005.10.010

LACHMAN, J; KOLIHOVÁ, D; MIHOLOVÁ, D; KOŠATA, J; TITĚRA, D; KULT, K (2007) Analysis of minority honey components: Possible use for the evaluation of honey quality. *Food Chemistry* 101(3): 973-979. http://dx.doi.org/10.1016/j.foodchem.2006.02.049

LEÓN-RUIZ, V; VERA, S; GONZÁLEZ-PORTO, A V; ANDRÉS, M P S (2011) Vitamin C and sugar levels as simple markers for discriminating Spanish honey sources. *Journal of Food Science* 76(3): C356-C361. http://dx.doi.org/10.1111/j.1750-3841.2011.02041.x

LEÓN-RUIZ, V; GONZÁLEZ-PORTO, A V.; AL-HABSI, N; VERA, S; SAN-ANDRÉS, M P; JAUREGI, P (2013) Antioxidant, antibacterial and ACE-inhibitory activity of four monofloral honeys in relation to their chemical composition. *Food & Function* 4(11): 1617. http://dx.doi.org/10.1039/c3fo60221d

LOUVEAUX, J; MAURIZIO, A; VORWOHL, G (1978) Methods of melissopalynology. *Bee World* 59(4): 139-157. http://dx.doi.org/10.1080/0005772X.1978.11097714

LUPANO, C E (1997) DSC study of honey granulation stored at various temperatures. *Food Research International* 30(9): 683-688.

MALACALZA, N H; CACCAVARI, M A; FAGÚNDEZ, G; LUPANO, C E (2005) Unifloral honeys of the province of Buenos Aires, Argentine. *Journal of the Science of Food and Agriculture* 85(8): 1389-1396. http://dx.doi.org/10.1002/jsfa.2105

MANIKIS, I; THRASIVOULOU, A (2001) The relation of physicochemical characteristics of honey and the crystallization sensitive parameters. *Apiacta* 36(2): 106-112.

MARINI, F; MAGRÌ, A L; BALESTRIERI, F; FABRETTI, F; MARINI, D (2004) Supervised pattern recognition applied to the discrimination of the floral origin of six types of Italian honey samples. *Analytica Chimica Acta* 515(1): 117-125. http://dx.doi.org/10.1016/j.aca.2004.01.013

MARTINS, R C; LOPES, V V.; VALENTÃO, P; CARVALHO, J C M F; ISABEL, P; AMARAL, M T; BATISTA, M T; ANDRADE, P B; SILVA, B M (2008) Relevant principal component analysis applied to the characterisation of Portuguese heather honey. *Natural Product Research* 22(17): 1560-1582. http://dx.doi.org/10.1080/14786410701825004

MATEO, R; BOSCH-REIG, F (1997) Sugar profiles of Spanish unifloral honeys. *Food Chemistry* 60(1): 33-41. http://dx.doi.org/10.1016/S0308-8146(96)00297-X

MATEO, R; BOSCH-REIG, F (1998) Classification of Spanish unifloral honeys by discriminant analysis of electrical conductivity, color, water content, sugars, and pH. *Journal of Agricultural and Food Chemistry* 46(2): 393-400. http://dx.doi.org/10.1021/jf970574w

MATO I, HUIDOBRO JF, SIMAL-LOZANO J, SANCHO MT (2003) Significance of nonaromatic organic acids in honey. *Journal of Food Protection* 66(12):2371-2376.

MATO, I; HUIDOBRO, J F; SIMAL-LOZANO, J; SANCHO, M T (2006) Rapid determination of nonaromatic organic acids in honey by capillary zone electrophoresis with direct ultraviolet detection. *Journal of Agricultural and Food Chemistry* 54(5): 1541-1550. http://dx.doi.org/10.1021/jf051757i

MAURIZIO, A (1975) Microscopy of honey. In *Crane, E (Ed). Honey. A comprehensive Survey*. Heinemann; London, UK. pp. 240-257.

MOISE, A; MĂRGHITAŞ, A L; DEZMIREAN, D; BOBIS, O (2013) Nutraceutical properties of Romanian heather honey. *Nutrition & Food Science* 43(3): 218-227. http://dx.doi.org/10.1108/00346651311327864

NANDA, V; SARKAR, B C; SHARMA, H K; BAWA, A S (2003) Physico-chemical properties and estimation of mineral content in honey produced from different plants in Northern India. *Journal of Food Composition and Analysis* 16(5): 613-619. http://dx.doi.org/10.1016/S0889-1575(03)00062-0

NOZAL, M J; BERNAL, J L; TORIBIO, L; ALAMO, M; DIEGO, J C; TAPIA, J (2005) The use of carbohydrate profiles and chemometrics in the characterization of natural honeys of identical geographical origin. *Journal of Agricultural and Food Chemistry* 53(8): 3095-3100. http://dx.doi.org/10.1021/jf0489724

NOZAL-NALDA, M J; BERNAL-YAGÜE, J L; DIEGO-CALVA, J C; MARTÍN-GÓMEZ, M T (2005) Classifying honeys from the Soria Province of Spain via multivariate analysis. *Analytical and Bioanalytical Chemistry* 382(2): 311-319. http://dx.doi.org/10.1007/s00216-005-3161-0

OJEC-OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES (2002) Council Directive 2001/110/EC of 20 December 2001 relating to honey. L 10: 47-52. Luxembourg. European Union.

OJEDA DE RODRÍGUEZ, G; SULBARÁN DE FERRER, B; FERRER, A; RODRÍGUEZ, B (2004) Characterization of honey produced in Venezuela. *Food Chemistry* 84(4): 499-502. http://dx.doi.org/10.1016/S0308-8146(02)00517-4

OJEU-OFFICIAL JOURNAL OF THE EUROPEAN UNION (2007) Commission Regulation EC 868/2007 entering a designation in the Register of protected designations of origin and protected geographical indications "Miel de Galicia" or "Mel de Galicia" (PGI). L192: 11-18. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32007R0868&from=EN

OUCHEMOUKH, S; SCHWEITZER, P; BACHIR-BEY, M; DJOUDAD-KADJI, H; LOUAILECHE, H (2010) HPLC sugar profiles of Algerian honeys. *Food Chemistry* 121(2): 561-568. http://dx.doi.org/10.1016/j.foodchem.2009.12.047

ÖZBALCI, B; BOYACI, İ H; TOPCU, A; KADILAR, C; TAMER, U (2013) Rapid analysis of sugars in honey by processing Raman spectrum using chemometric methods and artificial neural networks. *Food Chemistry* 136(3/4): 1444-1452. http://dx.doi.org/10.1016/j.foodchem.2012.09.064

ÖZCAN, M M; ÖLMEZ, Ç (2014) Some qualitative properties of different monofloral honeys. *Food Chemistry* 163: 212-218. http://dx.doi.org/10.1016/j.foodchem.2014.04.072

PASINI, F; GARDINI, S; MARCAZZAN, G L; CABONI, M F (2013) Buckwheat honeys: Screening of composition and properties. *Food Chemistry* 141(3): 2802-2811. http://dx.doi.org/10.1016/j.foodchem.2013.05.102

PÉREZ, R A; IGLESIAS, M T; PUEYO, E; GONZALEZ, M; DE LORENZO, C (2007) Amino acid composition and antioxidant capacity of Spanish honeys. *Journal of Agricultural and Food Chemistry* 55(2): 360-365. http://dx.doi.org/10.1021/jf062055b

PÉREZ-ARQUILLUÉ, C; CONCHELLO, P; ARIÑO, A; JUAN, T; HERRERA, A (1994) Quality evaluation of Spanish rosemary (*Rosmarinus oficinalis*) honey. *Food Chemistry* 51(2): 207-210. http://dx.doi.org/10.1016/0308-8146(94)90258-5

PÉREZ-ARQUILLUÉ, C; CONCHELLO, P; ARIÑO, A; JUAN, T; HERRERA, A (1995) Physicochemical attributes and pollen spectrum of some unifloral Spanish honeys. *Food Chemistry* 54(2): 167-172. http://dx.doi.org/10.1016/0308-8146(95)00022-B

PÉREZ-MARTÍN, R A; VELA-HORTIGÜELA, L; LORENZO-LOZANO, P; ROJO-CORTINA, M D; LORENZO-CARRETERO, C (2008) In vitro antioxidant and antimicrobial activities of Spanish honeys. *International Journal of Food Properties* 11(4): 727-737. http://dx.doi.org/10.1080/10942910701586257

PERSANO-ODDO, L; BALDI, E; ACCORTI, M (1990) Diastatic activity in some unifloral honeys. *Apidologie* 21(1): 17-24. http://dx.doi.org/10.1051/apido:19900103

PERSANO-ODDO, L; PIAZZA, M G; ZELLINI, G (1995) Caratteristiche cromatiche dei mieli uniflorali. *Apicoltura* 10: 109-120.

PERSANO-ODDO, L; PIRO, R (2004) Main European unifloral honeys: descriptive sheets. *Apidologie* 35(Suppl. 1): S38-S81. http://dx.doi.org/10.1051/apido:2004049

PIANA, M L; PERSANO-ODDO, L; BENTABOL, A; BRUNEAU, E; BOGDANOV, S; GUYOT-DECLERCK, C (2004) Sensory analysis applied to honey: state of the art. *Apidologie* 35(Suppl. 1): S26-S37. http://dx.doi.org/10.1051/apido:2004048

PIRES, J; ESTEVINHO, M L; FEÁS, X; CANTALAPIEDRA, J; IGLESIAS, A (2009) Pollen spectrum and physico-chemical attributes of heather (*Erica* sp.) honeys of north Portugal. *Journal of the Science of Food and Agriculture* 89(11): 1862-1870. http://dx.doi.org/10.1002/jsfa.3663

PRIMORAC, L; FLANJAK, I; KENJERIC, D; BUBALO, D; TOPOLNJAK, Z (2011) Specific rotation and carbohydrate profile of Croatian unifloral honeys. *Czech Journal of Food Science* 29(5): 515-519.

RICCIARDELLI-D'ALBORE, G (1998) Mediterranean melissopalynology. *Universita degli studi di Perugia, Faculta di Agraria, Perugia*.

RODRÍGUEZ-FLORES, M S; ESCUREDO-PÉREZ, O; SEIJO-COELLO, M C (2014) Characterization of *Eucalyptus Globulus* honeys produced in the Eurosiberian Area of the Iberian Peninsula. *International Journal of Food Properties* 17(10): 2177-2191. http://dx.doi.org/10.1080/10942912.2013.790050

RODRÍGUEZ-FLORES, M S; ESCUREDO, O; SEIJO, M C (2015) Assessment of physicochemical and antioxidant characteristics of *Quercus pyrenaica* honeydew honeys. *Food Chemistry* 166: 101-106. http://dx.doi.org/10.1016/j.foodchem.2014.06.005

RUOFF, K; BOGDANOV, S (2004) Authenticity of Honey and Other Bee Products. *Apiacta* 38: 317-327.

RYBAK-CHMIELEWSKA, H; SZCZĘSNA, T; WAŚ, E; JAŚKIEWICZ, K; TEPER, D (2013) Characteristics of Polish unifloral honeys IV. Honeydew honey, mainly *Abies Alba* L. *Journal of Apicultural Science* 57(1): 51-59. http://dx.doi.org/10.2478/jas-2013-0006

SÁNCHEZ, M P; HUIDOBRO, J F; MATO, I; MUNIATEGUI, S; SANCHO, M T (2001) Evolution of invertase activity in honey over two years. *Journal of Agricultural and Food Chemistry* 49(1): 416-422. http://dx.doi.org/10.1021/jf0003350

SANCHO, M T; MUNIATEGUI, S; HUIDOBRO J F; SIMAL, J (1991a) Correlation between the electrical conductivity of honey in humid and in dry matter. *Apidologie* 22(3): 221-227.

SANCHO, M T; MUNIATEGUI, S; HUIDOBRO J F; SIMAL, J (1991b) Mieles del País Vasco II: Indices de formol y prolina. *Anales de Bromatología* XLIII(1): 87-99.

SANCHO, M T; MUNIATEGUI, S; HUIDOBRO, J F; SIMAL, J (1991c) Mieles del País Vasco VI: Elementos de mielada. *Anales de Bromatología* XLIII(2/3): 165-172.

SANCHO, M T; MUNIATEGUI, S; HUIDOBRO, J F; SIMAL, J (1991d) Mieles del País Vasco X: Tendencia a la granulación. *Anales de Bromatología* XLIII(2/3): 283-292.

SANZ, S; PÉREZ, C; HERRERA, A; SANZ, M; JUAN, T (1995) Application of a statistical approach to the classification of honey by geographic origin. *Journal of the Science of Food and Agriculture* 69(2): 135-140. http://dx.doi.org/10.1002/jsfa.2740690202

ŠARIĆ, G; MATKOVIĆ, D; HRUŠKAR, M; VAHČIĆ, N (2008) Characterisation and classification of Croatian honey by physicochemical parameters. *Food Technology and Biotechnology* 46(4): 355-367. http://www.scopus.com/inward/record.url?eid=2-s2.0-56849097587&partnerID=tZOtx3y1

SEIJO, M C; JATO, M V; AIRA, M J; IGLESIAS, I (1997) Unifloral honeys of Galicia (North-West Spain). *Journal of Apicultural Research* 36(3/4):133-139. http://dx.doi.org/10.1080/00218839.1997.11100939

SEISONEN, S; KIVIMA, E; VENE, K (2015) Characterisation of the aroma profiles of different honeys and corresponding flowers using solid-phase microextraction and gas chromatography-mass spectrometry /olfactometry. *Food Chemistry* 169: 34-40. http://dx.doi.org/10.1016/j.foodchem.2014.07.125

SERRA-BONVEHÍ, J (1988) Determinación de antranilato de metilo en la miel de citricos (*Citrus* sp.) del Levante Español, y su influencia en la actividad diastasica de la miel, *Alimentaria* 197: 37-40.

SERRA-BONHEVI, J. (1989) Estudio de la validez de los índices que predicen la cristalización de la miel. *Revista de Agroquímica y Tecnología de Alimentos* 29(1): 47-62.

SERRA-BONVEHÍ, J; GÓMEZ-PAJUELO, J; GONELL-GALINDO, J (1987) Composición, propiedades fisico-químicas y espectro polínico de algunas mieles monoflorales de España. *Alimentaria* XXIV(185): 61-84.

SERRA-BONVEHÍ, J; GRANADOS-TARRÉS, E (1993) Physicochemical properties, composition and pollen spectrum of ling heather (Calluna vulgaris (L) Hull) honey produced in Spain. *Apidologie* 24(6): 586-596. http://dx.doi.org/10.1051/apido:19930606

SERRA-BONVEHI, J S; VENTURA-COLL, F (1993) Physico-chemical properties, composition and pollen spectrum of french lavender (*Lavandula stoechas* L.) honey produced in Spain. *Lebensmittel-Wissenschaft Und-Technologie* 196(6): 511-517.

SERRANO, S; VILLAREJO, M; ESPEJO, R; JODRAL, M (2004) Chemical and physical parameters of Andalusian honey: classification of Citrus and Eucalyptus honeys by discriminant analysis. *Food Chemistry* 87(4): 619-625. http://dx.doi.org/10.1016/j.foodchem.2004.01.031

SHIN, H-S; USTUNOL, Z (2005) Carbohydrate composition of honey from different floral sources and their influence on growth of selected intestinal bacteria: An in vitro comparison. *Food Research International* 38(6): 721-728. http://dx.doi.org/10.1016/j.foodres.2005.01.007

SILICI, S; KARAMAN, K (2014) Chemometric approaches for the characterization of Turkish rhododendron and honeydew honeys depending on amino acid composition. *Journal of Liquid Chromatography & Related Technologies* 37(6): 864-877. http://dx.doi.org/10.1080/10826076.2012.758149

SILVA, L R; VIDEIRA, R; MONTEIRO, A P; VALENTÃO, P; ANDRADE, P B (2009) Honey from Luso region (Portugal): Physicochemical characteristics and mineral contents. *Microchemical Journal* 93(1): 73-77. http://dx.doi.org/10.1016/j.microc.2009.05.005

SILVANO, M F; VARELA, M S; PALACIO, M A; RUFFINENGO, S; YAMUL, D K (2014) Physicochemical parameters and sensory properties of honeys from Buenos Aires region. *Food Chemistry* 152: 500-507. http://dx.doi.org/10.1016/j.foodchem.2013.12.011

SINGH, N; BATH, P K (1997) Quality evaluation of different types of Indian honey. *Food Chemistry* 58(1): 129-133. http://dx.doi.org/10.1016/S0308-8146(96)00231-2

SMANALIEVA, J; SENGE, B (2009) Analytical and rheological investigations into selected unifloral German honey. *European Food Research and Technology* 229(1): 107-113. http://dx.doi.org/10.1007/s00217-009-1031-2

SORIA, A C; GONZÁLEZ, M; DE LORENZO, C; MARTÍNEZ-CASTRO, I; SANZ, J (2004) Characterization of artisanal honeys from Madrid (Central Spain) on the basis of their melissopalynological, physicochemical and volatile composition data. *Food Chemistry* 85(1): 121-130. http://dx.doi.org/10.1016/j.foodchem.2003.06.012

SORIA, A C; GONZÁLEZ, M; DE LORENZO, C; MARTÍNEZ-CASTRO, I; SANZ, J (2005) Estimation of the honeydew ratio in honey samples from their physicochemical data and from their volatile composition obtained by SPME and GC-MS. *Journal of the Science of Food and Agriculture* 85(5): 817-824. http://dx.doi.org/10.1002/jsfa.1890

STATGRAPHICS CENTURION XVI.II (2010) Statpoint Technologies, Inc. Warrenton, VA (USA).

SUÁREZ-LUQUE, S; MATO, I; HUIDOBRO, J F; SIMAL-LOZANO, J; SANCHO, M T (2002) Rapid determination of minority organic acids in honey by high-performance liquid chromatography. *Journal of Chromatography A* 955(2): 207-214. http://dx.doi.org/10.1016/S0021-9673(02)00248-0

SUBDIRECCIÓN GENERAL DE PRODUCTOS GANADEROS (2015) El sector de la miel en cifras. Principales indicadores económicos en 2014. http://www.magrama.gob.es/es/ganaderia/temas/produccion-y-mercados-ganaderos/indicadoreseconomicossectordelamiel2014_tcm7-381460.pdf (Accessed 05/09/2015)

TABOURET, T (1979) Rôle de l'activité de l'eau dans la cristallisation du miel. *Apidologie* 10(4): 341-358. http://dx.doi.org/10.1051/apido:19790403

TAN, S T; WILKINS, A L; HOLLAND, P T; MCGHIE, T K (1989) Extractives from New Zealand unifloral honeys. 2. Degraded carotenoids and other substances from heather honey. *Journal of Agricultural and Food Chemistry* 37(5): 1217-1221. http://dx.doi.org/10.1021/jf00089a004

TERRAB, A; VEGA-PÉREZ, J M; DÍEZ, M J; HEREDIA, F J (2001) Characterisation of northwest Moroccan honeys by gas chromatographic-mass spectrometric analysis of their sugar components. *Journal of the Science of Food and Agriculture* 82(2): 179-185. http://dx.doi.org/10.1002/jsfa.1011

TERRAB, A; DÍEZ, M J; HEREDIA, F J (2002) Characterisation of Moroccan unifloral honeys by their physicochemical characteristics. *Food Chemistry* 79(3): 373-379. http://dx.doi.org/10.1016/S0308-8146(02)00189-9

TERRAB, A; DÍEZ, M J; HEREDIA, F J (2003a) Palynological, physico-chemical and colour characterization of Moroccan honeys: I. River red gum (Eucalyptus camaldulensis Dehnh) honey. *International Journal of Food Science and Technology* 38(4): 379-386. http://dx.doi.org/10.1046/j.1365-2621.2003.00715.x

TERRAB, A; GONZÁLEZ, A G; DÍEZ, M J; HEREDIA, F J (2003b) Characterisation of Moroccan unifloral honeys using multivariate analysis. *European Food Research and Technology* 218(1): 88-95. http://dx.doi.org/10.1007/s00217-003-0797-x

TERRAB, A; PONTES, A; HEREDIA, F J; DÍEZ, M J (2004) Characterisation of Spanish thyme honeys by their physicochemical characteristics and mineral contents. *Food Chemistry* 88(4): 537-542. http://dx.doi.org/10.1016/j.foodchem.2004.01.068

TERRADILLOS, L A; MUNIATEGUI, S; SANCHO, M T; HUIDOBRO, J F; SIMAL-LOZANO, J (1994) An alternative method for analysis of honey sediment. *Bee Science* 3(2): 86-93.

THRASYVOULOU, A; MANIKIS, J (1995) Some physicochemical and microscopic characteristics of Greek unifloral honeys. *Apidologie* 26(6): 441-452. http://dx.doi.org/10.1051/apido:19950601

TOMÁS-BARBERÁN, F A.; MARTOS, I; FERRERES, F; RADOVIC, B S; ANKLAM, E (2001) HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *Journal of the Science of Food and Agriculture* 81(5): 485-496. http://dx.doi.org/10.1002/jsfa.836

VALENCIA-BARRERA, R M; HERRERO, B; MOLNAR, T (2000) Pollen and organoleptic analysis of honeys in Leon province (Spain). *Grana* 39(2/3): 133-140. http://dx.doi.org/10.1080/001731300300045283

VANHANEN, L P; EMMERTZ, A; SAVAGE, G P (2011) Mineral analysis of mono-floral New Zealand honey. *Food Chemistry* 128(1): 236-240. http://dx.doi.org/10.1016/j.foodchem.2011.02.064

VELA, L; DE LORENZO, C; PÉREZ, R A (2007) Antioxidant capacity of Spanish honeys and its correlation with polyphenol content and other physicochemical properties. *Journal of the Science of Food and Agriculture* 87(6): 1069-1075. http://dx.doi.org/10.1002/jsfa.2813

VIÑAS, P; BALSALOBRE, N; LÓPEZ-ERROZ, C; HERNÁNDEZ-CÓRDOBA, M (2004) Liquid chromatographic analysis of riboflavin vitamers in foods using fluorescence detection. *Journal of Agricultural and Food Chemistry* 52(7): 1789-1794. http://dx.doi.org/10.1021/jf030756s

VON DER OHE W, DUSTMANN J H, VON DER OHE, K (1991) Prolin als Kriterium der Reife des Honigs. *Deutsche Lebensmittel-Rundschau* 87: 383-386.

VON DER OHE, W; PERSANO-ODDO, L; PIANA, M L; MORLOT, M; MARTINS, P (2004) Harmonized methods of melissopalynology. *Apidologie* 35(Suppl. 1): S18-S25. http://dx.doi.org/10.1051/apido:2004050

WAŚ, E; RYBAK-CHMIELEWSKA, H; SZCZESNA, T; KACHANIUK, K; TEPER, D (2011) Characteristics of Polish unifloral honeys. III. Heather honey (*Calluna vulgaris* L.). *Journal of Apicultural Science* 55(1): 129-137.

WESTON, R J; BROCKLEBANK, L K (1999) The oligosaccharide composition of some New Zealand honeys. *Food Chemistry* 64(1): 33-37. http://dx.doi.org/10.1016/S0308-8146(98)00099-5

WHITE, J W JR. (1978) Honey. *Advances in Food Research* 24: 287-374. http://dx.doi.org/10.1016/S0065-2628(08)60160-3

WHITE, J W JR. (1979a) Spectrophotometric method for hydroxymethylfurfural in honey. *Journal of the AOAC International* 62(3): 509.

WHITE, J W JR. (1979b) Physical characteristics of honey. In *Crane, E (Ed). Honey: A comprehensive survey (2nd Edition)*. Heinemann; London, UK. pp. 207-239.

WHITE, J W JR.; MAHER, J (1953) Transglucosidation by honey invertase. *Archives of Biochemistry and Biophysics* 42(2): 360-367.

WHITE, J W JR.; RIETHOF, M L; SUBERS, M H; KUSHNIR, I (1962) Composition of American honeys. *USA Department of Agriculture Technical Bulletin* 1261: 1-124.

WHITE, J W; MELOY, R; PROBST, J; HUSER, W (1986) Sugars containing galactose occur in honey. *Journal of Apicultural Research* 25(3): 182-185.

YANG, Y; BATTESTI, M J; PAOLINI, J; MUSELLI, A; TOMI, P; COSTA, J (2012) Melissopalynological origin determination and volatile composition analysis of Corsican 'Erica arborea spring maquis' honeys. *Food Chemistry* 134(1): 37-47. http://dx.doi.org/10.1016/j.foodchem.2012.02.026

YÜCEL, Y; SULTANOĞLU, P (2013) Characterization of Hatay honeys according to their multi-element analysis using ICP-OES combined with chemometrics. *Food Chemistry* 140(1/2): 231-237. http://dx.doi.org/10.1016/j.foodchem.2013.02.046

ZHOU, J; SUO, Z; ZHAO, P; CHENG, N; GAO, H; ZHAO, J; CAO, W (2013) Jujube honey from China: physicochemical characteristics and mineral contents. *Journal of Food Science* 78(3): C387-C394. http://dx.doi.org/10.1111/1750-3841.12049

CHAPTER 5

CRITICAL ASSESSMENT OF ANTIOXIDANT-RELATED PARAMETERS OF HONEY

CRITICAL ASSESSMENT OF ANTIOXIDANT-RELATED PARAMETERS OF HONEY

ABSTRACT

This chapter corresponds to the part of the setting up and optimization of trolox equivalent antioxidant capacity (TEAC) and total flavonoid content (TFC) analyses, research that is included within the paper accepted in the International Journal of Food Science and Technology (Sancho *et al.*, in press; Annex 2/Article 1). In this study, several antioxidant-related parameters were researched on 56 Spanish honeys. Solid phase extraction (SPE) was used to obtain honeys' phenolic extracts. Total phenolics, total flavonoids and trolox equivalent antioxidant capacity (TEAC) were determined in both honeys and extracts. It was verified that total flavonoids determination in neutral media must be carried out on extracts instead of on honeys, because of sugars' interferences; likewise, extracts' colours must be corrected in this assay. The end-point for honeys' trolox equivalent antioxidant capacity was researched. Significant linear relationships were found between TEAC values of honeys and honeys' phenolic extracts, as well as between the results of TEAC measured at different times. Therefore, it would be possible to reliably calculate TEAC at 60 minutes (end-point), measuring the absorbance at 6 minutes, thus saving analysis time and reducing costs.

1. Introduction

Honey has a wide range of phenolic compounds and therefore, it has been reported to possess an antioxidant ability, which greatly depends on its composition that is, in turn, conditioned by the botanical source of this foodstuff. Studies about the antioxidant potential of various unifloral, multifloral and honeydew honeys are interesting in order to later check if some honeys have actually antioxidant effects when they are ingredients of other food products, and/or within the body after consumption (*in vivo* assays). The latter research is of particular interest since the European Food Safety Authority denied the health claims with regard to antioxidant-related properties of honey because this food *"…has not been sufficiently characterized in relation to the claimed effects"* (European Food Safety Authority, 2010, 2011). Flavonoids and other phenolics are the main compounds responsible for honey antioxidant activity (Malenica-Staver *et al.*, 2014). Honey flavonoids, as a whole, are usually determined by aluminum chloride chelation methods that must be carried out after sugars' removal, because these substances hamper proper chelation (Denni and Mammen, 2012). However, in most published papers, authors determine total flavonoids in neutral media directly on honeys, and sometimes, with no sample's colour correction. Trolox equivalent antioxidant capacity (TEAC) is a simple and widely used procedure to determine antioxidant activity of foods. Nevertheless, before using it to measure the antioxidant capacity of a

particular food, the endpoint of the assay should be previously researched (Van den Berg *et al.*, 1999; Prior *et al.*, 2005).

The aims of this work were: First, to study antioxidant-related features of honeys analyzing such parameters as total phenolics, total flavonoids, and TEAC. Second, to go in depth in the method for honeys' total flavonoids analysis carried out in neutral media. Finally, to research the endpoint for honey's TEAC determination, in order to set up a reliable honey's antioxidant activity analysis by TEAC method.

2. Material and methods

2.1. Honey samples

This work was carried out on 56 representative artisanal and unpasteurized Spanish honeys, whose botanical origins had been determined by melissopalinology (Von der Ohe *et al.*, 2004), with the result of 21 multifloral, 16 honeydew, 10 heather (*Erica* sp. and *Calluna vulgaris*), 5 lavender (*Lavandula* sp.), 3 clover (Leguminosae Type *Trifolium* sp.), and 1 sainfoin (Leguminosae Type *Onobrychis* sp.) honeys. Sensory analysis and other physicochemical parameters were taken into account for this classification. Sampling was carried out within the Castilla y León region, covering an area larger than 94,200 square kilometers. Samples were stored at 4ºC until analysis in dark conditions.

2.2. Phenolic extracts

Phenolic extracts were obtained by solid phase extraction (SPE). 10 g honey was mixed with 15 ml acidified water and loaded onto Strata-X SPE cartridge (Phenomenex®, Torrance, CA, USA) previously conditioned with methanol and water. Sugars and other polar honey's constituents were completely removed with acidified and ultrapure water. After vacuum drying, phenolic fractions were eluted from the cartridge with 3 ml 2:1 (v/v) methanol:acetonitrile (Bertoncelj *et al.*, 2011).

2.3. Total phenolic content

Total phenolic content in both honeys (TPCh) and extracts (TPCe) were determined by Folin-Ciocalteu method (Meda *et al.*, 2005). 0.5 ml of a filtered honey solution (100 mg/ml), or 0.5 ml of a diluted extract (40 µl/ml) were mixed with 2.5 ml of 0.2 N Folin Ciocalteu reagent. After 5 min, 2 ml of saturated sodium carbonate solution was added, and the mixtures were kept in the dark for 120 min. Then, the absorbance was read at 760 nm. Gallic acid was used to adjust the standard curve. Results were expressed in mg gallic acid (GAE)/100 g.

2.4. Total flavonoid content

Total flavonoid content was determined in the extracts (TFCe) by the Dowd aluminum chloride colorimetric assay in neutral media (Dowd, 1959; Meda *et al.*, 2005; Isla *et al.*, 2011), and adapted for the analysed samples. 1 ml of a diluted honey extract (60 µl/ml) was mixed with the same volume of 2% aluminium trichloride in methanol. After 10 minutes, absorbance (A_1) was read at 415 nm against a blank constituted by 1 ml of 2% $AlCl_3$ in methanol and 1 ml of methanol instead of the diluted honey extract. Colour of extracts was corrected by determining the absorbance (A_2) of a solution containing 1 ml of a diluted honey extract mixed with the same volume of methanol against a blank of methanol. A_2 was substracted from A_1 before calculating. Quercetin was used to adjust the standard curve that was read against a blank of methanol. The same procedure was also applied to 0.01 mg/ml honey solutions (TFCh). Results were expressed in mg quercetin (QE)/100 g.

2.5. TEAC antioxidant activity

TEAC antioxidant activity was determined by measuring the scavenging ability of antioxidants to the radical $ABTS^{\bullet+}$ (Re *et al.*, 1999). TEAC was analyzed in both honeys (TEACh) and extracts (TEACe), measuring the absorbance at 734 nm after 6, 30 and 60 minutes. The radical cation $ABTS^{\bullet+}$ was produced by the reaction of 7 mM ABTS stock solution with 2.45 mM potassium persulfate in the dark for 16 h. Then, the $ABTS^{\bullet+}$ solution was diluted to obtain an absorbance between 0.70 and 0.80 at 734 nm. For honey solutions (100 mg/ml), 10 µl of each honey solution was mixed with 990 µl of the diluted $ABTS^{\bullet+}$ solution. For honey extracts, first, 300 µl extract was diluted to 5 ml with methanol, and finally, 10 µl of each diluted extract was mixed with 990 µl of the diluted $ABTS^{\bullet+}$ solution. Blank was distilled water for honeys and methanol for honey extracts. Trolox (6-hydroxy-2,5,7,8-tetrametylchroman-2-carboxylic acid) was used to adjust the standard curve. Results were expressed in µmol trolox equivalent (TE)/g.

2.6. Statistical determinations

Statistical determinations were carried out with Statgraphics Centurion XVI (2010).
All analytical procedures were carried out in triplicate.

3. Results and discusion

The results of total phenolics, total flavonoids and TEAC antioxidant activity (measuring the absorbance at different times) are summarized in Table 1. Total flavonoids results are those corresponding to honey extracts, after verifying the unfeasibility of the method when diluted honeys were used instead of their extracts, because for more than 95% honeys the absorbance of the colour correction (1 ml of honey solution plus 1 ml of methanol), was higher than the absorbance of the sample (1 ml of honey solution plus 1 ml of 2% $AlCl_3$ in methanol).

Table 1. Mean, median, standard deviation, minimum and maximum values of total phenolics of honeys and extracts, total flavonoids of extracts and TEAC antioxidant activity of honeys and extracts.

				TEACh (µmol TE/g)			TEACe (µmol TE/g)		
	TPCh (mg GAE/100 g)	TPCe (mg GAE/100 g)	TFCe (mg QE/100 g)	*6 min*	*30 min*	*60 min*	*6 min*	*30 min*	*60 min*
Mean	119.24	26.29	3.44	4.35	5.90	6.92	1.92	2.34	2.58
Median	130.05	27.51	3.41	4.55	6.23	7.52	1.99	2.45	2.68
Standard deviation	39.42	8.95	1.15	1.88	2.35	2.57	0.68	0.79	0.85
Minimum	29.10	7.98	0.93	0.97	1.39	1.64	0.49	0.62	0.76
Maximum	183.35	60.30	6.98	7.46	9.49	10.65	3.16	3.85	4.43

3.1. Total phenolics

As expected, honeys' total phenolics were higher than extracts' total phenolics because the method of analysis (Meda *et al.*, 2005) actually determines total reducing capacity and, apart from phenolic compounds, honeys possess different reducing substances such as ascorbic acid, and reducing sugars, among others (Ferreira *et al.*, 2009).

3.2. Total flavonoids

With regard to flavonoids' contents, the values of this work were in general lower than those described for different honeys by other authors. After a thorough literature revision, it must be explained that in most published papers, the spectrophotometric assay based on aluminum complex formation conducted in neutral media was carried out directly on a honey solution with no sugars' removal and, in some cases, with no sample's colour correction. The reason is likely due to the fact that those papers followed previous references that were, in turn, based on procedures published for propolis extracts (Arvouet-Grand *et al.*, 1994; Popova *et al.*, 2005) in which there were no sugars' interferences. Thus, if the assay is carried out on a honey solution, the results depend on the specific flavonoid composition of the sample because, on the one hand, flavonoids do not react uniformly and, on the other hand, glycosylation prevent chelation of Al(III) with some flavonoids, but not with all of them (Pekal and Pyrzynska, 2014).

In addition, in the literature there is no agreement about the blank when total flavonoids are analyzed directly on a honey solution in neutral media. As it has been commented above, the assays of all manuscripts were based on others, being the common principle for all of them the initial spectrophotometric Dowd's procedure with some modifications. The original method (Dowd, 1959), used an aluminum chloride reagent blank. Nevertheless, for honey solutions, in some articles the blank employed was methanol, thereby neither the reagents nor the samples colour were corrected, so that the final flavonoids' values could be overestimated. Despite the fact that a blank of reagents (as in Dowd, 1959), is usual in spectrophotometric

measurements, very few authors employed such blank for the analysis of honeys' flavonoids in neutral media, and their manuscripts cited Isla *et al.* (2011) as a reference, which was in turn based on Popova *et al.* (2005) procedure that had been set up and applied to six poplar Turkish propolis, in which the colour of the extracts could have not interfered. Values of TFCh of those papers (based on the Popova *et al.*, 2005 manuscript), might also be overestimated because matrix interferences were not substracted. When a honey solution is used, a sample's colour correction is compulsory, since the results are based on the absorbance measurement at 415 nm (or 425 nm), and at this(these) wavelength(s), there is a colour interference of the honey itself, which is particularly important for dark samples. In most published papers about total flavonoids analysis on honey solutions, authors claimed that they followed Meda *et al.* (2005) procedure, based in turn on Arvouet-Grand *et al.* (1994) assay for propolis extracts, which used as blank a solution of the sample and the solvent, thus only correcting the colour of the samples, but not the interference in the absorbance recording due to the aluminum chloride. Therefore, data of TFCh of those manuscripts (based on Arvouet-Grand *et al.* 1994 paper), could be overestimated, as well.

Both for honeys' extracts and for honeys' solutions, we followed the procedure described in this manuscript, in which a blank of reagents was used, and then absorbance of the colour of the samples was substracted, in a similar way to that described in the official method of AOAC (2005) for the analysis of proline in honey.

We verified that, when using honey solutions (instead of honey extracts), for the vast majority of samples, the absorbance values at 415 nm (and also at 425 nm), were considerably higher for colour correction than for the sample with flavonoid-aluminium complex, showing the unreliability of the procedure if sugars and other interferences were not removed. Therefore, spectrophotometric analysis of honeys' flavonoids in neutral media must be always carried out after getting rid of sugars; otherwise the results could be specious.

3.3. TEAC

TEAC antioxidant activity (μmol trolox equivalent/g) of both honeys and extracts progressively increased with time up to 60 minutes. Significant linear relationships (90% confidence level), were found among all TEAC results (Table 2). Linear relationships were obtained between TEACh and TEACe, as well as between TEAC of honeys and extracts measuring absorbance at different times (Figure 1).

Table 2. Linear relationships among TEAC results of honeys and honeys' phenolic extracts.

Relationships	Correlation coefficient (r)
TEAC-Extracts = 0.59+0.30* TEAC-Honeys	0.9043
TEAC-Extracts-60 = 0.22 + 1.23*TEAC-Extracts-6	0.9879
TEAC-Extracts-60 = 0.08 + 1.07*TEAC-Extracts-30	0.9978
TEAC-Extracts-30 = 0.12 + 1.16*TEAC-Extracts-6	0.9937
TEAC-Honeys-60 = 1.00 + 1.35*TEAC-Honeys-6	0.9818
TEAC-Honeys-60 = 0.50 + 1.09*TEAC-Honeys-30	0.9959
TEAC-Honeys-30 = 0.49 + 1.24*TEAC-Honeys-6	0.9945
TEAC-HoneysandExtracts-60 = -0.03 + 1.52*TEAC-HoneysandExtracts-6	0.9817
TEAC-HoneysandExtracts-60 = 0.50 + 1.09*TEAC-HoneysandExtracts-30	0.9959
TEAC-HoneysandExtracts-30 = 0.49 + 1.24*TEAC-HoneysandExtracts-6	0.9945

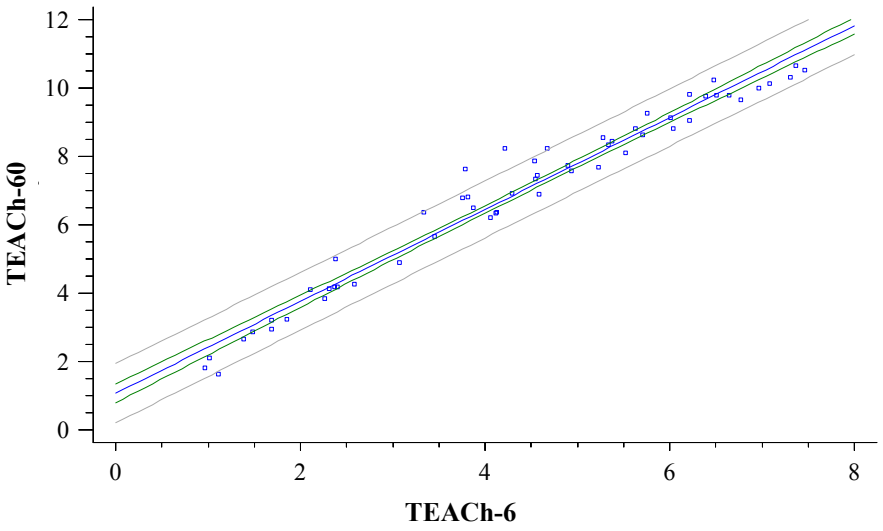

Figure 1. Relationships between TEAC values (µmol TE/g) of honeys at 6 and 60 minutes. "TEACh-60" is TEAC antioxidant activity measuring the absorbance at 60 minutes; "TEACh-6" is TEAC antioxidant activity measuring the absorbance at 6 minutes). Equation: TEACh-60 = 1.00 + 1.35*TEACh-6. Correlation coefficient = 0.9818.

With the results of honeys' TEAC at 6 minutes and the equations of Table 2, TEAC values at 60 minutes were calculated. Then, actual and calculated values of TEAC at 60 minutes were compared with t-test and One-way ANOVA (90% confidence level). Both procedures showed that there were no differences between actual TEAC values (µmol TE/g) of both honeys (Table 3) and extracts, measuring the absorbance at 60 minutes, and calculated TEAC values (µmol trolox equivalent/g) at 60 minutes, by measuring absorbance at 6 minutes.

Table 3. Summary of the results of t-test (90% confidence level) and variance check of one-way ANOVA (90% confidence level) applied to the results of honeys' TEAC actual values (µmol TE/g) measuring the absorbance at 60 minutes (TEACh-60), and the results of honeys' TEAC calculated values at 60 minutes (µmol TE/g), measuring the absorbance at 6 minutes (TEACh-6). t = 0.113464. P-value = 0.90987.

	Actual TEACh-60	Calculated TEACh-60
Sample size	56	56
Average	6.91875	6.86411
Standard deviation	2.56686	2.52967
Coefficient of variation	37.10%	36.85%
Minimum	1.64	2.31
Maximum	10.65	11.06
Range	9.01	8.75
Stnd. Skewness	-1.44897	-0.543526
Stnd. Kurtosis	-1.36357	-1.59705
Variance	6.58875	6.39924
Degrees of freedom	55	55

Variance Check		
	Test	*P-Value*
Levene's	0.010297	0.91936

Comparison	*Sigma1*	*Sigma2*	*F-Ratio*	*P-Value*
Actual TEACh-60 / Calculated TEACh-60	2.56686	2.52967	1.02961	0.9142

Our TEAC results were similar to the values described in the literature for Brazilian honeys (Sant'Ana *et al.*, 2012), and slightly lower than the antioxidant activities described for South African samples (Serem and Bester, 2012). Our TEAC data were also similar to those described in the literature for other honeys from different botanical and geographical origins that were analyzed by another method combined to a flow injection analysis (Álvarez-Suárez *et al.*, 2010a,b; Gorjanovic *et al.*, 2013).

In respect of TEAC antioxidant activity, in the literature no agreement was found regarding the proper end-point for the absorbance measurement. Some researchers determined the absorbance at 1, 4, 6 and 10 minutes (Baltrusaityté *et al.*, 2007; Escriche *et al.*, 2014), whereas other scientists considered the end-point at 1 minute (Tuberoso *et al.*, 2013), at 6 minutes (Vit *et al.*, 2009; Sant'Ana *et al.*, 2012), at 7 minutes (Habib *et al.*, 2014), at 10 minutes (Silva *et al.*, 2013), at 15 minutes (Socha *et al.*, 2009; Kowalski, 2013; Wilczynska, 2014), at 20 minutes (Lachman *et al.*, 2010), and at 30 minutes (Serem and Bester, 2012). Our work shows that absorbance values at different times change proportionally for both honeys from different botanical origins and their corresponding extracts, so that it would be possible to calculate the TEAC antioxidant activity at 60 minutes, measuring the absorbance at 6 minutes, thus saving analysis time and reducing costs. However, it would be necessary to study if similar relationships occur in other honeys from different origins and harvested in different years, in order to propose an appropriate analytical procedure for the determination of honey's TEAC antioxidant activity.

REFERENCES

ÁLVAREZ-SUÁREZ, J M; GONZÁLEZ-PARAMÁS, A M; SANTOS-BUELGA, C; BATTINO, M (2010a) Antioxidant characterization of native monofloral Cuban honeys. *Journal of Agricultural and Food Chemistry* 58(17): 9817-9824. http://doi.org/10.1021/jf1018164

ÁLVAREZ-SUÁREZ, J M; TULIPANI, S; DÍAZ, D; ESTÉVEZ, Y; ROMANDINI, S; GIAMPIERI, F; DAMIANI, E; ASTOLFI, P; BOMPADRE, S; BATTINO, M (2010b) Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. *Food and Chemical Toxicology* 48(8-9): 2490-2499. http://doi.org/10.1016/j.fct.2010.06.021

AOAC (2005). Proline in honey (method 979.20). In Official methods of analysis of AOAC International (edited by W. Horwitz). Pp. 25-37.Gaithersburg, Maryland, USA.

ARVOUET-GRAND, A; VENNAT, B; POURRAT, A; LEGRET, P (1994) Standardisation d'un extrait de propolis et identification des principaux constituants. *Journal de pharmacie de Belgique* 49(6): 462-468.

BALTRUSAITYTÉ, V; VENSKUTONIS, P R; ČEKSTERYTÉ, V (2007) Radical scavenging activity of different floral origin honey and beebread phenolic extracts. *Food Chemistry* 101(2): 502-514. http://doi.org/10.1016/j.foodchem.2006.02.007

BANSAL, V; SHARMA, H K; NANDA, V (2014) Optimisation of spray drying process parameters for low-fat honey-based milk powder with antioxidant activity. *International Journal of Food Science and Technology* 49(4): 1196-1202. http://doi.org/10.1111/ijfs.12416

BERTONCELJ, J; POLAK, T; KROPF, U; KOROSEC, M; GOLOB, T (2011) LC-DAD-ESI/MS analysis of flavonoids and abscisic acid with chemometric approach for the classification of Slovenian honey. *Food Chemistry* 127(1): 296-302. http://doi.org/10.1016/j.foodchem.2011.01.00

COMMISSION INTERNATIONALE DE L'ECLAIRAGE-CIE. (2004) Technical report. 3rd Edition. CIE 15:2004.

DENNI, M; MAMMEN, D (2012) A critical evaluation on the reliability of two aluminum chloride chelation methods for quantification of flavonoids. *Food Chemistry* 135(3): 1365-1368. http://doi.org/10.1016/j.foodchem.2012.05.109

DOWD, L E (1959) Spectrophotometric Determination of Quercetin. *Analytical Chemistry* 31(7): 1184-1187. http://doi.org/10.1021/ac60151a033

ESCRICHE, I; KADAR, M; JUAN-BORRAS, M; DOMENECH, E (2014) Suitability of antioxidant capacity, flavonoids and phenolic acids for floral authentication of honey. Impact of industrial thermal treatment. *Food Chemistry* 142: 135-143. http://doi.org/10.1016/j.foodchem.2013.07.033

EUROPEAN FOOD SAFETY AUTHORITY (2010) Scientific Opinion on the substantiation of health claims related to honey. *EFSA Journal* 8(2): 1484. http://doi.org/10.2903/j.efsa.2010.1484

EUROPEAN FOOD SAFETY AUTHORITY (2011) Scientific Opinion on the substantiation of health claims related to honey. EFSA Journal 9(6): 2243. http://doi.org/10.2903/j.efsa.2011.2243

FERREIRA, I C F R; AIRES, E; BARREIRA, J C M; ESTEVINHO, L M (2009) Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry* 114(4): 1438-1443. http://doi.org/10.1016/j.foodchem.2008.11.028

GAMBACORTA, E; SIMONETTI, A; GARRISI, N; INTAGLIETTA, I; PERNA, A (2014) Antioxidant properties and phenolic content of sulla (*Hedysarum* spp.) honeys from Southern Italy. *International Journal of Food Science and Technology* 49(10): 2260-2268. http://doi.org/10.1111/ijfs.12541

GONZÁLEZ-MIRET, M L; TERRAB, A; HERNANZ, D; FERNÁNDEZ-RECAMALES, M A; HEREDIA, F J (2005). Multivariate correlation between color and mineral composition of honeys and their botanical origin. *Journal of Agricultural and Food Chemistry* 53: 2574-2580. http://doi.org/10.1021/jf048207p

GORJANOVIC, S Ž; ÁLVAREZ-SUÁREZ, J M; NOVAKOVIC, M M; PASTOR, F T; PEZO, L; BATTINO, M; SUZNJEVIC, D Ž (2013) Comparative analysis of antioxidant activity of honey of different floral sources using recently developed polarographic and various spectrophotometric assays. *Journal of Food Composition and Analysis* 30(1): 13-18. http://doi.org/10.1016/j.jfca.2012.12.004

HABIB, H M; AL MEQBALI, F T; KAMAL, H; SOUKA, U D; IBRAHIM, W H (2014) Bioactive components, antioxidant and DNA damage inhibitory activities of honeys from arid regions. *Food Chemistry* 153: 28-34. http://doi.org/10.1016/j.foodchem.2013.12.044

ISLA, M I; CRAIG, A; ORDOÑEZ, R; ZAMPINI, C; SAYAGO, J; BEDASCARRASBURE, E; ALVAREZ, A; SALOMÓN, V; MALDONADO, L (2011) Physico chemical and bioactive properties of honeys from Northwestern Argentina. *LWT-Food Science and Technology* 44(9): 1922-1930. http://doi.org/10.1016/j.lwt.2011.04.003

KAMBOJ, R; BERA, M B; NANDA, V (2013) Evaluation of physico-chemical properties, trace metal content and antioxidant activity of Indian honeys. *International Journal of Food Science and Technology* 48(3): 578-587. http://doi.org/10.1111/ijfs.12002

KOWALSKI, S (2013) Changes of antioxidant activity and formation of 5-hydroxymethylfurfural in honey during thermal and microwave processing. *Food Chemistry* 141(2): 1378-1382. http://doi.org/10.1016/j.foodchem.2013.04.025

LACHMAN, J; ORSÁK, M; HEJTMÁNKOVÁ, A; KOVÁROVÁ, E (2010) Evaluation of antioxidant activity and total phenolics of selected Czech honeys. *LWT-Food Science and Technology* 43(1): 52-58. http://doi.org/10.1016/j.lwt.2009.06.008

MALENICA-STAVER, M; RATKAJ, I; BROZNIC, D; JERKOVIC, I; MARIJANOVIC, Z; ZELJEZIC, D; KRALJEVIC-PAVELIC, S (2014) Bioactivity of Satureja Montana L. honey extracts and their profile screening. *RSC Advances* 4(88): 47329-47340. http://doi.org/10.1039/C4RA08368G

MEDA, A; LAMIEN, C E; ROMITO, M; MILLOGO, J; NACOULMA, O G (2005) Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry* 91(3): 571-577. http://doi.org/10.1016/j.foodchem.2004.10.006

PEKAL, A; PYRZYNSKA, K (2014) Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay. *Food Analytical Methods* 7(9): 1776-1782. http://doi.org/10.1007/s12161-014-9814-x

PERNA, A; SIMONETTI, A; INTAGLIETTA, I; SOFO, A; GAMBACORTA, E (2012) Metal content of southern Italy honey of different botanical origins and its correlation with polyphenol content and antioxidant activity. *International Journal of Food Science and Technology* 47(9): 1909-1917. http://doi.org/10.1111/j.1365-2621.2012.03050.x

PETRETTO, G L; COSSU, M; ALAMANNI, M C (2015) Phenolic content, antioxidant and physico-chemical properties of Sardinian monofloral honeys. *International Journal of Food Science and Technology* 50(2): 482-491. http://doi.org/10.1111/ijfs.12652

PRIOR, R L; WU, X; SCHAICH, K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry* 53(10): 4290-4302. http://doi.org/10.1021/jf0502698

POPOVA, M; SILICI, S; KAFTANOGLU, O; BANKOVA, V (2005) Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. *Phytomedicine* 12(3): 221-228. http://doi.org/10.1016/j.phymed.2003.09.007

RE, R; PELLEGRINI, N; PROTEGGENTE, A; PANNAL, A; YANG, M; RICE-EVANS, C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* 26(9-10): 1231-1237. http://doi.org/10.1016/S0891-5849(98)00315-3

SANT'ANA, L D; SOUSA, J P L M; SALGUEIRO, F B; LORENZON, M C A; CASTRO, R N (2012) Characterization of monofloral honeys with multivariate analysis of their chemical profile and antioxidant activity. *Journal of Food Science* 77(1): C135-C140. http://doi.org/10.1111/j.1750-3841.2011.02490.x

SEREM, J C; BESTER, M J (2012) Physicochemical properties, antioxidant activity and cellular protective effects of honeys from southern Africa. *Food Chemistry* 133(4): 1544-1550. http://doi.org/10.1016/j.foodchem.2012.02.047

SILVA, I A A D; SILVA, T M S D; CAMARA, C A; QUEIROZ, N; MAGNANI, N; NOVAIS, J S D; SOLEDADE, L E B; LIMA, E D O; SOUZA, A L D; SOUZA, A G D (2013) Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas, Northern Brazil. *Food Chemistry* 141(4): 3552-3558. http://doi.org/10.1016/j.foodchem.2013.06.072

SOCHA, R; JUSZCZAK, L; PIETRZYK, S; FORTUNA, T (2009) Antioxidant activity and phenolic composition of herb honeys. *Food Chemistry* 113(2): 568-574. http://doi.org/10.1016/j.foodchem.2008.08.029

STATGRAPHICS CENTURION XVI. (2010) Statpoint Technologies, Inc. Warrenton, VA (USA).

TUBEROSO, C I G; BOBAN, M; BIFULCO, E; BUDIMIR, D; PIRISI, F M (2013) Antioxidant capacity and vasodilatory properties of Mediterranean food: the case of Cannonau wine, myrtle berries liqueur and strawberry-tree honey. *Food Chemistry* 140(4): 686-691. http://doi.org/10.1016/j.foodchem.2012.09.071

TUBEROSO, C I G; JERKOVIĆ, I; SARAIS, G; CONGIU, F; MARIJANOVIĆ, Z; KUŚ, P M (2014) Color evaluation of seventeen European unifloral honey types by means of spectrophotometrically determined CIE L*, C*ab, hºab chromaticity coordinates. *Food Chemistry* 145: 284-291. http://doi.org/10.1016/j.foodchem.2013.08.032

VAN DEN BERG, R; HAENEN, G R M M; VAN DEN BERG, H; BAST, A (1999) Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chemistry* 66(4): 511-517. http://doi.org/10.1016/S0308-8146(99)00089-8

VIT, P; RODRÍGUEZ-MALAVER, A; ROUBIK, D W; MORENO, E; ALMEIDA, S B; SANCHO, M T; FERNÁNDEZ-MUIÑO, M A; ALMEIDA-ANACLETO, D; MARCHINI, L C; GIL, F; GONZÁLEZ, C; AGUILERA, G; NIEVES, B (2009) Expanded parameters to assess the quality of honey from Venezuelan Apis mellifera. *Journal of ApiProduct and ApiMedical Science* 1(3): 72-81. http://doi.org/10.3896/IBRA.4.01.3.03

VON DER OHE, W; PERSANO-ODDO, L; PIANA, M L; MORLOT, M; MARTIN, P (2004) Harmonized methods of melissopalynology. *Apidologie* 35 (Suppl. 1): S18-S25. http://doi.org/10.1051/apido:2004050

WILCZYNSKA, A (2014) Effect of filtration on colour, antioxidant activity and total phenolics of honey. *LWT-Food Science and Technology* 57(2): 767-774. http://doi.org/10.1016/j.lwt.2014.01.034

CHAPTER 6

ANTIOXIDANT CAPACITY AND BIOACTIVE COMPOUNDS OF HONEYS FROM CASTILLA Y LEÓN (SPAIN)

ANTIOXIDANT CAPACITY AND BIOACTIVE COMPOUNDS OF HONEYS FROM CASTILLA Y LEÓN (SPAIN)

ABSTRACT

This research was focused on analysing by HPLC the polyphenol profiles of honeys from Castilla y León (Spain). Fourteen compounds were quantified: six phenolic acids and eight flavonoids. The main phenolic compounds quantified in these honeys were ellagic acid and pinocembrin. The results were discussed together with the data of colour, total phenolic content (TPC), total flavonoid content (TFC) and trolox equivalent antioxidant capacity (TEAC). Total phenolic contents and TEAC results were compared in honeys and their methanolic extracts. Dark honeys showed higher ellagic acid, phenolic acid content (PA), TPC and TEAC, and lower L*, quercetin and kaempferol contents than light ones. Significant correlations were found among some antioxidant-related parameters. However, there were no significant correlations between TFC and TPC, as well as between TFC and TEAC. Bioactive compounds and other antioxidant-related parameters proved to be useful for the discrimination of honeys from some botanical origins.

1. Introduction

Honey is a natural sweet substance produced by honeybees, and consumed since ancient times for its nutritive value and health-promoting effects. This food is a complex mixture of at least 200 substances, where carbohydrates (mainly fructose and glucose) and water are the main constituents. It also contains small amounts of other minor but interesting compounds (White, 1978; Küçük *et al.*, 2007). Honey composition is related to its botanical source, the geographical area of origin (environmental conditions and weather), the physiology of the bee and the post-harvest processing and storage conditions (Anklam, 1998; Gheldof and Engeseth 2002).

Honey has shown antioxidant activity. It contains bioactive compounds that play an interesting role in food preservation and human health (Chen *et al.*, 2000), avoiding the oxidative damage by acting as free radical scavengers (Ferreres *et al.*, 1993; Nagai *et al.*, 2001; Gheldof *et al.*, 2002; The National Honey Board, 2003). Most honey's bioactive compounds come from plants that are used by bees to collect nectar or honeydew. Those phytochemicals are then transferred into the honey (Baltrušaite *et al.*, 2007; Alvarez-Suarez *et al.*, 2010; Bertoncelj *et al.*, 2011b). Antioxidant compounds present in honey include enzymatic substances, organic acids, Maillard reaction products, amino acids, proteins, vitamins, carotenoid derivatives and polyphenolic compounds, such as flavonoids and phenolic acids (Gheldof *et al.*, 2002; Baltrušaite *et al.*, 2007). Many of these components have

been suggested as markers for botanical and/or geographical origin of honeys (Yao *et al.*, 2004).

Variations of honey's antioxidant activity depend on the quantitative and qualitative nature of phenolic constituents, because they have various chemical structures (Meda *et al.*, 2005; Michalak *et al.*, 2006) with different antioxidant power and, in addition some of them might not be antioxidant agents (Aljadi and Kamaruddin, 2004).

Because of sugars interferences, in the analysis of honey's phenolics, their isolation from the honey matrix is compulsory to get a proper identification and quantification (Ferreres *et al.*, 1991). For this purpose, several techniques have been employed. The first one was the liquid-liquid extraction (Amiot *et al.*, 1989), but lacks of uncompleted phenolics recoveries as well as significant interferences in the chromatographic determination were important drawbacks (Ferreres *et al.*, 1994c; Hennion, 1999; Rodríguez *et al.*, 2000).

Solid Phase Extraction (SPE) by using the non-ionic polymeric resin Amberlite XAD-2 was a widely employed method, followed by a purification step that includes filtration with Sephadex LH20 or liquid-liquid extraction with ethyl ether to guarantee polar compounds removal (Ferreres *et al.*, 1991; Tomás-Barberán *et al.*, 1993a; Ferreres *et al.*, 1994a,c). As the Amberlite method is time-consuming, needing high quantities of honey sample and toxic solvents, in the recent years a simple and selective SPE method using cartridges has been developed to overcome these disadvantages (Dimitrova *et al.*, 2007).

After phenolic compounds extraction and purification, reversed-phase HPLC with Diode Array Detector (DAD) has been the most used technique for phenolics' separation and quantification (Tuberoso *et al.*, 2013; Habib *et al.*, 2014).

The research on honeys' phenolic substances has been focused on their botanical and/or geographic characterization. Phenolics' profile can provide with a "fingerprint" of a specific origin, characterized by different profiles or the same phenolic pattern but with different relative amounts. Then, reliable chemical markers can be identified and quantified. Phenolics that have been suggested as markers so far, are: 1) compounds that are present in honeys from specific botanical origins and that have not been yet detected in honeys from other sources, such as hesperetin in citrus honey (Ferreres *et al.*, 1993); 2) substances that are common for different unifloral honeys, whose relative amounts are typical of specific origins, such as ellagic acid in heather honey (Ferreres *et al.*, 1996); 3) compounds present in several honeys from different floral origin, whose HPLC profiles are characteristics of specific botanical origins, such as myricetin, tricetin, quercetin, luteolin and kaempferol in eucalyptus honey (Martos *et al.*, 2000a,b). Pulcini *et al.* (2006) claimed that for the characterization of the botanical origin of a given honey, differences in the whole composition of the polyphenols could be more suitable than the use of a single specific compound.

This work is part of a research project about the study of representative samples of artisanal honeys produced in Castilla y León, region that covers the area of North Central Iberian Peninsula. Phenolics' profiles, total phenolics', total flavonoids' contents, TEAC antioxidant activities and colour parameters were analysed.

2. Material and methods

2.1. Samples

2.1.1. Honey samples

This study was carried out with 53 representative raw honey samples from all nine provinces of Castilla y León (Spain). Sampling area covered the most important artisanal honey production zones within the region (94,200 km^2). All samples were harvested in 2011, being stored at 4ºC in the dark until analysis. Botanical origins were determined by both mellisopalinology (Louveaux *et al.*, 1978; Terradillos *et al.*, 1994; Von der Ohe *et al.*, 2004) and sensory analyses, and then confirmed by other physicochemical determinations such as pH, electrical conductivity, sugar profiles and colour, among others. Botanical origins of samples showed that there were 18 honeydew honeys, 15 multifloral honeys rich in broom, clover, chestnut and/or sunflower, 9 heather honeys (*Erica* sp. and *Calluna vulgaris*), 4 chestnut honeys (*Castanea sativa*), 4 lavender honeys (*Lavandula* sp.) and 3 clover honeys (Leguminosae type *Trifolium* sp.). Chestnut honeys were classified using the criteria published by Spanish honeys labelled as chestnut within the Protected Geographical Indication "Miel de Galicia" (OJEU, 2007). Honeydew honeys were classified according to the criteria proposed by Mateo and Boch-Reig (1997, 1998).

2.1.2. Polyphenol methanolic extracts

In order to eliminate interfering compounds, the flavonoids and phenolic acids from the honey matrix were extracted following the SPE method described by Bertoncelj *et al.* (2011b) with some modifications that helped improve extracts' purification and pre-concentration (Chapter 5). First, the Strata-X SPE cartridges (200 mg/6 ml, supplied by Phenomenex, Torrance, CA, USA) were conditioned with 3 ml of methanol, followed by 3 ml of ultrapure water. Honey samples (10.00 g) were dissolved in 15 ml of water acidified to pH 2 with concentrated HCl and loaded onto the previously conditioned cartridges (Sergiel *et al.*, 2014). After removing sugars and other polar honeys' interferences by washing with 5 ml acidified ultrapure water and then with 15 ml ultrapure water, cartridges were vacuum dried during 3 minutes. Finally, the phenolic fraction retained on the cartridge was eluted with 3 ml of a mixture methanol:acetonitrile (2:1, v:v).

2.2. Flavonoids and phenolic acids profile

2.2.1. Preparation of standards and calibration curves

The standards of chlorogenic acid, caffeic acid, rutin, ellagic acid, coumaric acid, sinapic acid, ferulic acid, luteolin, quercetin, naringenin, kaempferol, chrysin, pinocembrin and galangin (purity higher than 98%) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

A methanolic stock solution with a concentration of 1 mg/ml for each standard was prepared and stored at -20ºC. A working standard solution of 100 ng/ml in water was also prepared and stored at 4ºC.

Calibration curves (0.5-30 ng/ml) were obtained plotting peak areas versus concentrations. In order to evaluate the matrix effect, calibration curves were prepared with the extracts obtained from 10.00 g of one honey sample spiked with the standard working solution. Then, calibration curves in solvent were compared with calibration curves in spiked honey. As there were differences between the calibration curves in solvent and calibration curves in spiked honey, calibration curves in spiked honey were used for quantification in all samples.

An internal quality control (standard working solution) was injected into the equipment as a first step, before each batch of samples, in order to ensure the quality of the results and evaluate the stability of the proposed method.

2.2.2. HPLC-DAD analysis

The extracts of flavonoids and phenolic acids were filtered through 0.45 µl membrane filters, and analysed using an HPLC system (Waters Alliance 2695) with a photodiode array detector (Waters 2996, USA). Flavonoids and phenolic compounds were separated on a Brisa LC2, C18 column (250 mm x 4.6 mm x 5 µm) (Teknokroma, Spain). The binary mobile phase consisted of solvent A (ACN) and solvent B (water and formic acid, 99:1). Binary gradient conditions were: initial, 90% B, linear gradient to 40% B from 0 min to 25 min, held for 1 min, and then a second linear gradient to 20% B from 26 min to 40 min. The temperature of the column was 30ºC. The flow-rate and the injection volume were 0.5 ml/min and 10 µl, respectively (Periche *et al.*, *in press*). Chromatograms were recorded at three wavelengths (290, 320 and 360 nm).

2.2.3. Identification and quantification of the flavonoids and phenolic acids

Flavonoids and phenolic acids were identified by comparison of chromatographic retention times and UV spectral characteristics of unknown compounds with those of standards and the available literature data (Merken and Beecher, 2000).

Quantification was performed by using the calibration curves at 0.5, 1, 2, 5, 7 and 10 mg/l. Results were expressed as µg of compound per g of honey.

2.2.4. Validation of the polyphenol analysis method

The guidelines established by the European Union Commission Decision (OJEC, 2002) were followed in order to validate the analytical methodology employed to analyse the flavonoids and phenolic acids. For this purpose, several parameters were studied: linearity, accuracy (established through recovery studies), and precision verified by repeatability or intraday precision and reproducibility or interday precision.

2.3. Total phenolic content

The Folin–Ciocalteu method was employed (Singleton *et al.*, 1999), following the procedure described by Meda *et al.* (2005). Measurements were carried out with a Varian/Agilent Cary 400 UV/VIS Bio double-beam Lab Spectrophotometer (Palo Alto, California), using gallic acid as standard (mg GAE/100 g). Samples consisted of 5.00 g honey diluted up to 50 ml with distilled water and filtered. Extract's samples consisted of 200 µl extracts filled up to 5 ml with methanol (Chapter 5).

2.4. Total flavonoid content

The total flavonoid content was determined on honeys' extracts by the aluminium trichloride procedure carried out in neutral media (Dowd, 1959), following the procedure detailed in Chapter 5. Each sample consisted of 300 µl extract diluted up to 5 ml of methanol. Measurements were carried out with a Varian/Agilent Cary 400 UV/VIS Bio double-beam Lab Spectrophotometer (Palo Alto, California), using quercetin as standard (mg QE/100 g). The flavonoid content was measured only in the honey methanolic extracts, because by using honey samples, their absorbance of the colour correction was sometimes higher than the absorbance of the sample due to matrix interferences (Chapter 5).

2.5. TEAC

TEAC antioxidant activity was determined by measuring the scavenging ability of antioxidants to the radical $ABTS^{\bullet+}$ according the method described by Re *et al.* (1999) with some modifications (Chapter 5). Honey samples consisted of 2.50 g honey diluted and filled up to 50 ml with distilled water. Measurements were carried out at 6 minutes (Chapter 5) with a Varian/Agilent Cary 400 UV/VIS Bio double-beam Lab Spectrophotometer (Palo Alto, California), using trolox (6-hydroxy-2,5,7,8-tetrametylchroman-2-carboxylic acid) as standard (µmol TE/g).

2.6. Colour

The honey surface colour was measured by the CIELAB system (Commission Internationale de L'eclairage, 2004) using a HunterLab colorimeter model Color Flex EZ (Reston, VA,

USA), recording the reflectance of the whole visible spectrum in a wavelength interval between 380 and 770 nm, with 45°/0° geometry, illuminant D65 and 10° observation angle.

The colour coordinates L* (lightness, 100 for white and 0 for black), a* (positive values for redness and negative values for greenness, +100/-100) and b* (positive values for yellowness and negative values for blueness, +100/-100) were measured. The L*C*h* colour space uses the same diagram as the L*a*b* colour space, but with Polar coordinates instead of Cartesian ones (Figure 1) (Tuberoso *et al.*, 2014). These values were calculated from L*, a* and b* coordinates (González-Miret *et al.*, 2005). C* is the chroma, saturation, vividness or purity of a colour that represents the amount of colour measuring the distance from the coordinates origin (+100/-100) ($C^*=(a^{*2}+b^{*2})^{1/2}$) and h* is the hue angle or tone, that is the perceived colour to human eye's (0º red, 90º yellow, 180º green and 270º blue) (h*=arctg b*/a*) (Tuberoso *et al.*, 2014).

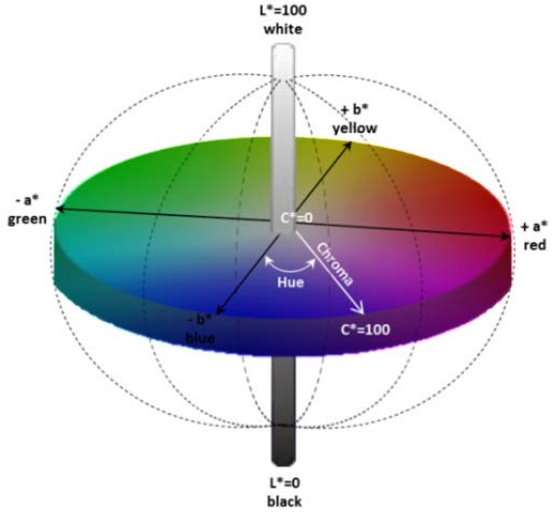

Figure 1. L*, a*, b*, C* and h* colour coordinates.

2.7. Statistical analysis

The statistical package software Statgraphics Centurion XVI.II (2010) was employed. When possible, analysis of variance (ANOVA) for the comparison of means was carried out. When normality assumption was not fulfilled, Kruskal-Wallis ANOVA was used. Possible correlations among the parameters were researched. Annex 1/Table 4 shows the Pearson's correlations (significant correlations $p<0.05\%$). Multivariate Principal Components Analysis (PCA) was also carried out.

3. Results and discussion

3.1. Colour

Honey colour is an important quality factor for consumers. Colour preferences depend on the geographical areas in which honeys are produced. It is closely related to the floral origin and

chemical composition of honey, primarily pigments such as carotenoids, flavonoids and phenolic acids, which also contribute to the antioxidant properties (Frankel *et al.*, 1998; Juszczak *et al.*, 2009; Eleazu *et al.*, 2013). In the literature, dark-coloured honeys showed higher total phenolic and total flavonoid contents, and consequently higher antioxidant activity than light honeys (Meda *et al.*, 2005; Alvarez-Suarez *et al.*, 2010). Different researchers discriminated different honey types on the basis of colour parameters. Nevertheless, it is important to bear in mind that small contribution of other nectars as well as heating and aging affect the honey colour (Kuś *et al.*, 2015).

Table 1. Averages, standard deviations and maximum and minimum values of the colour parameters of artisanal honeys from Castilla y León.

	Chestnut (n=4)	Clover (n=3)	Heather (n=9)	Honeydew (n=18)	Lavender (n=4)	Multifloral (n=15)
	Mean ± SD (min; max)	Mean ± SD (min; max)	Mean ± SD (min; max)	Mean ± SD (min; max)	Mean ± SD (min; max)	Mean ± SD (min; max)
L*	39.54 ± 1.70^{a} (38.23; 41.99)	$52.44 \pm 1.83^{b,c}$ (50.49; 54.12)	$44.50 \pm 5.52^{a,b}$ (39.10; 57.36)	41.53 ± 6.67^{a} (26.07; 58.26)	59.49 ± 11.71^{c} (43.16; 71.01)	56.70 ± 8.68^{c} (44.11; 66.19)
a*	$11.86 \pm 1.81^{a,b}$ (10.14; 14.41)	$7.59 \pm 1.01^{c,d}$ (6.58; 8.59)	12.53 ± 2.25^{a} (7.19; 14.57)	$9.85 \pm 1.48^{b,c}$ (5.73; 12.65)	4.51 ± 4.01^{d} (0.61; 9.70)	$6.72 \pm 2.58^{c,d}$ (3.20; 11.53)
b*	34.67 ± 2.61^{a} (31.69; 37.95)	33.33 ± 0.30^{a} (33.11; 33.67)	38.31 ± 2.59^{b} (33.78; 43.46)	32.25 ± 2.35^{a} (26.24; 36.91)	31.74 ± 4.14^{a} (26.88; 35.72)	34.31 ± 2.74^{a} (29.83; 38.70)
C*	36.65 ± 3.04^{a} (33.27; 40.59)	34.19 ± 0.51^{a} (33.76; 34.75)	40.34 ± 2.89^{b} (35.51; 45.84)	33.74 ± 2.43^{a} (28.35; 38.57)	32.20 ± 4.59^{a} (26.89; 36.14)	35.04 ± 2.94^{a} (30.64; 40.38)
h* (º)	71.19 ± 1.41 (69.21; 72.26)	77.18 ± 1.54 (75.69; 78.76)	71.96 ± 2.72 (69.53; 78.91)	73.02 ± 2.37 (67.78; 79.40)	82.49 ± 6.24 (74.33; 88.70)	79.01 ± 3.80 (73.41; 84.10)

Mean values within the same row having different lower-case letter are significantly different by Student-Newman-Kreus multiple range test (p<0.05). No letters mean that ANOVA could not be determined.

Table 1 shows that L* ranged between 26.07 (honeydew honey) to 71.01 (lavender honey). Chestnut honeys showed the lowest mean value of lightness, followed by honeydew and heather samples, being all of them dark-coloured honeys, in agreement with the results found in the literature (Bertoncelj *et al.*, 2007; González-Paramás *et al.*, 2007; Can *et al.*, 2015). All these dark honeys had L* values lower than 50, agreeing with González-Miret *et al.* (2005) research. High L* averages were those of light honeys, always with values higher than 50. Lavender samples were the brightest, followed by multifloral and clover honeys. a* (red component) and b* (yellow component) values were positive in all honeys, a* ranged from 0.61 (lavender honey) to 15.64 (heather honey), and b* between 26.24 (honeydew honey) and 43.46 (heather honey). Lavender honeys showed the lowest mean data of red and yellow colours, and heather honeys the highest ones. With regard to the chroma C*, that represents the colour intensity, the highest average (lower greyish tone) was found for heather samples and the lowest one for lavender. The rest of the samples showed similar C* mean values than lavender honeys. In respect of h*, our samples were located in a tight range of hue angles, from 67.78º to 88.70º. Light honeys (lavender, multifloral and clover) showed hue averages

higher than those of dark samples (chestnut, heather and honeydew), in accordance to the literature findings (González-Miret *et al.*, 2005; Tuberoso *et al.*, 2014). The information provided by the same researchers about C* values is contradictory. González-Miret *et al.* (2005) reported low C* values for dark honeys and high values for light ones, on the basis of the high values of b* colour coordinate found in light samples in comparison with those considerably lower found in dark ones. Conversely, Tuberoso *et al.* (2014) reported high C* values for dark samples, being the opposite for light ones.

Heather honeys showed means of C* and b* significantly higher than the other samples, so that C* and b* values could be potentially useful to help characterize heather honeys. L* values grouped honeydew and chestnut honeys, as well as lavender and multifloral honeys.

With regard to the correlations between the colour parameters, only significant ($p<0.05$) correlations were considered (Annex 1/Table 4). L*, a* and h* were correlated among them with $r>0.8467$. This shows that L* was higher in samples with less reddish colours and higher hue angles. On the other hand, C* was strongly correlated with b* ($r=0.9849$) and had a moderate significant relationship with a* ($r=0.6610$), showing that greyish samples (higher C* values), possessed higher yellowish and reddish colours.

In comparison with our results, for honeys from the same botanical origins, literature references described higher values for C*, b* and similar for L*, a* and h*, showing a considerably variability among data (Annex 1/Table 3), that makes it difficult compare results. Honeys' colour depends on many factors such as geographical origins (soil, weather) and accompanying flora (Almeida-Muradian *et al.*, 2014; Tuberoso *et al.*, 2014), as well as on the different methodology for colour measurement, with diverse data acquisition system (reflectance or transmittance), observer angle or illuminant and/or the physical state of honey that is usually not mentioned (Tuberoso *et al.*, 2014). Samples are usually liquefied in a water bath at <45 °C throughout 1 hour, so that sugar crystals are dissolved. Honey liquefaction is compulsory only if colour is measured by transmittance or analysed by Pfund procedure. In our study, we faced problems regarding heather samples liquefaction, in particular ling heather honeys and samples rich in *Calluna vulgaris*, probably due to the thixotropy of these honeys. The same drawback was also reported by Waś *et al.* (2011). After trying an increase of the water bath temperature and/or the heating time, we realised that total clarification of those samples was not possible. Moreover high temperatures or high processed time resulted in a darkening of the samples. In order to avoid heating-related problems, eventually, we decided to measure the colour of the honey surface by reflection in all unheated samples.

The colour of four honeys (light, medium, dark and very dark samples) in both liquid and crystallized state was also compared (Table 2). It was observed that the lightest honey possessed the highest L* and h* values, whereas the darkest sample had the lowest ones, both in liquid and crystallised states. Liquid light samples presented lower greyish, lower reddish and higher yellowish colours than liquid dark honeys, being these values similar in

crystallised samples. Comparing the physical state of honeys, crystallised samples were lighter (higher L*) and greyer (lower C* values) than liquid honeys. Red component and hue angle were similar in both liquid and crystallized light honeys at all states, but a* was higher in liquid dark samples and h* in crystallised dark samples. Yellow component was similar in both liquid and crystallized dark honeys being higher in liquid light samples.

Table 2. Comparison of colour parameters among light and dark honeys in liquid and crystallized states.

		LIGHT HONEYS		DARK HONEYS	
HONEY SAMPLE		Lavender	Multifloral	Heather	Honeydew
Liquid state	L*	54.07	49.89	26.27	22.20
	a*	5.01	12.75	23.77	22.44
	b*	53.77	67.75	41.16	34.11
	C*	54.00	68.94	47.53	40.82
	h*	84.68	79.34	59.99	56.66
Crystallized state	L*	60.79	51.14	38.77	23.79
	a*	5.56	11.18	12.84	12.54
	b*	37.70	46.05	38.77	27.69
	C*	38.11	47.39	39.78	30.40
	h*	81.61	76.35	71.17	65.64

3.2. Phenolic compounds profile

3.2.1. Validation of polyphenols and flavonoids analytical methodology

The results from the polyphenols and flavonoids validation procedure are available in table 3. The wavelength used for compounds' quantification and their retention times were also shown. In order to obtain the linearity value, an external standard calibration curve was made using 6 standard solutions with final concentration levels from 0.5 to 10 mg/l. Six replicates were made for each level. The calibration curves were obtained by plotting the peak area of the compound at each level versus the concentration. The linearity response observed from 0.5 to 10 mg/l was good because the correlation coefficient between peak areas and injected nominal concentrations was $r^2 \geq 0.996$.

The recovery studies were performed by adding known quantities of antioxidants to a sample (1, 5 and 10 mg/l). Six replicates of all the spiked sample levels were analysed using the HPLC method. Antioxidants' standards recoveries varied between 83.1% and 119.5% for the studied concentration range. The relative standard deviation (RSD) corresponding to recovery values was less than 20% in all cases (ranging from 0.4 to 16.1), confirming that the analytical method was accurate.

Repeatability was evaluated by performing the assay on six replicates of fortified honey samples, at the same levels (1, 5 and 10 μg/g), and it was carried out by the same operator on the same day. In order to evaluate reproducibility, the experiment was performed by 2 different operators on 3 consecutive days. The results were expressed as the percentage of

relative standard deviation. Intra-day precision (RSD_r) ranged from 0.3% to 15.5% and inter-day precision (RSD_R) from 3.0% to 16.6%. These RSD values were in complete agreement with those proposed by the European Union Commission Decision (OJEC, 2002) requirements since they were always lower than 20% for all the concentration levels assayed. Therefore, it can be concluded that the method used in this work had good precision.

The results of the validation proved that the analytical procedure carried out appropriately guarantees the quantitative values of polyphenols and flavonoids obtained in the samples analysed.

Table 3. Validation parameters (accuracy and precision) of antioxidants.

COMPOUND	λ	Retention time	Level (µg/g)	% recovery (SD)	RSD_r %	RSD_R %
Chlorogenic acid	320	14.58	1	93.9 (1.8)	2.0	10.3
			5	98.8 (6.3)	6.4	5.3
			10	108.5 (0.5)	0.6	10.1
Caffeic acid	320	17.00	1	110.4 (13.5)	12.2	13.7
			5	103.6 (3.5)	3.4	6.1
			10	91.3 (2.6)	2.8	12.6
Rutin	360	17.43	1	84.1 (5.4)	6.5	13.2
			5	95.2 (6.6)	7.0	5.5
			10	100.9 (0.7)	1.3	8.3
Ellagic acid	360	18.68	1	107.1 (11.3)	9.6	10.6
			5	92.8 (7.3)	8.0	6.6
			10	119.2 (6.6)	5.3	15.1
Coumaric acid	320	19.98	1	96.4 (7.1)	7.4	7.4
			5	95.1 (4.8)	5.0	6.2
			10	105.4 (3.2)	3.0	6.8
Sinapic acid	320	20.31	1	84.4 (8.2)	7.4	10.2
			5	94.6 (6.1)	6.4	6.9
			10	108.6 (10.1)	9.3	12.0
Ferulic acid	320	20.76	1	92.2 (7.8)	8.5	8.5
			5	85.9 (6.5)	7.6	8.1
			10	119.1 (0.4)	0.3	14.4
Luteolin	320	25.07	1	100.5 (5.7)	13.7	8.2
			5	96.8 (4.1)	4.3	3.0
			10	91.8 (8.1)	8.9	9.3
Quercetin	360	25.40	1	119.5 (6.9)	5.1	5.8
			5	92.4 (7.9)	8.6	12.2
			10	104.5 (16.1)	15.5	13.2

COMPOUND	λ	Retention time	Level (μg/g)	% recovery (SD)	RSD_r %	RSD_R %
Naringenin	290	28.05	1	76.9 (13.6)	14.8	15.1
			5	99.6 (0.9)	0.9	1.9
			10	99.1 (1.4)	1.4	7.5
Kaempferol	360	28.42	1	95.1 (3.9)	4.1	12.4
			5	93.3 (5.2)	5.5	13.3
			10	100.8 (13.5)	13.4	16.6
Chrysin	320	33.34	1	90.8 (9.4)	10.4	11.8
			5	98.4 (4.5)	4.5	7.2
			10	96.3 (2.7)	2.8	8.2
Pinocembrin	290	33.42	1	86.1 (0.5)	0.6	7.9
			5	83.1 (11.1)	13.4	12.2
			10	98.9 (1.5)	1.6	9.1
Galangin	290	33.62	1	114.6 (4.3)	4.4	10.4
			5	97.5 (1.1)	1.2	5.3
			10	103.3 (3.2)	3.1	7.7

3.2.2. Polyphenols profile

In this study, fourteen phenolic compounds were identified and quantified: six phenolic acids (chlorogenic, caffeic, ellagic, coumaric, sinapic and ferulic) and eight flavonoids (rutin, luteolin, quercetin, naringenin, kaempferol, chrysin, pinocembrin and galangin). The summatory of the total phenolic acids (PA_{HPLC}) and the total flavonoids (TFC_{HPLC}), as well as the total phenolic compounds (TPC_{HPLC} as summatory of PA_{HPLC} and TFC_{HPLC}) were also calculated (Table 4).

ANOVA and Kruskal-Wallis could be applied to six compounds (because the other phenolics did not meet the required assumptions) that could not help characterize the analysed honeys by their botanical origins. These six phenolic compounds were the sinapic acid and the flavonoids naringenin, pinocembrin, chrysin, galangin and luteolin (Table 4).

The comparison of our values with those of literature is difficult, probably due to the different sample preparation, extraction conditions or HPLC procedure. A deep screening of the literature shows a considerable variability in the concentration of phenolic compounds (Annex 1/Table 3), probably because these substances derive from nectar, pollen and propolis, whose chemical composition is also related to the geographical origin, seasonal climatic changes, vegetation conditions, processing, handling and storage (Gheldof *et al.*, 2002; Kenjeric *et al.*, 2007; Lachman *et al.*, 2010b; Perna *et al.*, 2012; Petretto *et al.*, 2015). Similarities and differences were reported in terms of phenolic composition among honeys from same floral origins but different geographical locations.

Table 4. Averages, standard deviations, and maximum and minimum values of the phenolic compounds of artisanal honeys from Castilla y León.

	Chestnut (n=4)	Clover (n=3)	Heather (n=9)	Honeydew (n=18)	Lavender (n=4)	Multifloral (n=15)
	Mean ± SD (min; max)	Mean ± SD (min; max)	Mean ± SD (min; max)	Mean ± SD (min; max)	Mean ± SD (min; max)	Mean ± SD (min; max)
Chlorogenic acid (µg/g)	0.41 ± 0.47 (ND; 1.05)	0.17 ± 0.29 (ND; 0.51)	0.2 ± 0.2 (ND; 0.46)	0.23 ± 0.46 ND; 1.79)	1.25 ± 1.14 (ND; 2.43)	1.61 ± 2.13 (ND; 6.63)
Caffeic acid (µg/g)	6.05 ± 1.39 (5.17; 8.12)	6.96 ± 1 (6.24; 8.1)	5.74 ± 1.03 (4.68; 8.1)	5.28 ± 2.22 (1.55; 9.78)	4.95 ± 0.96 (4.33; 6.36)	7.38 ± 5.41 (ND; 20.32)
Rutin (µg/g)	1.23 ± 0.25 (0.93; 1.54)	1.06 ± 0.34 (0.67; 1.29)	1.23 ± 1.97 (0.09; 6.32)	1.14 ± 1.21 (0.2; 5.68)	0.44 ± 0.66 (0.02; 1.42)	1.26 ± 0.73 (0.3; 2.7)
Ellagic acid (µg/g)	60.97 ± 13.34 (44.96; 72.5)	33.52 ± 2.04 (32.2; 35.87)	64.55 ± 48.3 (ND; 116.83)	92.48 ± 34.58 (36.18; 148.64)	16.32 ± 32.64 (ND; 65.28)	38.93 ± 33.14 (ND; 90.37)
Coumaric acid (µg/g)	14.97 ± 11.41 (4.05; 30.76)	3.58 ± 0.37 (3.27; 3.99)	5.15 ± 6.23 (ND; 17.51)	12.05 ± 11.98 (ND; 43.76)	4.56 ± 1.52 (2.94; 6.37)	7.66 ± 5.87 (ND; 20.27)
Sinapic acid (µg/g)	ND*	ND	1.04 ± 2.33* (ND; 6.86)	ND	ND	0.31 ± 1.2* (ND; 4.65)
Ferulic acid (µg/g)	3.54 ± 1.87 (0.77; 4.75)	ND	0.88 ± 1.4 (ND; 3.74)	0.64 ± 1.26 (ND; 4.31)	0.04 ± 0.05 (ND; 0.11)	4.89 ± 7.21 (ND; 18.52)
Luteolin (µg/g)	1.36 ± 0.36* (0.95; 1.68)	1.24 ± 0.07* (1.19; 1.31)	0.92 ± 0.59* (ND; 1.91)	0.78 ± 0.71* (ND; 1.98)	0.96 ± 0.35* (0.64; 1.31)	0.71 ± 0.59* (ND; 2.1)
Quercetin (µg/g)	1.47 ± 0.45 (0.9; 1.86)	2.31 ± 1.86 (0.17; 3.56)	1.93 ± 1.67 (ND; 3.99)	1.64 ± 1.6 (ND; 6.65)	3.39 ± 0.43 (3.03; 3.97)	3.93 ± 3.04 (0.42; 9.86)
Naringenin (µg/g)	ND	ND	0.75 ± 2.18* (ND; 6.55)	1.15 ± 2.75* (ND; 8.81)	ND	0.82 ± 2.19* (ND; 6.97)
Kaempferol (µg/g)	0.28 ± 0.56 (ND; 1.13)	3.26 ± 0.18 (3.1; 3.45)	2.26 ± 0.95 (0.1; 3.41)	0.81 ± 1.3 (ND; 4.72)	5.41 ± 6.73 (1.52; 15.49)	2.2 ± 1.49 (ND; 5.17)
Chrysin (µg/g)	5.97 ± 3.49* (1.79; 10.32)	4.07 ± 0.2* (3.88; 4.28)	9.79 ± 3.19* (4.14; 14.15)	6.24 ± 3.78* (0.4; 14.26)	7.08 ± 2.02* (4.48; 8.7)	6.72 ± 2.68* (3.05; 11.84)
Pinocembrin (µg/g)	7.08 ± 3.34* (2.9; 11.06)	6.77 ± 0.97* (6.04; 7.87)	14.8 ± 6.27* (5.37; 23.87)	9.1 ± 5.17* (2.01; 19.46)	10.5 ± 3.98* (6.01; 14.03)	9.7 ± 4.68* (3.77; 19.32)
Galangin (µg/g)	3.79 ± 1.32* (2.22; 5.12)	ND	1.84 ± 2.95* (ND; 7.35)	2.02 ± 2.5* (ND; 8.32)	0.9 ± 1.8* (ND; 3.6)	1.74 ± 2.23* (ND; 5.56)
PA_{HPLC} (mg/100 g)	8.59 ± 2.22 $^{a.b}$ (5.97; 11.37)	4.42 ± 0.17 ^{a} (4.24; 4.57)	7.76 ± 4.75 $^{a.b}$ (0.62; 12.27)	11.07 ± 2.78 ^{b} (5.58; 15.78)	2.71 ± 3.32 ^{a} (0.98; 7.68)	6.08 ± 4.05 $^{a.b}$ (0.95; 14.26)
TFC_{HPLC} (mg/100 g)	2.12 ± 0.86* (1.06; 3.16)	1.87 ± 0.33* (1.51; 2.15)	3.35 ± 1* (1.71; 4.49)	2.29 ± 1.23* (0.46; 5.5)	2.87 ± 0.57* (2.14; 3.52)	2.71 ± 0.96* (1.14; 4.52)
TPC_{HPLC} (mg/100 g)	10.71 ± 2.68$^{a.b}$ (7.03; 13.43)	6.3 ± 0.29^{a} (5.97; 6.52)	11.11 ± 4.77$^{a.b}$ (3.78; 15.79)	13.36 ± 3.05^{b} (7.69; 17.62)	5.58 ± 3.77^{a} (3.18; 11.21)	8.79 ± 4.02$^{a.b}$ (2.39; 16.69)

ND: Not Detected. Mean values within the same row having different lower-case letter are significantly different by Student-Newman-Kreus multiple range test (p<0.05). Medians values within the same row having different capital letter are significantly different by the median notch in the Box-and-Whisker Plot (p<0.05).* There are not statistically significant differences between the means or medians at the 95.0% confidence level (p-value greater than or equal to 0.05). No letters mean that ANOVA could not be determined.

Typical HPLC polyphenol profiles at wavelength of 290, 320 and 360 nm are represented in Figure 2.

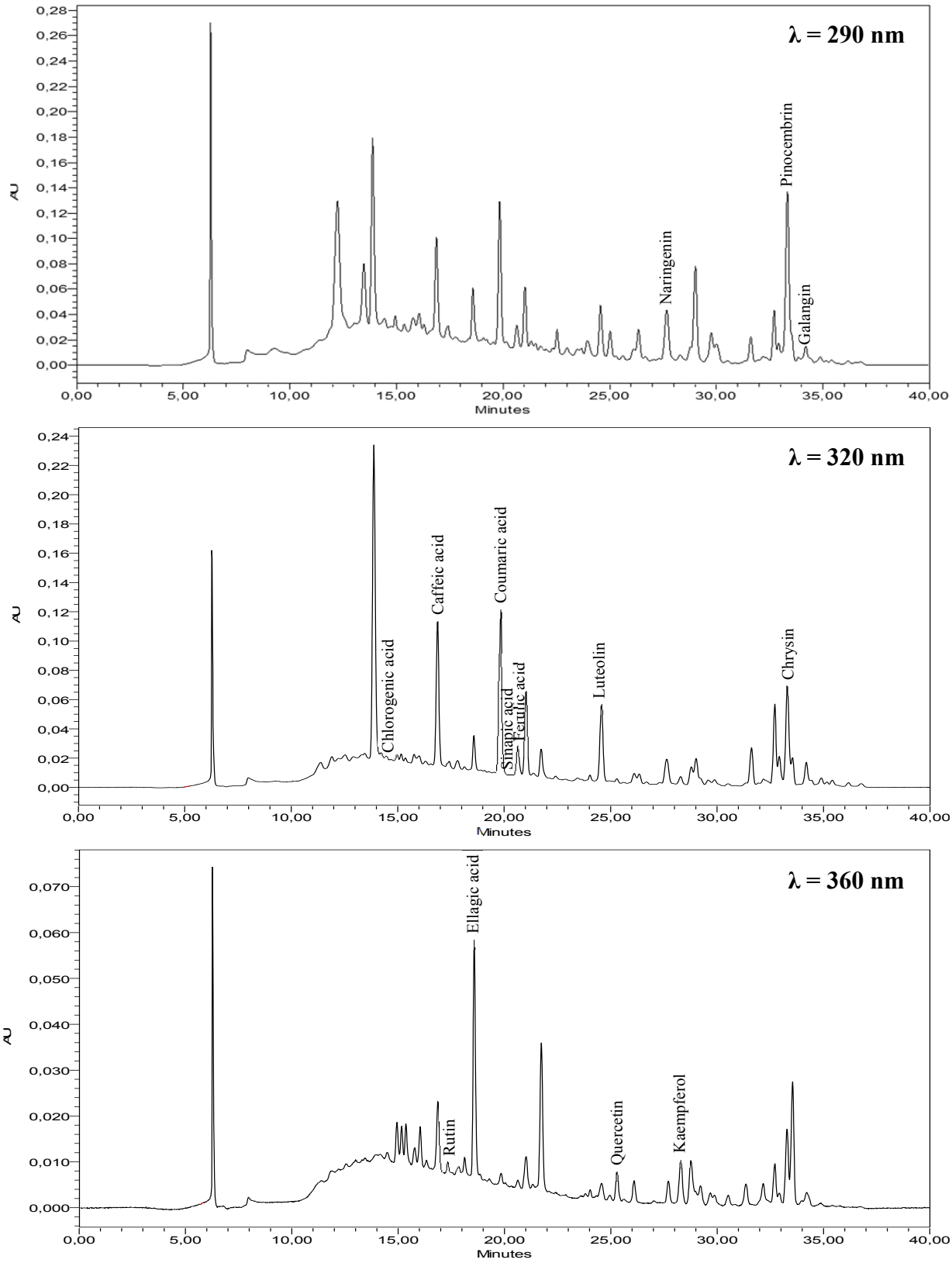

Figure 2. HPLC chromatograms of polyphenols at the wavelengths recorded (290, 320 and 360 nm).

Ellagic acid was the phenolic compound that was found in the highest concentrations in our samples. Kilik *et al*. (2014) reported the antioxidant and anticarcinogenic activity of this acid. Honeydew honeys exhibited the highest ellagic acid average (92.48 μg/g), followed by heather and chestnut samples. Only one out of the four lavender honeys contained ellagic acid, with a value considerably lower than the averages of honeys from the other botanical origins (16.32 μg/g). A moderate correlation between ellagic acid and the colour parameter L* was found (r=-0.5289), indicating that the darker the honeys (lower L* values) were, the higher ellagic acid content the samples had.

Ferreres *et al.* (1996) suggested ellagic acid as marker of heather honeys. But in our case, although high quantities were found in heather samples (64.55 μg/g), the highest amounts were obtained for honeydew honeys, being also ellagic acid the main phenolic compound of the vast majority of honeys of our study. Ferreres *et al.* (1996) also described that the presence or absence of ellagic acid could be a special feature of different heather species, but in our study no differences were found between heather honeys from *Erica* sp. and *Calluna vulgaris* species in respect of ellagic acid contents. Apart from heather honeys, ellagic acid was also found in Australian honeys such as jelly bush (*Leptospermum polygalifolium*), manuka (*Leptospermum scoparium),* kanuka (*Kunzea ericoides*), tea tree (*Melaleuca quinquenervia*), brush box (*Lophostemon conferta*) and *Eucalyptus* (Martos *et al.*, 2000a,b; Yao *et al.*, 2003; Yaoa *et al.*, 2005; Stephens *et al.*, 2010), Tunisian thyme and multifloral honeys (Martos *et al.*, 1997), acacia (Tomás-Barberán *et al.*, 2001), *Rubus* (Escuredo *et al.*, 2012) and honeydew honeys (Trautvetter *et al.*, 2009).

D'Arcy (2005) reported that the different quantities of ellagic acid found in honeys of different studies could be due to the different phenolics' extraction procedures, because it was observed that Amberlite resin retained neither ellagic nor gallic acids, and for other phenolics such as coumaric, caffeic, chlorogenic and ferulic acids, this resin yielded low recoveries. On the contrary, Bertoncelj *et al.* (2007) claimed that Amberlite resin gave the highest recoveries for phenolic acids. Ferreres *et al.* (1996) observed that after Amberlite extraction, a purification step using Sephadex LH-20 chromatography was necessary to further detect ellagic acid. In the literature, Ferreres *et al.* (1996), Tomás-Barberán *et al.* (2001) and Jasicka-Misiak *et al.* (2012) that had employed Amberlite for the extraction of phenolic compounds, quantified lower values of ellagic acid in heather honeys than those found in our study (averages of 2.91, 7.16 and 20.06 μg/g, respectively). However, those researchers pointed out that ellagic acid was the highest phenolic compound of heather honeys, as well. Ellagic acid was also the main polyphenol found in *Rubus* honeys from North Western Spain (Escuredo *et al.*, 2012).

Coumaric acid was the second most important phenolic acid in quantity. Chestnut (14.97 μg/g), followed by honeydew honeys (12.05 μg/g) showed the highest averages, whereas the lowest mean value was found in clover samples (3.58 μg/g). Four out of the six

samples in which coumaric acid was not detected belonged to the group of heather samples. The ranges observed for coumaric acid were similar to those found in the literature for honeys from different floral and geographical origins, with maximum averages of 11.80 μg/g (Gheldof *et al.*, 2002), 11.50 μg/g (Biesaga and Pyrzynska, 2009), 15.95 μg/g (Alvarez-Suarez *et al.,* 2010) and 15.58 μg/g (Can *et al.*, 2015). Some researchers reported coumaric acid as one of the predominant phenolic acids in honeys (Yaoa *et al.*, 2005; Socha *et al.*, 2011). In comparison with our study, in the literature (Annex 1/Table 3) lower averages of coumaric acid were described for lavender honeys (ranging from 0.40 to 2.56 μg/g) and higher for heather samples (2.98-14.90 μg/g) from different geographical origins.

Coumaric acid and other hydroxycinnamic-related compounds such as caffeic and ferulic acids were suggested as markers for chestnut honeys (Tomás-Barberán *et al.*, 2001). In our study, the highest average for the sum of hydroxycinnamic-related compounds was observed in chestnut honeys, agreeing with literature data (D'Arcy, 2005; Dimitrova *et al.*, 2007; Perna *et al.*, 2013). Conversely, Can *et al.* (2015) reported higher averages of hydroxycinnamic-related compounds in Turkish oak honeys than in chestnut honeys (15.95, 26.78 and 4.19 μg/g versus 5.52, 4.83 and 1.64 μg/g, respectively).

In our study, a significant correlation was found between caffeic and ferulic acids (r=0.6891). Silici *et al.* (2013) reported a significant correlation between caffeic and coumaric acids (r=0.893). Although in our study chestnut honeys showed high values for caffeic and ferulic compounds (6.05 μg/g and 3.54 μg/g, respectively), multifloral honeys possessed the highest results (7.38 and 4.89 μg/g, respectively). But within the multifloral group, the honeys rich in chestnut from León province showed the highest caffeic and ferulic averages (13.62 and 15.48 μg/g, respectively). On the other hand, the multifloral honey rich in chestnut from Zamora province, which had the lowest content of *Castanea sativa* pollen, also possessed low caffeic acid value (4.38 μg/g), being ferulic acid not detected.

Finally, chlorogenic and sinapic acids were found in low concentrations. Multifloral honeys showed the highest chlorogenic acid average (1.61 μg/g). Sinapic acid was only detected in three samples, being two of which heather honeys. Regarding literature data for honeys from the same floral origins than those of our research, low values were reported for chlorogenic acid, whereas no data were found about sinapic acid (Annex 1/Table 3). Silici *et al.* (2013) reported significant correlations between chlorogenic acid with caffeic (r=0.908) and coumaric (r=0.836) acids. In our study, chlorogenic acid was correlated with the colour parameters L* (r=0.6233), a* (r=-0.5522) and h* (r=0.6143), indicating that darker honeys with high reddish colours' values had lower chlorogenic acid contents. In contrast, Sergiel *et al.* (2014) only detected chlorogenic acid in dark Polish honeys.

In respect of flavonoids, pinocembrin was the main one, being after ellagic acid the second most important phenolic compound. It was, together with the flavonoids chrysin and rutin, the

only phenolic compound found in all the samples. Heather honeys showed the highest pinocembrin and chrysin (the second main flavonoid) averages (14.80 and 9.79 μg/g, respectively), whereas clover presented the lowest ones (6.77 and 4.07 μg/g, respectively). Tomás-Barberán *et al.* (2001) also observed that pinocembrin and chrysin were the main flavonoids in honey. In our study, a significant correlation was found between the results of pinocembrin and chrysin (r=0.9141), in agreement with other researchers (Bertoncelj *et al.*, 2011b; Gašić *et al.*, 2015). The wide range of values found for both pinocembrin and chrysin showed that these flavonoids were not useful to botanically characterize our samples. Tomás-Barberán *et al.* (2001) had already reported that pinocembrin and chrysin, together with galangin, were not useful markers of floral origin, because they are the main flavonoids in propolis, thus their content in honey depends on the degree of propolis contamination. Other honey's flavonoids could be originated from nectar and/or pollen, so that they might be appropriate botanical markers (Kečkeš *et al.*, 2013). Dark-coloured honeys possessed higher galangin averages than light ones, being galangin absent in our clover honeys. No significant correlations were found between galangin with pinocembrin and chrysin, in contrast to other researchers observations (Bertoncelj *et al.*, 2011b; Gašić *et al.*, 2015). Similar average ranges as those obtained by us, were found in the literature for pinocembrin and galangin: between Not Detected (ND)-10.60 μg/g and ND-3.99 μg/g in honeys from California, USA (Gheldof *et al.*, 2002), and between 0.20-16.00 μg/g and 0.40-5.80 μg/g in Italian honeys, respectively (Petretto *et al.*, 2015). On the contrary, lower values than ours were reported for chrysin, ranged between ND-3.95 μg/g in honeys from California, USA (Gheldof *et al.*, 2002), 0.30 μg/g and 1.10 μg/g in Czech honeys (Lachman *et al.*, 2010a), and 0.14 μg/g and 0.73 μg/g in Greek honeys (Karabagias *et al.*, 2014). This values showed that propolis-derived compounds could be useful for geographical origin determinations of honey (Tomás-Barberán *et al.*, 1993a,b). In our study, propolis-derived flavonoids represented 71.61% of the TFC_{HPLC}, ranged between 58.61% (clover) and 78.49% (heather), meanwhile Tomás-Barberán *et al.* (1993b) reported a contribution of approximately 50% in other Spanish honeys. In general, other researchers found lower propolis-derived flavonoids content in honeys from the same floral origin that those studied for us (Annex 1/Table 3).

After chrysin, the quantitatively most important flavonoids of our study were quercetin and kaempferol. Both flavonoids showed higher values in light honeys (lavender and clover), than in dark ones (chestnut and honeydew). Quercetin was moderately correlated with the colour parameters L* (r=0.6143), a* (r=-0.5640) and h* (r=0.5988). The lowest averages for both quercetin and kaempferol were found in chestnut honeys (1.47 μg/g and 0.28 μg/g, respectively). Multifloral samples had the highest quercetin average (3.93 μg/g) and lavender the highest kaempferol mean value (5.41 μg/g). On the one hand, quercetin was detected in all samples but two honeydew and two heather honeys. On the other hand, kaempferol was not detected in three out of the four chestnut honeys, in ten out of the eighteen honeydew samples

and in two multifloral honeys. Low values for quercetin and kaempferol were reported by other researchers (Annex 1/Table 3). In comparison with our results, Petretto *et al.* (2015) found similar quercetin averages in honeys from Italy, possessing also lavender honey the highest quercetin average in analogous quantities (3.5 μg/g). In contrast, Petrus *et al.* (2011) reported lower quercetin amounts in Croatian lavender honeys (0.3 μg/g). Perna *et al.* (2013) observed the highest amount of quercetin in chestnut honeys, but their values were similar to those found for the chestnut samples in our study (1.50 μg/g). Quercetin was suggested as a marker for sunflower honeys (Ferreres *et al.*, 1992; Soler *et al.*, 1995; Tomás-Barberán *et al.*, 2001) and in agreement with this claim, the three multifloral honeys of our study that were rich in sunflower pollen contained higher quercetin amounts than the rest of the samples (average of 7.32 μg/g).

Finally, in the analysed samples from Castilla y León, we found luteolin, rutin and naringenin in low quantities. Multifloral honeys had the lowest luteolin average and the highest rutin average (0.71 μg/g and 1.26 μg/g, respectively). Chestnut samples possessed the highest luteolin mean value and lavender honeys the lowest rutin average. Naringenin was detected only in eleven out of fifty three samples: four heather, four honeydew and three multifloral honeys. Although the flavonoids luteolin and naringenin were described as useful markers for lavender honeys characterization (Ferreres *et al.*, 1994b; Andrade *et al.*, 1997b), in our study neither luteolin nor naringenin proved to be suitable markers for lavender honeys. In fact, naringenin was not detected in any lavender samples, being our results in agreement with those of other researchers (Petrus *et al.*, 2011; Campillo *et al.*, 2015). In general, low values for these three flavonoids (luteolin, rutin and naringenin) were reported in the literature (Annex 1/Table 3).

Apart from the correlations among phenolic acids and among flavonoids, in this study significant correlations were found between phenolic acids and flavonoids, the most important of which were between chlorogenic acid and quercetin (r=0.7281), and between coumaric acid and galangin (r=0.5637). Gašić *et al.* (2015) described correlations between caffeic acid and galangin, and between chrysin and pinocembrin (r>0.691).

Table 5 compiles, in descending order of concentration, the phenolic compounds found in our research, as well as those reported in the literature for honeys from the same botanical origins.

Table 5. Compilation, in descending order of concentration, of the phenolic compounds found in our study and the phenolic compounds described in the literature for honeys from the same botanical origins.

CHESTNUT HONEY			
AUTHOR	**LOCATION**	**FLORAL ORIGIN**	**PHENOLIC COMPOUNDS (in descending order of concentration)**
Our study	**Spain**	***Castanea sativa***	**Ellagic, coumaric, pinocembrin, caffeic, chrysin, galangin, ferulic, quercetin, luteolin, rutin, chlorogenic, kaempferol (ND: naringenin, sinapic)**
Tomás-Barberán *et al.*, 2001	Europe	*Castanea sativa*	Pinocembrin, coumaric, chrysin, kaempferol, caffeic

Dimitrova *et al.*, 2007	Europe	*Castanea sativa*	Ferulic, caffeic, coumaric
Pichichero *et al.*, 2009	Italy	*Castanea sativa*	Chlorogenic, coumaric, galangin, caffeic, kaempferol, chrysin, quercetin
Perna *et al.*, 2013	Italy	*Castanea sativa*	Ferulic, caffeic, coumaric, chlorogenic, rutin, quercetin
Campillo *et al.*, 2015	Spain	*Castanea sativa*	Quercetin, kaempferol, chrysin, naringenin
Can *et al.*, 2015	Turkey	*Castanea sativa*	Coumaric, caffeic, quercetin, ferulic (ND: chlorogenic, kaempferol, rutin)
Petretto *et al.*, 2015	Italy	*Castanea sativa*	Galangin, pinocembrin (ND: chlorogenic, ferulic, kaempferol, quercetin, rutin)
CLOVER HONEY			
AUTHOR	**LOCATION**	**FLORAL ORIGIN**	**PHENOLIC COMPOUNDS (in descending order of concentration)**
Our study	**Spain**	***Leguminosae* type *Trifolium***	**Ellagic, caffeic, pinocembrin, chrysin, coumaric, kaempferol, quercetin, luteolin, rutin, chlorogenic (ND: ferulic, galangin, naringenin, sinapic)**
Gheldof *et al.*, 2002	USA	*Melilotus* sp.	Pinocembrin, coumaric, kaempferol, chrysin, galangin, quercetin
Can *et al.*, 2015	Turkey	*Trifolium* sp.	Caffeic (ND: chlorogenic, coumaric, ferulic, rutin, quercetin, kaempferol)
HEATHER HONEY			
AUTHOR	**LOCATION**	**FLORAL ORIGIN**	**PHENOLIC COMPOUNDS (in descending order of concentration)**
Our study	**Spain**	**Heather**	**Ellagic, pinocembrin, chrysin, caffeic, coumaric, kaempferol, quercetin, galangin, rutin, sinapic, luteolin, ferulic, naringenin, chlorogenic**
Ferreres *et al.*, 1996	Portugal	*Erica* sp.	Ellagic, quercetin, kaempferol
Andrade *et al.*, 1997a	Portugal	*Erica* sp.	Coumaric, ferulic, chlorogenic, ellagic, caffeic
Tomás-Barberán *et al.*, 2001	Europe	*Erica* sp.	Ellagic, pinocembrin, chrysin, kaempferol, caffeic, ferulic, coumaric
Dimitrova *et al.*, 2007	Europe	Heather	Caffeic, coumaric, ferulic
Michalkiewicz *et al.*, 2008	Poland	Heather	Rutin, caffeic, quercetin, kaempferol
Jasicka-Misiak *et al.*, 2012	Poland	*Calluna vulgaris*	Ellagic, chlorogenic, coumaric, ferulic, caffeic, quercetin, kaempferol, chrysin, galangin
Sergiel *et al.*, 2014	Poland	Heather	Coumaric, ferulic, quercetin, caffeic, chlorogenic, luteolin, kaempferol, rutin
Campillo *et al.*, 2015	Spain	Heather	Chrysin, kaempferol, quercetin (ND: naringenin)
Can *et al.*, 2015	Turkey	*Calluna vulgaris*	Quercetin, kaempferol, caffeic (ND: kaempferol, rutin, ferulic, chlorogenic)
HONEYDEW HONEYS			
AUTHOR	**LOCATION**	**FLORAL ORIGIN**	**PHENOLIC COMPOUNDS (in descending order of concentration)**
Our study	**Spain**	**Oak**	**Ellagic, coumaric, pinocembrin, chrysin, caffeic, galangin, quercetin, naringenin, rutin, kaempferol, luteolin, ferulic, chlorogenic (ND: sinapic).**
Biesaga and Pyrizynska, 2009	Poland	Honeydew	Ferulic, coumaric, caffeic, chlorogenic, rutin, quercetin (ND: naringenin)
Pichichero *et al.*, 2009	Italy	Honeydew	Chlorogenic, galangin, coumaric, chrysin, caffeic, quercetin (ND: kaempferol)
Silici *et al.*, 2013	Turkey	Honeydew	Coumaric, chlorogenic, ferulic, caffeic (ND: quercetin, pinocembrin)
Karabagias *et al.*, 2014	Greece	Fir and Pine	Kaempferol, quercetin, chrysin
Can *et al.*, 2015	Turkey	Oak	Rutin, caffeic, coumaric, ferulic (ND: kaempferol, quercetin, chlorogenic)
Can *et al.*, 2015	Turkey	Pine	Quercetin, rutin, kaempferol, coumaric, caffeic, ferulic (ND: chlorogenic)

LAVENDER HONEYS			
AUTHOR	**LOCATION**	**FLORAL ORIGIN**	**PHENOLIC COMPOUNDS (in descending order of concentration)**
Our study	**Spain**	***Lavender* sp.**	**Ellagic, pinocembrin, chrysin, kaempferol, caffeic, coumaric, quercetin, chlorogenic, luteolin, galangin, rutin, ferulic (ND: naringenin, sinapic)**
Andrade *et al.*, 1997a	Portugal	*Lavandula stoechas*	Chlorogenic, ferulic, caffeic, coumaric
Truchado *et al.*, 2009	Italy	*Lavandula* sp.	Pinocembrin, chrysin, galangin, kaempferol (ND: quercetin)
Campillo *et al.*, 2015	Spain	*Lavandula* sp.	Chrysin (ND: naringenin, quercetin, kaempferol)
Can *et al.*, 2015	Turkey	*Lavandula stoechas*	Rutin, caffeic, coumaric (ND: chlorogenic, ferulic, quercetin, kaempferol)
Petretto *et al.*, 2015	Italy	*Lavandula stoechas*	Pinocembrin, rutin, quercetin, galangin, luteolin, chlorogenic (ND: kaempferol)

ND: Not Detected.

As Table 5 shows, the different methodology, geographical origins and associated flora influence the concentration and the distribution of the different phenolic compounds in honeys from the same botanical origins. In addition, it is important to consider the differences between various species that are designated under the same name (within heather, lavender and clover groups, for example) and honeydew honeys from different floral origins.

The total phenolic content was calculated as the sum of the compounds quantified by HPLC (TPC_{HPLC}). TPC_{HPLC} ranged between 2.39 mg/100 g and 17.62 mg/100 g. Honeydew honeys were characterized by the highest TPC_{HPLC} average (13.36 mg/100 g), decreasing the rest of TPC_{HPLC} in the following order: heather > chestnut > multifloral > clover > lavender (5.58 mg/100 g) honeys. Despite the fact that similar general TPC_{HPLC} values than those obtained in our study were reported by Silici *et al.* (2013), these researchers obtained higher amounts of phenolic compounds in nectar (16.71 mg/100 g) than in honeydew honeys (7.26 mg/100 g).

Phenolic acids (PA_{HPLC}) represented 69.23% of the TPC_{HPLC}, with an averages' range from 38.69% (lavender) to 82.71% (honeydew). Only in lavender honeys the average for the total flavonoid content, calculated as the sum of all the flavonoids quantified by HPLC (TFC_{HPLC}), was higher than the PA_{HPLC}. Ferreres *et al.* (1994a) described high content of flavonoids and low amount of phenolic acids in light honeys. On the other hand, they reported high content of phenolic acid derivatives and small amount of flavonoids in heather honeys. In our study, although heather samples possessed lower TFC_{HPLC} average than PA_{HPLC} average, these honeys contained the highest TFC_{HPLC} average (3.35 mg/100 g). Isla *et al.* (2011) reported the highest TFC_{HPLC} values in the multifloral darkest honeys. Escuredo *et al.* (2012) reported the highest TFC_{HPLC} values in *Rubus* honeys, ranging between 0.55 and 3.59 mg/100 g. Sergiel *et al.* (2014) reported that dark honeys contained higher flavonoid contents than light ones. In our study, lavender honeys had higher TFC_{HPLC} than dark-coloured honeydew and chestnut honeys, but lower than heather samples.

Significant correlations were found among ellagic acid, PA_{HPLC} and TPC_{HPLC} (r>0.8800). On the other hand, chrysin, pinocembrin and quercetin were significantly correlated with TFC_{HPLC} (r=0.9123, r=0.8420 and r=0.5420, respectively).

As expected, dark coloured samples had a significantly higher content of phenolic compounds and phenolic acids than light ones, in agreement with the literature results (Estevinho *et al.*, 2008; Ferreira *et al.*, 2009; Escuredo *et al.*, 2012). In our study, medium and low significant correlations among the colour parameters L*, h* and a*, and PA_{HPLC} and TPC_{HPLC} were found. The highest influence was showed between lightness and PA_{HPLC} (r=-0.5191). The main phenolic compounds related to the honey colour, as mentioned above, were ellagic acid, chlorogenic acid and quercetin. Escuredo *et al.* (2012) found significant correlations between colour and p-coumaric acid and colour and kaempferol in *Rubus* honeys. Isla *et al.* (2011) reported high positive correlations between colour with TFC_{HPLC} and TPC_{HPLC} (r =0.99 and r=0.96, respectively).

3.3. Antioxidant related parameters

The following table shows the values of the antioxidant-related parameters of the honeys from Castilla y León (Table 6).

Table 6. Averages, standard deviations, and maximum and minimum values of the antioxidant-related parameters of honeys from Castilla y León.

	Chestnut (n=4)	Clover (n=3)	Heather (n=9)	Honeydew (n=18)	Lavender (n=4)	Multifloral (n=15)
	Mean ± SD (min; max)	Mean ± SD (min; max)	Mean ± SD (min; max)	Mean ± SD (min; max)	Mean ± SD (min; max)	Mean ± SD (min; max)
Total polyphenols honey (mg GAE/100 g)	134.45 ± 11.58^{a} (119.04; 147.13)	99.89 ± 12.71^{b} (87.71; 113.07)	155.11 ± 23.65^{a} (112.32; 183.35)	140.85 ± 17.59^{a} (97.95; 168.44)	77.03 ± 20.59^{b} (51.52; 101.48)	88.96 ± 32.63^{b} (44.30; 148.93)
Total polyphenols extract (mg GAE/100 g)	31.48 ± 5.33^{a} (23.67; 35.63)	$27.85 \pm 8.53^{b,c}$ (20.2; 37.05)	27.63 ± 6.11^{b} (15.62; 37.79)	30.22 ± 3.55^{a} (23.42; 36.52)	17.43 ± 7.36^{c} (11.49; 27.34)	$21.58 \pm 6.70^{b,c}$ (12.13; 34.02)
Total flavonoids extract (mg QE/100 g)	3.69 ± 0.62* (3.02; 4.49)	3.37 ± 0.77* (2.57; 4.11)	4.24 ± 0.57* (3.41; 5.36)	3.18 ± 0.86* (1.61; 5.22)	2.91 ± 1.11* (1.92; 4.49)	3.47 ± 1.15* (1.3; 5.3)
TEAC honey (µmol TE/g)	5.63 ± 1.01^{a} (4.21; 6.48)	$2.88 \pm 0.69^{b,c}$ (2.11; 3.45)	4.25 ± 1.15^{b} (2.38; 6.51)	6.04 ± 0.98^{a} (3.87; 7.46)	2.23 ± 1.39^{c} (1.01; 4.13)	$3.17 \pm 1.33^{b,c}$ (1.48; 5.28)
TEAC extract (µmol TE/g)	2.34 ± 0.35^{a} (1.83; 2.61)	1.75 ± 0.22^{b} (1.59; 2.00)	1.76 ± 0.42^{b} (1.03; 2.47)	2.54 ± 0.25^{a} (2.08; 2.96)	1.17 ± 0.62^{b} (0.71;2.04)	1.48 ± 0.46^{b} (0.82; 2.34)

Mean values within the same row having different lower-case letter are significantly different by Student-Newman-Kreus multiple range test (p<0.05).* There is not statistically significant differences between the means or medians at the 95.0% confidence level (p-value greater than or equal to 0.05).

3.3.1. Total phenolic compounds

As expected, the total phenolic content in the entire honeys (TPCh) was higher than in the extracts (TPCe) (Table 6 and Figure 3). This fact has been also reported by other authors (Ferreira *et al.*, 2009; Montenegro *et al.*, 2013). These differences are probably due to an overestimation of the TPCh produced by the low specificity of the Folin-Ciocalteau assay. Folin-Ciocalteau reagent not only measures total polyphenols, but it can also oxidize other non-phenolic reducing compounds, such as monosaccharides, amino acids and vitamins, among others, increasing the absorbance values and leading to positive errors in the determination of TPC (Meda *et al.*, 2005; Alvarez-Suarez *et al.*, 2009; Ferreira *et al.*, 2009; Perna *et al.*, 2012; Ciappini and Stoppani, 2014; Meinen *et al.*, 2014; Petretto *et al.*, 2015). For this reason, measuring total phenolic content by Folin-Ciocalteau assay in the phenolic extracts is the proper procedure, because interferences have been previously removed, separating the polyphenol fraction from other reducing substances (Alvarez-Suarez *et al.*, 2009; Ferreira *et al.*, 2009; Petretto *et al.*, 2015). In this study, the moderately significant relationship found between total phenolics of honeys and extracts is interesting (r=0.6270), because it shows that the proportion of total reducing substances in honeys and their corresponding extracts appears to be somehow constant (Chapter 5).

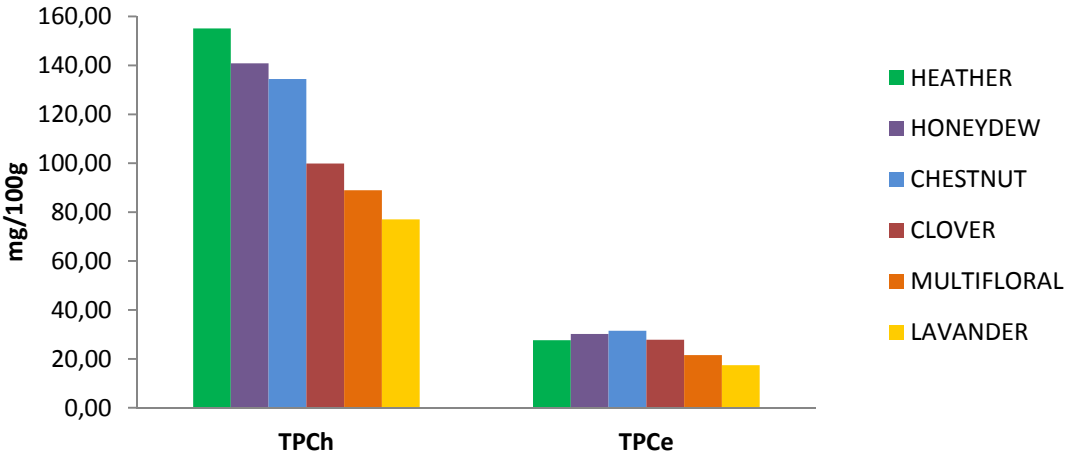

Figure 3. Comparison between TPCh and TPCe measured by spectrophotometry.

In order to avoid overestimated results, some researchers used a blank with honey and polyvinylpolypyrrolidine (Gheldof and Engeseth, 2002; Gomes *et al.*, 2011) and other authors quantified the sugars interferences developing the assay with a blank of artificial honey, whose quantitative composition included the most important honey carbohydrates (Rodríguez *et al.*, 2012; Maksimović and Nedić, 2013; Sant'Ana *et al.*, 2014). Although the total phenolic content in the sugar analogue was very low or even not detected, it does not mean that in a complex matrix such as honey, these compounds could not interfere.

In our research, TPCh decreased in the following order: heather (155.11 mg GAE/100 g) < honeydew < chestnut < clover < lavender (77.03 mg GAE/100 g). In this case, dark honeys

(heather, honeydew and chestnut) showed a statistically significantly higher TPCh (p-value<0.05) than light honeys (lavender, multifloral and clover), in agreement with the literature (Bertoncelj *et al.*, 2007; Can *et al.*, 2015). Escuredo *et al.* (2013b) also reported that heather, followed by oak honeydew and chestnut honeys, were the samples with the highest TPCh. Their values were similar to ours. Regarding TPCe, chestnut and honeydew samples (both dark honeys) had the maximum mean values (31.48 and 30.22 mg GAE/100 g). Lavender honeys that were the lightest samples, possessed the lowest average (17.43 mg GAE/100 g). TPCh and TPCe were significantly correlated with the colour parameters L*, a* and h* (r>-0.7527, r>0.7181 and r>-0.7840, respectively). Other researchers had also reported significant correlations between TPC and colour (Bertoncelj *et al.*, 2007; Alvarez-Suarez *et al.*, 2010; Monirruzaman *et al.*, 2014).

Most literature references report that dark honeys, such as "forest", chestnut, honeydew and heather contain the highest values of TPCh and TPCe (Pulcini *et al.*, 2006; Küçük *et al.*, 2007; Pérez *et al.*, 2007; Bertoncelj *et al.*, 2011a; Tezcan *et al.*, 2011; Alves *et al.*, 2013; Cimpoiu *et al.*, 2013), whereas light honeys, such as lavender, citrus, rosemary, and clover have the lowest values for these parameters (Ferreira *et al.*, 2009; Alves *et al.*, 2013; Özcan and Ölmez al., 2014). However, León-Ruiz *et al.* (2013) found higher TPCh average in amber coloured thyme honeys than in the dark coloured chestnut honeys, which was attributed to the fact that thyme honeys possessed higher contents of vitamin C than other honeys, so that as reducing agent, ascorbic acid could interfere with the Folin-Ciocalteu reagent.

It is important to highlight that in our study the average of TPCe for heather honeys was similar than TPCe for clover honeys. In contrast, the average of TPCh for heather honeys was the highest, being significantly higher than those found in clover ones. It could be due to the fact that heather honeys contain more reducing compounds than honeys from other botanical origins.

Literature shows that TPCh varies widely. TPCh values described in the references for honeys from other countries were in general lower than those obtained by us in this study (Annex 1/Table 3). Alvarez-Suarez *et al.* (2010) reported a range of 21.4-59.6 mg GAE/100 g for Cuban honeys, Bertoncelj *et al.* (2011a) of 4.5-23.3 mg GAE/100 g for Slovenian honeys and Perna *et al.* (2012) of 10.8-14.7 mg GAE/100 g for Italian honeys. On the other hand, our results were similar to those described in the literature for different Spanish honeys with ranges of 57.0-113.0 mg GAE/100 g (Vela *et al.*, 2007), 78.4-181.0 mg GAE/100 g (Escuredo *et al.*, 2013b) and 20.3-121.4 mg GAE/100 g (León-Ruiz *et al.*, 2013). In particular, our data were in agreement with those reported by Escuredo *et al.* (2013b) and León-Ruiz *et al.* (2013) for Spanish chestnut honeys, Chang *et al.* (2011) and Ciappini and Stoppani (2014) for Chinese and Argentinian clover honeys, Escuredo *et al.* (2013b) and Wilczynska (2014) for Polish and Spanish heather honeys, Escuredo *et al.* (2013b) and Rodríguez-Flores *et al.* (2015) for Spanish oak honeydew honeys, and León-Ruiz *et al.* (2013) for Spanish lavender

honeys. Therefore, geographical origins seem to have a decisive influence on TPC for honeys from the same botanical origins (Aazza *et al.*, 2013; Alves *et al.*, 2013).

Some researchers quantified TPC on honey methanolic extracts. Our data were similar to the range 13.2-20.4 mg GAE/100 g reported in Portuguese honeys (Ferreira *et al.*, 2009), 9.0-21.5 mg GAE/100 g in Czech honeys (Lachman *et al.*, 2010b), 41.1-49.8 mg GAE/100 g in Algerian honeys (Khalil *et al.*, 2012), 7.9-52.3 mg GAE/100 g in Mexican honeys (Rodríguez *et al.*, 2012), 5.5-32.1 mg GAE/100 g in Chilean honeys (Montenegro *et al.*, 2013) and 6.6-38.9 mg GAE/100 g in Italian honeys (Petretto *et al.*, 2015). Higher TPCe averages, similar than our TPCh results, were observed by Tenore *et al.* (2012), between 41.1 mg GAE/100 g and 139.2 mg GAE/100 g in Italian honeys, Dong *et al.* (2013), between 10.4 mg GAE/100 g and 149.6 mg GAE/100 g in Chinese honeys and Can *et al.* (2015), between 16.0 mg GAE/100 g and 120.0 mg GAE/100 g in Turkish honeys. Other authors calculated TPCe on the basis of the extract weight instead of the honey weight, being impossible to compare the results, due to the fact that the relation extract/honey (w/w) was unknown (Kumazawa *et al.*, 2012; Almeida da Silva *et al.*, 2013; Silva *et al.*, 2013). Regarding the TPCe in respect of the floral origin, similar averages were found by Petretto *et al.* (2015) in Italian chestnut honeys, Can *et al.* (2015) in Turkish clover honeys and Ferreira *et al.* (2009) in Portuguese heather honeys. In comparison with our values, Can *et al.* (2015) reported higher values for Turkish oak honeydew and lavender honeys, whereas Lachman *et al.* (2010b) and Petretto *et al.* (2015) found lower values for Czech honeydew and Italian lavender honeys, respectively (Annex 1/Table 3).

TPCh and TPCe were significantly correlated with some phenolic compounds, being the most important correlations those of TPCh with ellagic acid (r=0.6093), TPCh with chlorogenic acid (r=-0.5778) and TPCe with quercetin (r=-0.5599). It means that honeys with high TPC, also contained higher amounts of ellagic acid, and less quantities of both chlorogenic acid and quercetin. Socha *et al.* (2011) observed a positive significant correlation between TPCh and caffeic acid content (r=0.928), but we did not find this correlation in our study.

3.3.2. Total flavonoids

Although heather honeys exhibited the highest total flavonoid average (4.24 mg QE/100 g) and lavender samples the lowest one (2.11 mg QE/100 g), there were not statistically significant differences between their results.

Some studies showed that flavonoids are pigments that influence honey's colour (Frankel *et al.*, 1998), being such dark-coloured honeys as honeydew (Meda *et al.*, 2005; Mărghitaş *et al.*, 2009; Lachman *et al.*, 2010a; Oroian *et al.*, 2012; Escuredo *et al.*, 2013b), and chestnut (Perna *et al.*, 2013; Can *et al.*, 2015), those that have higher TFC. Several researchers found significant correlations between flavonoids and honey colour (Alvarez Suarez *et al.*, 2010; Kamboj *et al.*, 2013). In our case, we obtained weak correlations between TFC and the colour

parameters b* (r=0.4196) and C* (r=0.4226), so our results show that for the honeys harvested in Castilla y León, phenolic acids and/or other non-flavonoids phenolic compounds have more influence than flavonoids on honeys' colour.

The aluminium chloride method in neutral media (Dowd's method) used in our study for TFC determination, was described as specific only for flavonols (such as quercetin, rutin, quercetin, galangin, morin, kaempferol) and the flavone luteolin (Chang *et al.*, 2002; Pękal and Pyrzynska, 2014). Our TFCe results were moderately correlated with the sum of all flavonoids by HPLC (r=0.6256) and chrysin values (r=0.5596), so that the proportion of total flavonoids measured in neutral media and chrysin appears to be somehow constant for honeys from different botanical origins. No correlation was found between TFC and TPC. Literature references show that some researchers did not find significant correlations between TFC and TPC either (Meda *et al.*, 2005; Kamboj *et al.*, 2013), other authors found weak correlations (Özkök *et al.*, 2010; Chang *et al.*, 2011; Perna *et al.*, 2012) and some scientists obtained significant correlations (Pichichero *et al.*, 2009; Islam *et al.*, 2012; Oroian *et al.*, 2012; Moise *et al.*, 2013; Monirruzaman *et al.*, 2013).

The comparison of our values with other data reported in the literature was difficult for different reasons:

1) First of all, some researchers measured TFC in neutral media by the same method than us, but directly on a honey solution, without removing interferences and/or without sample's colour correction, thus overestimating the results.
2) Second, other scientists used the $AlCl_3$ procedure in alkaline medium with $NaNO_2$ addition, which was described as a less selective method for TFC determination. The $AlCl_3$ assay in alkaline medium allows the join quantification of catechin, rutin and luteolin, as well as other non-flavonoid components, such as phenolic acids (Pękal and Pyrzynska, 2014), so that the result of total flavonoids' content could also be overestimated.
3) Finally, literature references show that for TFC quantification, different standard substances were used for calibration curves and expression of results, complicating even more the comparison of data. For TFC determination in neutral media, quercetin, galangin and rutin were used, meanwhile for TFC in alkaline media, catechin was proposed as the more adequate standard (Pękal and Pyrzynska, 2014). Therefore, it is necessary to standardize all procedures used to quantify the TFC of honeys.

Our TFC results (ranging from 1.30 to 5.36 mg QE/100 g) were comparable to those of the literature references: Alvarez-Suarez *et al.* (2010) in Cuban honeys (1.09-2.52), Khalil *et al.* (2011) in Algerian honeys (1.15-3.46 mg QE/100 g), Escuredo *et al.* (2013b) in Spanish honeys (4.30-9.60 mg QE/100 g), Kamboj *et al.* (2013) in Indian honeys (4.79-7.18),

Boussaid *et al.* (2014) in Turkish honeys (0.96-2.25 mg QE/100 g) and Ulloa *et al.* (2015) in Portuguese honeys (4.09-5.77 mg QE/100 g).

Regarding the values for honeys from the same botanical origins than those studied in our research, higher averages were found for Italian chestnut honeys by Pichichero *et al.* (2009) and Perna *et al.* (2013) (4.40 and 12.50 mg QE/100 g, respectively), and a similar average was reported by Escuredo *et al.* (2013b) for Spanish chestnut samples (7.60 mg QE/100 g). Ciappini and Stoppani (2014) reported similar mean values in *Trifolium* sp. honeys from Argentina (3.29 mg QE/100 g), whereas 18.0 mg QE/100 g was the value found by Kumazawa *et al.* (2012) in a Japanese clover honey. Although higher values were reported for honeydew honeys, in general, the averages found in the literature were similar to the mean value of our study (Annex 1/Table 3). Aazza *et al.* (2013) reported lower TFC mean value for lavender honeys from Northeast Portugal (3.10 mg QE/100 g) and Escuredo *et al.* (2013b) found a similar average for Spanish heather honeys (6.00 mg QE/100 g).

3.3.3. TEAC

The antioxidant capacity of honeys has been attributed to different compounds with reducing capacity, among them polyphenols. Honeys' antioxidant activity depends on the botanical source, phytochemical composition and external factors such as season, environment and geographical origin (Gómez-Caravaca *et al.*, 2006; Alvarez-Suarez *et al.*, 2010).

There is no official analytical method for the determination of honey antioxidant capacity, and several assays have been employed for this purpose, whose results have often shown significant correlations (Alvarez Suarez *et al.*, 2010; Perna *et al.*, 2012). It would be interesting to select, set up and apply a standardized procedure for the determination of antioxidant activity of honeys. TEAC was proposed as one of the most reliable methods to assess antioxidant capacity (Prior *et al.*, 2005), being a fast and simple assay, so that in this research TEAC was the method of choice for the honeys from Castilla y León. TEAC was applied to both entire honeys (TEACh) and their corresponding methanolic extracts (TEACe), observing high significant correlation between both measurements (Chapter 5). Rodríguez *et al*. (2012) did not find significant correlations between antioxidant activity (measured by different methods) of honeys and extracts, claiming that in Mexican samples, other antioxidant compounds could contribute to honeys' antioxidant activity.

Lavender samples showed the lowest averages of TEACh and TEACe (2.23 μmol TE/g and 1.17 μmol TE/g, respectively) and honeydew and chestnut possessed the highest mean values (6.04 μmol TE/g and 5.63 μmol TE/g for entire honeys, as well as 2.54 μmol TE/g and 2.34 μmol TE/g for extracts, respectively). As expected, dark honeys (honeydew and chestnut) showed higher antioxidant activity than light ones (clover and lavender). Literature references reported relationships between honey colour and antioxidant capacity (Beretta *et al.*, 2005; Küçük *et al.*, 2007; Alvarez-Suarez *et al.*, 2010; Bertoncelj *et al.*, 2011a; Escuredo

et al., 2013a; Sagdic *et al.*, 2013; Kuś *et al.*, 2014; Pontis *et al.*, 2014; Wilczynska, 2014), where dark honeys (honeydew, chestnut, heather, buckwheat and strawberry tree, among others) always possessed higher total antioxidant activity than light ones (citrus, lavender, sulla, rhododendron, rape, ailanthus, orange and blank locust, among others). Socha *et al.* (2011) found high antioxidant activities in the methanolic extracts of dark-coloured honeys. In our samples, colour parameters L*, a* and h* were significantly correlated with TEACh and TEACe ($r>0.5931$).

With regard to botanical origins, Perna *et al.* (2012) measured the antioxidant activity of honeys from different floral origins by different methods, observing that chestnut honeys had always the highest antioxidant activities. Aazza *et al.* (2013) described citrus and lavender, as the honeys with the lowest antioxidant activities. Can *et al.* (2015) reported the highest antioxidant activity in chestnut and oak honeydew honeys, followed by heather and pine honeydew honeys. Other researchers observed higher antioxidant capacities in honeydew honeys than in nectar ones (Bertoncelj *et al.*, 2007; Vela *et al.*, 2007; Lachman *et al.*, 2010b).

In our study, total phenolic compounds (TPCh, TPCe and TPC_{HPLC}) possessed significant correlations with TEACh and TEACe (Annex 1/Table 4). The highest correlations, as expected, were found between TPCh and TEACh ($r=0.7574$) and between TPCe and TEACe ($r=0.8507$). Many researchers had already observed that high polyphenol levels were correlated to high antioxidant properties (Vela *et al.*, 2007; Zalibera *et al.*, 2008; Ferreira *et al.*, 2009; Pichichero *et al.*, 2009; Tenore *et al.*, 2012; Sagdic *et al.*, 2013), thereby considering that TPC measurement would be as well a suitable test to determine the antioxidant capacity of honey (Can *et al.*, 2015). On the contrary, Meda *et al.* (2005) did not find significant correlations between total phenolics and antioxidant activities of honeys, highlighting the importance of other non-polyphenolic compounds on antioxidant activity of honeys. In our research, between 31.7% and 90.1% antioxidant activity was due to polyphenol content. The remaining antioxidant activity percentage could be related to other compounds that are not extracted with methanol, such as enzymes, organic acids, Maillard reaction products, aminoacids and some vitamins, among others (Gheldof *et al.*, 2002; Baltrušaityte *et al.*, 2007; Bertoncelj *et al.*, 2007).

In our study, total flavonoid contents were not related to TEAC values, in agreement with the results of other authors (Meda *et al.*, 2005), and in contrast to the results of several researchers (Sant'Ana *et al.*, 2012; Kamboj *et al.*, 2013), who found significant correlations between total flavonoid contents and antioxidant activities. The lack of correlation revealed in our study could be due to the fact that in our samples, other phenolic compounds different from flavonoids were more responsible for the antioxidant activity of the analysed honeys. In fact, we found weak negative correlations between TEAC and the contents of quercetin and kaempferol (Annex 1/Table 4), in opposition to the positive correlations of antioxidant activity with quercetin, luteolin, kaempferol and chrysin described by Tenore *et al.* (2012).

Our results of TEACh were significantly and positively correlated to the values of the sum of phenolic acids analysed by HPLC (PA_{HPLC}) (r=0.7044), as well as to the data of ellagic acid (r=0.7087). In contrast, TEACh was significantly and negatively correlated to chlorogenic acid (r=-0.5200). However, our values did not agree with those of Gambacorta *et al.* (2014), who found positive correlations between honeys' FRAP antioxidant activity and chlorogenic acid. In the literature, other high correlations were observed between the antioxidant capacity and individual phenolic compounds such as gallic acid (Socha *et al.*, 2011; Tenore *et al.*, 2012), caffeic acid (Socha *et al.*, 2011), coumaric and ferulic acids (Gambacorta *et al.*, 2014) and weak correlations between antioxidant capacity and ferulic and sinapic acids (Tenore *et al.*, 2012).

In order to optimize honeys' antioxidant activity determination, different researchers recommended a previous extraction of honey antioxidants. Thus, such interferences as reducing sugars would be removed and the determination would be more precise and reliable (Singleton *et al.*, 1999; Alvarez-Suarez *et al.*, 2009). Some scientists studied the contribution of carbohydrates on antioxidant activity, using an artificial honey composed by the main honey carbohydrates in a similar proportion that those found in real honey (Gheldof *et al.*, 2002; Alvarez-Suarez *et al.*, 2010). For those artificial honeys, low antioxidant activities were reported.

The comparison of our TEAC results with those reported by other authors was difficult because:

1) First, although μmol trolox/g is the most common unit used to express the TEAC, other different units were also used, being the most important of which inhibition percentage (Perna *et al*., 2012), IC50 (concentration of substrate that causes half loss of ABTS radical scavenging activity) (Aazza *et al*., 2013) and other concentration units of trolox (Ciappini *et al*., 2014).
2) Second, instead of trolox, some researchers employed such standards as gallic acid or ascorbic acid (Lachman *et al.*, 2010a; León-Ruiz *et al.*, 2013).
3) Finally, the time for TEAC absorbance measurement was also different in literature references (Wilczynska, 2010; Perna *et al*., 2012; Ciappini *et al*., 2014), so that as absorbance decreases with time, it would be necessary to set up a harmonized TEAC procedure applied to honeys. In this regard, it was demonstrated that the end-point for TEAC determination could be calculated from the measurement at 6 minutes (Chapter 5).

TEACh values in our study ranged between 1.01 μmol TE/g and 7.46 μmol TE/g and TEACe between 0.71 μmol TE/g and 2.96 μmol TE/g. Lower values were reported for Rodríguez *et al.* (2012) in Mexican honeys and extracts, ranging from 0.91 μmol TE/g to 2.93 μmol TE/g and 0.24 μmol TE/g to 1.68 μmol TE/g, respectively. However, these researchers did not indicate the time in which absorbance was measured. Other TEACh values at the end time of 6 minutes ranged between 0.40-0.58 μmol TE/g in Chinese honeys (Chang *et al.*, 2011), and

between 0.70-7.00 µmol TE/g and 0.31-1.38 µmol TE/g respectively in honeys from Brazil (Sant'Ana *et al.*, 2012; Salgueiro *et al.*, 2014).

3.4. Principal components analysis

PCA was carried out with the results of antioxidant-related parameters of unifloral honeys. Four components were extracted, since they had eigenvalues greater than or equal to 1.0. Together they account for 89.84% of the variability in the original data. As Figure 4 shows, the first component explains the separation between light honeys (clover and lavender) from the rest of samples; meanwhile the second component explains the separation between heather honeys and the rest of samples.

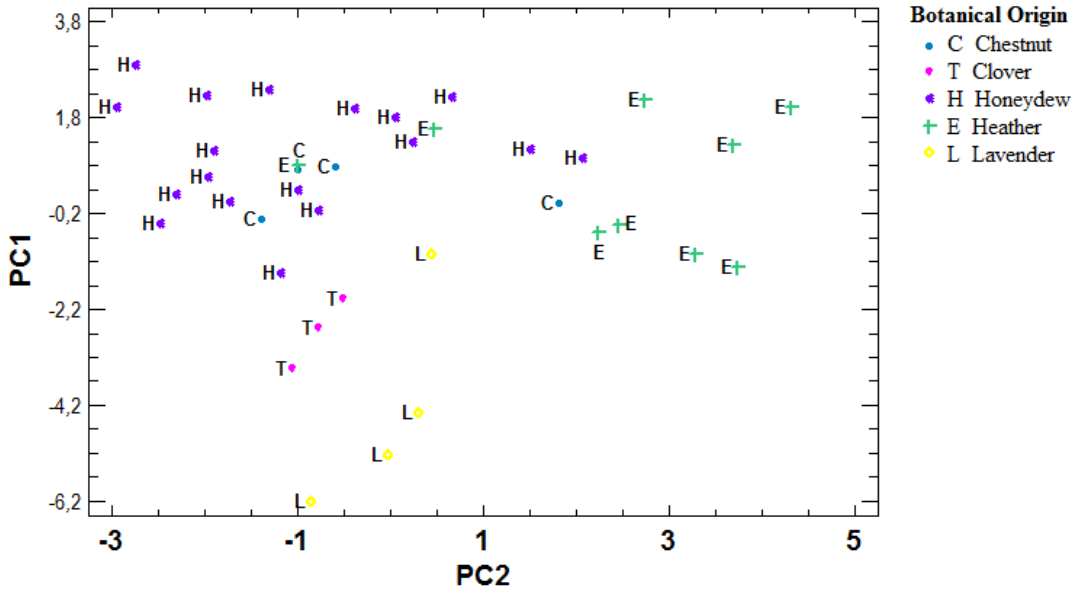

Figure 4. Principal Component Analysis scare plot.

REFERENCES

AAZZA, S; LYOUSSI, B; ANTUNES, D; MIGUEL, M G (2013) Physicochemical characterization and antioxidant activity of commercial portuguese honeys. *Journal of Food Science* 78(8): C1159-C1165. http://dx.doi.org/10.1111/1750-3841.12201

ALVES, A; RAMOS, A; GONÇALVES, M M; BERNARDO, M; MENDES, B (2013) Antioxidant activity, quality parameters and mineral content of Portuguese monofloral honeys. *Journal of Food Composition and Analysis* 30(2): 130-138. http://dx.doi.org/10.1016/j.jfca.2013.02.009

ALJADI, A M; KAMARUDDIN, M Y (2004) Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chemistry* 85(4): 513-518. http://dx.doi.org/10.1016/S0308-8146(02)00596-4

ALMEIDA DA SILVA, I A; DA SILVA, T M S; CAMARA, C A; QUEIROZ, N; MAGNANI, M; SANTOS DE NOVAIS, J; BASTOS-SOLEDADE, L E; DE OLIVEIRA-LIMA, E; DE SOUZA, A L; GOUVEIA DE SOUZA, A (2013) Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas, Northern Brazil. *Food Chemistry* 141: 3552-3558. http://dx.doi.org/10.1016/j.foodchem.2013.06.072

ALMEIDA-MURADIAN, L B; STRAMM, K M; ESTEVINHO, L M (2014) Efficiency of the FT-IR ATR spectrometry for the prediction of the physicochemical characteristics of *Melipona subnitida* honey and study of the temperature's effect on those properties. *International Journal of Food Science & Technology* 49(1): 188-195. http://dx.doi.org/10.1111/ijfs.12297

ALVAREZ-SUAREZ, J; TULIPANI, S; ROMANDINI, S; VIDAL, A; BATTINO, M (2009) Methodological aspects about determination of phenolic compounds and in vitro evaluation of antioxidant capacity in the honey: A review. *Current Analytical Chemistry* 5(4): 293-302. http://dx.doi.org/10.2174/157341109789077768

ALVAREZ-SUAREZ, J M; GONZÁLEZ- PARAMÁS, A M; SANTOS-BUELGA, C; BATTINO, M (2010) Antioxidant characterization of native monofloral Cuban honeys. *Journal of Agricultural and Food Chemistry* 58(17): 9817-9824. http://dx.doi.org/10.1021/jf1018164

AMIOT, M J; AUBERT, S; GONNET, M; TACCHINI, M (1989) Les composes phénoliques des miels: étude préliminaire sur l'identification et la quantification par familles. *Apidologie* 20(2): 115-125. http://dx.doi.org/10.1051/apido:19890202

ANDRADE, P; FERRERES, F; AMARAL, M T (1997a) Analysis of honey phenolic acids by hplc, its application to honey botanical characterization. *Journal of Liquid Chromatography & Related Technologies* 20(14): 2281-2288. http://dx.doi.org/10.1080/10826079708006563

ANDRADE, P; FERRERES, F; GIL, M I; TOMÁS-BARBERÁN, F A (1997b) Determination of phenolic compounds in honeys with different floral origin by capillary zone electrophoresis. *Food Chemistry* 60(1): 79-84. http://dx.doi.org/10.1016/S0308-8146(96)00313-5

ANKLAM, E (1998) A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry* 63(4): 549-562. http://dx.doi.org/10.1016/S0308-8146(98)00057-0

BALTRUŠAITYTĖ, V; VENSKUTONIS, P R; ČEKSTERYTĖ, V (2007) Radical scavenging activity of different floral origin honey and beebread phenolic extracts. *Food Chemistry* 101(2): 502-514. http://dx.doi.org/10.1016/j.foodchem.2006.02.007

BERETTA, G; GRANATA, P; FERRERO, M; ORIOLI, M; MAFFEI FACINO, R (2005) Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytica Chimica Acta* 533(2): 185-191. http://dx.doi.org/10.1016/j.aca.2004.11.010

BERTONCELJ, J; DOBERSEK, U; JAMNIK, M; GOLOB, T (2007) Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chemistry* 105(2): 822-828. http://dx.doi.org/10.1016/j.foodchem.2007.01.060

BERTONCELJ, J; GOLOB, T; KROPF, U; KOROŠEC, M (2011a) Characterisation of Slovenian honeys on the basis of sensory and physicochemical analysis with a chemometric approach. *International Journal of Food Science & Technology* 46(8): 1661-1671. http://dx.doi.org/10.1111/j.1365-2621.2011.02664.x

BERTONCELJ, J; POLAK, T; KROPF, U; KOROSEC, M; GOLOB, T (2011b) LC-DAD-ESI/MS analysis of flavonoids and abscisic acid with chemometric approach for the classification of Slovenian honey. *Food Chemistry* 127: 296-302. http://dx.doi.org/10.1016/j.foodchem.2011.01.003

BIESAGA, M; PYRZYNSKA, K (2009) Liquid chromatography/tandem mass spectrometry studies of the phenolic compounds in honey. *Journal of Chromatography A* 1216(38): 6620-6626. http://dx.doi.org/10.1016/j.chroma.2009.07.066

BOUSSAID, A; CHOUAIBI, M; REZIG, L; HELLAL, R; DONSÌ, F; FERRARI, G; HAMDI, S (2014) Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. *Arabian Journal of Chemistry* 10. http://dx.doi.org/10.1016/j.arabjc.2014.08.011

CAMPILLO, N; VIÑAS, P; FÉREZ-MELGAREJO, G; HERNÁNDEZ-CÓRDOBA, M (2015) Dispersive liquid–liquid microextraction for the determination of flavonoid aglycone compounds in honey using liquid chromatography with diode array detection and time-of-flight mass spectrometry. *Talanta* 131: 185-191. http://dx.doi.org/10.1016/j.talanta.2014.07.083

CAN, Z; YILDIZ, O; SAHIN, H; AKYUZ-TURUMTAY, E; SILICI, S; KOLAYLI, S (2015) An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles. *Food Chemistry* 180: 133-141. http://dx.doi.org/10.1016/j.foodchem.2015.02.024

CHANG, C-C; YANG, M-H; WEN, H-M; CHERN, J-C (2002) Estimation of total flavonoid content in propolis by two complementary colourimetric methods. *Journal of Food and Drug Analysis* 10(3): 17-182.

CHANG, X; WANG, J; YANG, S; CHEN, S; SONG, Y (2011) Antioxidative, antibrowning and antibacterial activities of sixteen floral honeys. *Food & Function* 2(9): 541. http://dx.doi.org/10.1039/c1fo10072f

CHEN, L; MEHTA, A; BERENBAUM, M; ZANGERL, A R; ENGESETH, N J (2000) Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. *Journal of Agricultural and Food Chemistry* 48(10): 4997-5000. http://dx.doi.org/10.1021/jf000373j

CIAPPINI, M C; STOPPANI, F (2014) Determination of antioxidant capacity, flavonoids, and total phenolic content in eucalyptus and clover honeys. *Journal of Apicultural Science* 58(1): 103-111.

CIMPOIU, C; HOSU, A; MICLAUS, V; PUSCAS, A (2013) Determination of the floral origin of some Romanian honeys on the basis of physical and biochemical properties. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 100: 149-154. http://dx.doi.org/10.1016/j.saa.2012.04.008

COMMISSION INTERNATIONALE DE L'ECLAIRAGE-CIE. (2004) Technical report. 3rd Edition. CIE 15:2004.

D'ARCY, B R (2005) Antioxidants in Australian floral honeys. Identification of health enhancing nutrient components. *Rural Industries Research and Development Corporation. Australian Government*. Publication 05/040.
http://tasmanianmanukahoney.com.au/wp-content/uploads/2013/10/AUS-Gov-RIRDC-Pub-2005-040.pdf

DIMITROVA, B; GEVRENOVA, R; ANKLAM, E (2007) Analysis of phenolic acids in honeys of different floral origin by solid-pase extraction and high-performance liquid chromatography. *Phytochemical Analysis* 18(1): 24-32. http://dx.doi.org/10.1002/pca.948

DONG, R; ZHENG, Y; XU, B (2013) Phenolic profiles and antioxidant capacities of Chinese unifloral honeys from different botanical and geographical sources. *Food and Bioprocess Technology* 6(3): 762-770.
http://doi.org/10.1007/s11947-011-0726-0

DOWD, L E (1959) Spectrophotometric determination of quercetin. *Analytical Chemistry* 31(7): 1184-1187.
http://doi.org/10.1021/ac60151a033

ELEAZU, C O; IROAGANACHI, M A; OKORONKWO, J O (2013) Determination of the physico-chemical composition, microbial quality and free radical scavenging activities of some commercially sold honey samples in Aba, Nigeria: 'The Effect of Varying Colors'. *Journal of Nutrition & Food Science* 3(2): 7 pp. http://dx.doi.org/10.4172/2155-9600.1000189

ESCUREDO, O; SILVA, L R; VALENTÃO, P; SEIJO, M C; ANDRADE, P B (2012) Assessing Rubus honey value: Pollen and phenolic compounds content and antibacterial capacity. *Food Chemistry* 130(3): 671-678.
http://dx.doi.org/10.1016/j.foodchem.2011.07.107

ESCUREDO, O; FERNÁNDEZ-GONZÁLEZ, M; RODRÍGUEZ-FLORES, M S; SEIJO-RODRIGUEZ, A; SEIJO-COELLO, M C (2013a) Influence of the botanical origin of honey from North western Spain in some antioxidant components. *Journal of Apicultural Science* 57(1): 5-14. http://dx.doi.org/10.2478/jas-2013-0001

ESCUREDO, O; MÍGUEZ, M; FERNÁNDEZ-GONZÁLEZ, M; CARMEN SEIJO, M (2013b) Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. *Food Chemistry* 138(2-3): 851-856.
http://dx.doi.org/10.1016/j.foodchem.2012.11.015

ESTEVINHO, L; PEREIRA, A P; MOREIRA, L; DIAS, L G; PEREIRA, E (2008) Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food and Chemical Toxicology* 46(12): 3774-3779. http://dx.doi.org/10.1016/j.fct.2008.09.062

FEÁS, X; PIRES, J; ESTEVINHO, M L; IGLESIAS, A; PINTO DE ARAUJO, J P (2010a) Palynological and physicochemical data characterisation of honeys produced in the Entre-Douro e Minho region of Portugal. *International Journal of Food Science & Technology* 45(6): 1255-1262. http://dx.doi.org/10.1111/j.1365-2621.2010.02268.x

FERREIRA, I C F R; AIRES, E; BARREIRA, J C M; ESTEVINHO, L M (2009) Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry* 114(4): 1438-1443. http://dx.doi.org/10.1016/j.foodchem.2008.11.028

FERRERES, F; TOMÁS-BARBERÁN, F A; GIL, M I; TOMÁS-LORENTE, F (1991) An HPLC technique for flavonoid analysis in honey. *Journal of the Science of Food and Agriculture* 56(1): 49-56. http://dx.doi.org/10.1002/jsfa.2740560106

FERRERES, F; ORTIZ, A; SILVA, C; GARCÍA-VIGUERA, C; TOMÁS-BARBERÁN, F A; TOMÁS-LORENTE, F (1992) Flavonoids of "La Alcarria" honey: A study of their botanical origin. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* 194(2): 139-143. http://dx.doi.org/10.1007/BF01190185

FERRERES, F; GARCÍA-VIGUERA, C; TOMÁS-LORENTE, F; TOMÁS-BARBERÁN, F A (1993) Hesperetin: A marker of the floral origin of citrus honey. *Journal of the Science of Food Agriculture* 61(1): 121-123. http://dx.doi.org/10.1002/jsfa.2740610119

FERRERES, F; ANDRADE, P; TOMÁS-BARBERÁN, F A (1994a) Flavonoids from Portuguese heather honey. *Zeitschrift für Lebensmittel-Untersuchung und Forshung* 199(1): 32-37. http://dx.doi.org/10.1007/BF01192949

FERRERES, F; BLÁZQUEZ, M A; GIL, M I; TOMÁS-BARBERÁN, F A (1994b) Separation of honey flavonoids by micellar electrokinetic capillary chromatography. *Journal of Chromatography A* 669(1-2): 268-274. http://dx.doi.org/10.1016/0021-9673(94)80359-5

FERRERES, F; TOMÁS-BARBERÁN, F A; SOLER, C; GARCÍA-VIGUERA, C; ORTIZ, A; TOMÁS-LORENTE, F A (1994c) A simple extractive technique for honey flavonoid HPLC analysis. *Apidologie* 25(1): 21-30. http://dx.doi.org/10.1051/apido:19940103

FERRERES, F; ANDRADE, P; GIL, M I; TOMÁS-BARBERÁN, F A (1996) Floral nectar phenolics as biochemical markers for the botanical origin of heather honey. *Zeitschrift für Lebensmittel-Untersuchung und Forshung* 202(1): 40-44. http://dx.doi.org/10.1007/BF01229682

FRANKEL, S; ROBINSON, G E; BERENBAUM, M R (1998) Antioxidant capacity and correlated characteristics of 14 unifloral honeys. *Journal of Apicultural Research* 37: 27-31. http://dx.doi.org/10.1080/00218839.1998.11100951

GAMBACORTA, E; SIMONETTI, A; GARRISI, N; INTAGLIETTA, I; PERNA, A (2014) Antioxidant properties and phenolic content of sulla (*Hedysarum* spp.) honeys from Southern Italy. *International Journal of Food Science & Technology* 49(10): 2260-2268. http://dx.doi.org/10.1111/ijfs.12541

GAŠIĆ, U M; NATIĆ, M M; MIŠIĆ, D M; LUŠIĆ, D V; MILOJKOVIĆ-OPSENICA, D M; TEŠIĆ, Z LJ; LUŠIĆ, D (2015) Chemical markers for the authentication of unifloral *Salvia officinalis* L. honey. *Journal of Food Composition and Analysis* 44: 128-138. http://dx.doi.org/10.1016/j.jfca.2015.08.008

GHELDOF, N; ENGESETH, N J (2002) Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *Journal of Agricultural and Food Chemistry* 50(10): 3050-3055. http://dx.doi.org/10.1021/jf0114637

GHELDOF, N; WANG, X H; ENGESETH, N J (2002) Identification and quantification of antioxidant components of honeys from various floral sources. *Journal of Agricultural and Food Chemistry* 50(21): 5870-5877. http://dx.doi.org/10.1021/jf0256135

GOMES, T; FEÁS, X; IGLESIAS, A; ESTEVINHO, L M (2011) Study of organic honey from the Northeast of Portugal. *Molecules* 16(12): 5374-5386. http://dx.doi.org/10.3390/molecules16075374

GÓMEZ-CARAVACA, A M; GÓMEZ-ROMERO, M; ARRÁEZ-ROMÁN, D; SEGURA-CARRETERO, A; FERNÁNDEZ-GUTIÉRREZ, A (2006) Advances in the analysis of phenolic compounds in products derived from bees. *Journal of Pharmaceutical and Biomedical Analysis* 41(4): 1220-1234. http://dx.doi.org/10.1016/j.jpba.2006.03.002

GONZÁLEZ-MIRET, M L; TERRAB, A; HERNANZ, D; FERNÁNDEZ-RECAMALES, M A.; HEREDIA, F J (2005) Multivariate correlation between colour and mineral composition of honeys and by their botanical origin. *Journal of Agricultural and Food Chemistry* 53: 2574-2580. http://dx.doi.org/10.1021/jf048207p

GONZÁLEZ-PARAMÁS, A M; GARCÍA-VILLANOVA, R J; GÓMEZ BÁREZ, J A; SÁNCHEZ SÁNCHEZ, J; ARDANUY ALBAJAR, R (2007) Botanical origin of monovarietal dark honeys (from heather, holm oak, pyrenean oak and sweet chestnut) based on their chromatic characters and amino acid profiles. *European Food Research and Technology* 226(1-2): 87-92. http://dx.doi.org/10.1007/s00217-006-0512-9

HABIB, H M; AL MEQBALI, F T; KAMAL, H; SOUKA, U D; IBRAHIM, W H (2014) Physicochemical and biochemical properties of honeys from arid regions. *Food Chemistry* 153: 35-43. http://dx.doi.org/10.1016/j.foodchem.2013.12.048

HENNION, M C (1999) Solid-phase extraction: method development, sorbents, and coupling with liquid chromatography. *Journal of Chromatography A* 856(1-2): 3-54. http://dx.doi.org/10.1016/S0021-9673(99)00832-8

ISLA, M I; GRAIG, A; ORDOÑEZ, R; ZAMPINI, C; SAYAGO, J; BEDASCARRASBURE, E; ALVAREZ, A; SALOMÓN, V; MALDONADO, L (2011) Physico chemical and bioactive properties of honeys from Northwestern Argentina. *LWT-Food Science and Technology* 44(9): 1922-1930. http://dx.doi.org/10.1016/j.lwt.2011.04.003

ISLAM, A; KHALIL, I; ISLAM, N; MONIRUZZAMAN, M; MOTTALIB, A; SULAIMAN, S A; GAN, S H (2012) Physicochemical and antioxidant properties of Bangladeshi honeys stored for more than one year. *BMC Complementary and Alternative Medicine* 12(1): 177-187. http://dx.doi.org/10.1186/1472-6882-12-177

JASICKA-MISIAK, I; POLIWODA, A; DEREŃ, M; KAFARSKI, P (2012) Phenolic compounds and abscisic acid as potential markers for the floral origin of two Polish unifloral honeys. *Food Chemistry* 131(4): 1149-1156. http://dx.doi.org/10.1016/j.foodchem.2011.09.083

JUSZCZAK, L; SOCHA, R; ROŻNOWSKI, J; FORTUNA, T; NALEPKA, K (2009) Physicochemical properties and quality parameters of herbhoneys. *Food Chemistry* 113(2): 538-542. http://dx.doi.org/10.1016/j.foodchem.2008.07.098

KARABAGIAS, I K; VAVOURA, M V; NIKOLAOU, C; BADEKA, A V; KONTAKOS, S; KONTOMINAS, M G (2014) Floral authentication of Greek unifloral honeys based on the combination of phenolic compounds, physicochemical parameters and chemometrics. *Food Research International* 62: 753-760. http://dx.doi.org/10.1016/j.foodres.2014.04.015

KAMBOJ, R; BERA, M B; NANDA, V (2013) Evaluation of physico-chemical properties, trace metal content and antioxidant activity of Indian honeys. *International Journal of Food Science & Technology* 48(3): 578-587. http://dx.doi.org/10.1111/ijfs.12002

KEČKEŠ, S; GAŠIĆ, U; VELIČKOVIĆ, T Ć; MILOJKOVIĆ-OPSENICA, D; NATIĆ, M; TEŠIĆ; Ž (2013) The determination of phenolic profiles of Serbian unifloral honeys using ultra-high-performance liquid chromatography/high resolution accurate mass spectrometry. *Food Chemistry* 138(1): 32-40. http://dx.doi.org/10.1016/j.foodchem.2012.10.025

KENJERIC, D; MANDIC, M; PRIMORAC, L; BUBALO, D; PERL, A (2007) Flavonoid profile of *Robinia* honeys produced in Croatia. *Food Chemistry* 102(3): 683-690. http://dx.doi.org/10.1016/j.foodchem.2006.05.055

KHALIL, M I; ALAM, N; MONIRUZZAMAN, M; SULAIMAN, S A; GAN, S H (2011) Phenolic acid composition and antioxidant properties of Malaysian honeys. *Journal of Food Science* 76(6): C921-C928. http://dx.doi.org/10.1111/j.1750-3841.2011.02282.x

KHALIL, M I; MONIRUZZAMAN, M; BOUKRAÂ, L; BENHANIFIA, M; ISLAM, M A; ISLAM, M N; SULAIMAN, S A; GAN, S H (2012) Physicochemical and antioxidant properties of Algerian honey. *Molecules* 17(12): 11199-11215. http://dx.doi.org/10.3390/molecules170911199

KILIC, I; YEŞILOĞLU, Y; BAYRAK, Y (2014) Spectroscopic studies on the antioxidant activity of ellagic acid. *Spectrochimica Acta-Part A: Molecular and Biomolecular Spectroscopy* 130: 447-452. http://dx.doi.org/10.1016/j.saa.2014.04.052

KÜÇÜK, M; KOLAYLI, S; KARAOĞLU, Ş; ULUSOY, E; BALTACI, C; CANDAN, F (2007) Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chemistry* 100(2): 526-534. http://dx.doi.org/10.1016/j.foodchem.2005.10.010

KUMAZAWA, S; OKUYAMA, Y; MURASE, M; AHN, M-R; NAKAMURA, J; TATEFUJI, T (2012) Antioxidant activity in honeys of various floral origins: Isolation and identification of antioxidants in peppermint honey. *Food Science and Technology Research* 18(5): 679-685. http://dx.doi.org/10.3136/fstr.18.679

KUŚ, P M; CONGIU, F; TEPER, D; SROKA, Z; JERKOVIC, I; TUBEROSO, C I G (2014) Antioxidant activity, color characteristics, total phenol content and general HPLC fingerprints of six polish unifloral honey types. *LWT - Food Science and Technology* 55(1): 124-130. http://dx.doi.org/10.1016/j.lwt.2013.09.016

KUŚ, P M; VAN RUTH, S (2015) Discrimination of Polish unifloral honeys using overall PTR-MS and HPLC fingerprints combined with chemometrics. *LWT - Food Science and Technology* 62(1): 69-75. http://dx.doi.org/10.1016/j.lwt.2014.12.060

LACHMAN, J; HEJTMÁNKOVÁ, A; SÝKORA, J; KARBAN, J; ORSÁK, M; RYGEROVÁ, B (2010a) Contents of major phenolic and flavonoid antioxidants in selected Czech honey. *Czech Journal of Food Sciences* 28(5): 412-426.

LACHMAN, J; ORSÁK, M; HEJTMÁNKOVÁ, A; KOVÁŘOVÁ, E (2010b) Evaluation of antioxidant activity and total phenolics of selected Czech honeys. *LWT-Food Science and Technology* 43(1): 52-58. http://dx.doi.org/10.1016/j.lwt.2009.06.008

LEÓN-RUIZ, V; GONZÁLEZ-PORTO, A V.; AL-HABSI, N; VERA, S; SAN ANDRÉS, M P; JAUREGI, P (2013) Antioxidant, antibacterial and ACE-inhibitory activity of four monofloral honeys in relation to their chemical composition. *Food & Function* 4(11): 1617-1624. http://dx.doi.org/10.1039/c3fo60221d

LOUVEAUX, J; MAURIZIO, A; VORWOHL, G (1978) Methods of melissopalynology. *Bee World* 59(4): 139-157. http://dx.doi.org/10.1080/0005772X.1978.11097714

MAKSIMOVIĆ, Z; NEDIĆ, N (2013) In vitro antioxidant activity of honeydew and multifloral types of honey from Serbia. *Acta Periodica Technologica* 321(44): 269-277. http://dx.doi.org/10.2298/APT1344269M

MĂRGHITAŞ, L A; DEZMIREAN, D; BOBIS, O; LASLO, L; BOGDANOV, S (2009) Physico-chemical and bioactive properties of different floral origin honeys from Romania. *Food Chemistry* 112(4): 863-867. http://dx.doi.org/10.1016/j.foodchem.2008.06.055

MARTOS, I; COSSENTINI, M; FERRERES, F; TOMÁS-BARBERÁN, F A (1997) Flavonoid composition of Tunisian honeys and propolis. *Journal of Agricultural and Food Chemistry* 45(8): 2824-2829. http://dx.doi.org/10.1021/jf9609284

MARTOS, I; FERRERES, F; TOMÁS-BARBERÁN, F A (2000a) Identification of flavonoid markers for the botanical origin of Eucalyptus honey. *Journal of Agricultural and Food Chemistry* 48(5): 1498-1502. http://dx.doi.org/10.1021/jf991166q

MARTOS, I; FERRERES, F; YAO, L; D'ARCY, B; CAFFIN, N; TOMÁS-BARBERÁN, F A (2000b) Flavonoids in monospecific *Eucalyptus* honeys from Australia. *Journal of Agricultural and Food Chemistry* 48(10): 4744-4748. http://dx.doi.org/10.1021/jf000277i

MATEO, R; BOSCH-REIG, F (1997) Sugar profiles of Spanish unifloral honeys. *Food Chemistry* 60(1): 33-41. http://dx.doi.org/10.1016/S0308-8146(96)00297-X

MATEO, R; BOSCH-REIG, F (1998) Classification of Spanish unifloral honeys by discriminant analysis of electrical conductivity, colour, water content, sugars, and pH. *Journal of Agricultural and Food Chemistry* 46(2): 393-400. http://dx.doi.org/10.1021/jf970574w

MEDA, A; LAMIEN, C E; ROMITO, M; MILLOGO, J; NACOULMA, O G (2005) Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry* 91(3): 571-577. http://dx.doi.org/10.1016/j.foodchem.2004.10.006

MEINEN, N; CAMILLERI, L; ATTARD, E (2014) The antioxidant activity of Maltese honey. *Journal of Apicultural Science* 58(1): 51-60. http://dx.doi.org/10.2478/jas-2014-0004

MERKEN, H M; BEECHER G R (2000) Measurement of food flavonoids by high-performance liquid chromatography: A review. *Journal of Agricultural and Food Chemistry* 48(3): 577-599.

MICHALAK, A (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies* 15(4): 523-530.

MICHALKIEWICZ, A; BIESAGA, M; PYRZYNSKA, K (2008) Solid-phase extraction procedure for determination of phenolic acids and some flavonols in honey. *Journal of Chromatography A* 1187(1-2): 18-24. http://dx.doi.org/10.1016/j.chroma.2008.02.001

MOISE, A; MĂRGHITAŞ, L A; DEZMIREAN, D; BOBIS, O (2013) Nutraceutical properties of Romanian heather honey. *Nutrition & Food Science* 43(3): 218-227. http://dx.doi.org/10.1108/00346651311327864

MONIRUZZAMAN, M; SULAIMAN, S A; KHALIL, M I; GAN, S H (2013) Evaluation of physicochemical and antioxidant properties of sourwood and other Malaysian honeys: a comparison with manuka honey. *Chemistry Central Journal* 7(1): 138-150. http://dx.doi.org/10.1186/1752-153X-7-138

MONIRUZZAMAN, M; YUNG-AN, C; VISWESWARA-RAO, P; HAWLADER, M N I; AZLAN, S A B M; SULAIMAN, S A; GAN, S H (2014) Identification of phenolic acids and flavonoids in monofloral honey from Bangladesh by High Performance Liquid Chromatography: Determination of antioxidant capacity. *BioMed Research International* Volume 2014, Article ID 737490: 11 pp.

MONTENEGRO, G; SANTANDER, F; JARA, C; NUÑEZ, G; FREDES, C (2013) Actividad antioxidante y antimicrobiana de mieles monoflorales de plantas nativas chilenas. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* 12(3): 257-268.

NAGAI, T; SAKAI, M; INOUE, R; INOUE, H; SUZUKI, N (2001) Antioxidative activities of some commercially honeys, royal jelly, and propolis. *Food Chemistry* 75: 237-240.

OJEC-OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES (2002) Commission Decision 2002/657/EC of 12 August, Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. L 221: 8-36. Brusels, Belgium. European Union. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32002D0657&from=EN

OJEU-OFFICIAL JOURNAL OF THE EUROPEAN UNION (2007) Commission Regulation EC 868/2007 entering a designation in the Register of protected designations of origin and protected geographical indications "Miel de Galicia or Mel de Galicia (PGI). L192: 11-18. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32007R0868&from=EN

OROIAN, M (2012) Physicochemical and rheological properties of Romanian honeys. *Food Biophysics* 7(4): 296-307. http://dx.doi.org/10.1007/s11483-012-9268-x

ÖZCAN, M M; ÖLMEZ, Ç (2014) Some qualitative properties of different monofloral honeys. *Food Chemistry* 163: 212-218. http://dx.doi.org/10.1016/j.foodchem.2014.04.072

ÖZKÖK, A; D'ARCY, B; SORKUN, K (2010) total phenolic acid and total flavonoid content of Turkish pine honeydew honey. *Journal of ApiProduct & ApiMedical Science* 2(2): 65-71.

PEKAL, A; PYRZYNSKA, K (2014) Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Analytical Methods* 7(9): 1776-1782. http://doi.org/10.1007/s12161-014-9814-x

PÉREZ, R A; IGLESIAS, M T; PUEYO, E; GONZALEZ, M; DE LORENZO, C (2007) Amino acid composition and antioxidant capacity of Spanish honeys. *Journal of Agricultural and Food Chemistry* 55(2): 360-365. http://dx.doi.org/10.1021/jf062055b

PERICHE, A; CASTELLÓ, M L; HEREDIA, A; ESCRICHE I (*In press*) Effect of different drying methods on the phenolic, flavonoid and volatile compounds of *Stevia rebaudiana* leaves. *Flavour and Fragrance Journal.* http://dx.doi.org/10.1002/ffj.3298

PERNA, A; SIMONETTI, A; INTAGLIETTA, I; SOFO, A; GAMBACORTA, E (2012) Metal content of southern Italy honey of different botanical origins and its correlation with polyphenol content and antioxidant activity. *International Journal of Food Science & Technology* 47(9): 1909-1917. http://dx.doi.org/10.1111/j.1365-2621.2012.03050.x

PERNA, A; INTAGLIETTA, I; SIMONETTI, A; GAMBACORTA, E (2013) A comparative study on phenolic profile, vitamin C content and antioxidant activity of Italian honeys of different botanical origin. *International Journal of Food Science & Technology* 48(9): 1899-1908. http://dx.doi.org/10.1111/ijfs.12169

PETRETTO, G L; COSSU, M; ALAMANNI, M C (2015) Phenolic content, antioxidant and physico-chemical properties of Sardinian monofloral honeys. *International Journal of Food Science & Technology* 50(2): 482-491. http://dx.doi.org/10.1111/ijfs.12652

PETRUS, K; SCHWARTZ, H; SONTAG, G (2011) Analysis of flavonoids in honey by HPLC coupled with coulometric electrode array detection and electrospray ionization mass spectrometry. *Analytical and Bioanalytical Chemistry* 400(8): 2555-2563. http://dx.doi.org/10.1007/s00216-010-4614-7

PICHICHERO, E; CANUTI, L; CANINI, A (2009) Characterisation of the phenolic and flavonoid fractions and antioxidant power of Italian honeys of different botanical origin. *Journal of the Science of Food and Agriculture* 89(4): 609-616. http://dx.doi.org/10.1002/jsfa.3484

PONTIS, J A; DA COSTA, L A M A; SILVA, S J R; FLACH, A (2014) Colour, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. *Food Science and Technology* 34(1): 69-73. http://dx.doi.org/10.1590/S0101-20612014005000015

PRIOR, R L; WU, X; SCHAICH, K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry* 53(10): 4290-4302. http://dx.doi.org/10.1021/jf0502698

PULCINI, P; ALLEGRINI, F; FESTUCCIA, N (2006) Fast SPE extraction and LC-ESI-MS-MS analysis of flavonoids and phenolic acids in honey. *Apiacta* 41: 21-27.

RE, R; PELLEGRINI, N; PROTEGGENTE, A; PANNAL, A; YANG, M; RICE-EVANS, C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* 26(9-10): 1231-1237. http://doi.org/10.1016/S0891-5849(98)00315-3

RODRÍGUEZ, B A; MENDOZA, S; ITURRIGA, M H; CASTAÑO-TOSTADO, E (2012) Quality parameters and antioxidant and antibacterial properties of some Mexican honeys. *Journal of Food Science* 77(1): C121-C127. http://dx.doi.org/10.1111/j.1750-3841.2011.02487.x

RODRÍGUEZ, I; LLOMPART, M P; CELA, R (2000) Solid-phase extraction of phenols. *Journal of Chromatography A* 885(1-2): 291-304. http://dx.doi.org/10.1016/S0021-9673(00)00116-3

RODRÍGUEZ-FLORES, M S; ESCUREDO, O; SEIJO, M C (2015) Assessment of physicochemical and antioxidant characteristics of Quercus pyrenaica honeydew honeys. *Food Chemistry* 166: 101-106. http://dx.doi.org/10.1016/j.foodchem.2014.06.005

SAGDIC, O; SILICI, S; EKICI, L (2013) Evaluation of the phenolic content, antiradical, antioxidant, and antimicrobial activity of different floral sources of honey. *International Journal of Food Properties* 16(3): 658-666. http://dx.doi.org/10.1080/10942912.2011.561463

SALGUEIRO, F B; LIRA, A F; RUMJANEK, V M; CASTRO, R N (2014) Phenolic composition and antioxidant properties of Brazilian honeys. *Química Nova* 37(5): 821-826.http://dx.doi.org/10.5935/0100-4042.20140132

SANT'ANA, L D'O; SOUSA, J P L M; SALGUEIRO, F B; LORENZON, M C A; CASTRO, R N (2012) Characterization of monofloral honeys with multivariate analysis of their chemical profile and antioxidant activity. *Journal of Food Science* 77(1): C135-C140. http://dx.doi.org/10.1111/j.1750-3841.2011.02490.x

SANT'ANA, L D'O; BUARQUE-FERREIRA, A B; AFFONSO-LORENZON, M C; LOURO-BERBARA, R L; CASTRO, R N (2014) Correlation of total phenolic and flavonoid contents of Brazilian honeys with colour and antioxidant capacity. *International Journal of Food Properties* 17(1): 65-76. http://dx.doi.org/10.1080/10942912.2011.614368

SEREM, J C; BESTER, M J (2012) Physicochemical properties, antioxidant activity and cellular protective effects of honeys from southern Africa. *Food Chemistry* 133(4): 1544-1550. http://dx.doi.org/10.1016/j.foodchem.2012.02.047

SERGIEL, I; POHL, P; BIESAGA, M (2014) Characterization of honeys according to their content of phenolic compounds using high performance liquid chromatography/tandem mass spectrometry. *Food Chemistry* 145: 404-408. http://dx.doi.org/10.1016/j.foodchem.2013.08.068

SINGLETON, V L; ORTHOFER, R; LAMUELA-RAVENTOS, R M (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Methods in Enzymology* 299: 152-178. http://dx.doi.org/10.1016/S0076-6879(99)99017-1

SILICI, S; SARIOGLU, K; KARAMAN, K (2013) Determination of polyphenols of some Turkish honeydew and nectar honeys using HPLC-DAD. *Journal of Liquid Chromatography & Related Technologies* 36(July 2014): 2330-2341. http://dx.doi.org/10.1080/10826076.2012.720332

SILVA, T M S; DOS SANTOS, F P; EVANGELISTA-RODRIGUES, A; DA SILVA, E M S; DA SILVA, G S; DE NOVAIS, J S; DOS SANTOS, F A R; CAMARA, C A (2013) Phenolic compounds, melissopalynological, physicochemical analysis and antioxidant activity of jandaíra (*Melipona subnitida*) honey. *Journal of Food Composition and Analysis* 29(1): 10-18. http://dx.doi.org/10.1016/j.jfca.2012.08.010

SOCHA, R; JUSZCZAK, L; PIETRZYK, S; GAŁKOWSKA, D; FORTUNA, T; WITCZAK, T (2011) Phenolic profile and antioxidant properties of Polish honeys. *International Journal of Food Science & Technology* 46(3): 528-534. http://dx.doi.org/10.1111/j.1365-2621.2010.02517.x

SOLER, C; GIL, M; GARCÍA-VIGUERA, C; TOMÁS-BARBERÁN, F A(1995) Flavonoid patterns of French honeys with different floral origin. *Apidologie* 26(1986): 53-60.

STATGRAPHICS CENTURION XVI.II (2010) Statpoint Technologies, Inc. Warrenton, VA (USA).

STEPHENS, J M; SCHLOTHAUER, R C; MORRIS, B D; YANG, D; FEARNLEY, L; GREENWOOD, D R; LOOMES, K M (2010) Phenolic compounds and methylglyoxal in some New Zealand manuka and kanuka honeys. *Food Chemistry* 120(1): 78-86. http://dx.doi.org/10.1016/j.foodchem.2009.09.074

TENORE, G C; RITIENI, A; CAMPIGLIA, P; NOVELLINO, E (2012) Nutraceutical potential of monofloral honeys produced by the Sicilian black honeybees (Apis mellifera ssp. sicula). *Food and Chemical Toxicology* 50(6): 1955-1961. http://dx.doi.org/10.1016/j.fct.2012.03.067

TERRADILLOS, L A; MUNIATEGUI, S; SANCHO, M T; HUIDOBRO, J F; SIMAL-LOZANO, J (1994) An alternative method for analysis of honey sediment. *Bee Science* 3(2): 86-93.

TEZCAN, F; KOLAYLI, S; SAHIN, H; ULUSOY, E; ERIM, F B (2011) Evaluation of organic acid, saccharide composition and antioxidant properties of some authentic Turkish honeys. *Journal of Food and Nutrition Research* 50(1): 33-40.

THE NATIONAL HONEY BOARD (2003) Honey-Health and Therapeutic Qualities. The National Honey Board, 390 Lashley Street Longmont, CO 80501-6045, USA. http://www.honey.com

TOMÁS-BARBERÁN, F A; FERRERES, F; BLÁZQUEZ, M A; GARCIA-VIGUERA, C; TOMÁS-LORENTE, F (1993a) High-Performance Liquid Chromatography of honey flavonoids. *Journal of Chromatography A* 634(7): 41-46. http://dx.doi.org/10.1016/0021-9673(93)80310-5

TOMÁS-BARBERÁN, F A; FERRERES, F; GARCÍA-VIGUERA, C; TOMÁS-LORENTE, F (1993b) Flavonoids in honey of different geographical origin. *Zeitshrift für Lebensmittel-Untersuchung und-Forschung* 196(1): 38-44. http://dx.doi.org/10.1007/BF01192982

TOMÁS-BARBERÁN, F A; MARTOS, I; FERRERES, F; RADOVIC, B S; ANKLAM, E (2001) HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *Journal of the Science of Food and Agriculture* 81(5): 485-496. http://dx.doi.org/10.1002/jsfa.836

TRAUTVETTER, S; KOELLING-SPEER, I; SPEER, K (2009) Confirmation of phenolic acids and flavonoids in honeys by UPLC-MS. *Apidologie* 40(2): 140-150. http://dx.doi.org/10.1051/apido/2008072

TRUCHADO, P; FERRERES, F; TOMÁS-BARBERÁN, F A (2009) Liquid Chromatography–tandem Mass Spectrometry reveals the widespread occurrence of flavonoid glycosides in honey, and their potential as floral origin markers. *Journal of Chromatography A* 1216(43): 7241-7248. http://dx.doi.org/10.1016/j.chroma.2009.07.057

TUBEROSO, C I G; BOBAN, M; BIFULCO, E; BUDIMIR, D; PIRISI, F M (2013) Antioxidant capacity and vasodilatory properties of Mediterranean food: the case of Cannonau wine, myrtle berries liqueur and strawberry-tree honey. *Food Chemistry* 140: 686-691. http://dx.doi.org/10.1016/j.foodchem.2012.09.071

TUBEROSO, C I G; JERKVIĆ, I; SARAIS, G; CONGIU, F; MARIJANOVIĆ, Z; KÚS, P M (2014) Color evaluation of seventeen European unifloral honey types by means of spectrophotometrically determined CIE L* C^*_{ab} h°_{ab} chromaticity coordinates. *Food chemistry* 145: 284-291. http://dx.doi.org/10.1016/10.1016/j.foodchem.2013.08.032

ULLOA, P A; MAIA, M; BRIGAS, A F (2015) Physicochemical parameters and bioactive compounds of strawberry tree (*Arbutus unedo* L.) honey. *Journal of Chemistry* Volume 2015, Article ID 602792: 10 pp. http://dx.doi.org/10.1155/2015/602792

VELA, L; DE LORENZO, C; PÉREZ, R A (2007) Antioxidant capacity of Spanish honeys and its correlation with polyphenol content and other physicochemical properties. *Journal of the Science of Food and Agriculture* 87(6): 1069-1075. http://dx.doi.org/10.1002/jsfa.2813

VON DER OHE, W; PERSANO-ODDO, L; PIANA, M L; MORLOT, M; MARTINS, P (2004) Harmonized methods of melissopalynology. *Apidologie* 35(Suppl. 1): S18-S25. http://dx.doi.org/10.1051/apido:2004050

WAŚ, E; RYBAK-CHMIELEWSKA, R; SZCZĘSNA, T; KACHANIUK, K; TEPER, D (2011) Characteristics of Polish unifloral honeys. III: Heather honey (*Calluna vulgaris* L). *Journal of Apicultural Science* 55: 129-137.

WHITE, J W JR. (1978) Honey. *Advances in Food Research* 24: 287-374. http://dx.doi.org/10.1016/S0065-2628(08)60160-3

WILCZYNSKA, A (2014) Effect of filtration on colour, antioxidant activity and total phenolics of honey. *LWT-Food Science and Technology* 57(2): 767-774. http://dx.doi.org/10.1016/j.lwt.2014.01.034

YAO, L; DATTA, N; TOMÁS-BARBERÁN, F A; FERRERES, F; MARTOS, I; SINGANUSONG, R (2003) Flavonoids, phenolic acids and abscisic acid in Australian and New Zealand *Leptospermum* honeys. *Food Chemistry* 81(2): 159-168. http://dx.doi.org/10.1016/S0308-8146(02)00388-6

YAO, L; JIANG, Y; SINGANUSONG, R; D'ARCY, B; DATTA, N; CAFFIN, N; RAYMONT, K (2004) Flavonoids in Australian *Melaleuca, Guioa, Lophostemon, Banksia* and *Helianthus* honeys and their potential for floral authentication. *Food Research International* 37(2): 166-174. http://dx.doi.org/10.1016/j.foodres.2003.11.004

YAOA, L; JIANG, Y; SINGANUSONG, R; DATTA, N; RAYMONT, K (2005) Phenolic acids in Australian Melaleuca, Guioa, Lophostemon, Banksia and Helianthus honeys and their potential for floral authentication. *Food Research International* 38(6): 651-658. http://dx.doi.org/10.1016/j.foodres.2005.01.002

ZALIBERA, M; STAŠKO, A; ŠLEBODOVÁ, A; JANČOVIČOVÁ, V; ČERMÁKOVÁ, T; BREZOVÁ, V (2008) Antioxidant and radical-scavenging activities of Slovak honeys-An electron paramagnetic resonance study. *Food Chemistry* 110(2): 512-521. http://dx.doi.org/10.1016/j.foodchem.2008.02.015

CHAPTER 7

COMPARISON OF METHODS TO DETERMINE ANTIBACTERIAL ACTIVITY OF HONEYS AGAINST *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Nowadays, researching potentially functional properties of honey such as antimicrobial activity is interesting due to the overwhelming problem of bacteria strains resistant to antibiotics, and the expected higher value for honey that consumers constantly demand. In this research, we compared three different methods (agar dilution, broth dilution, as well as agar well diffusion), to analyse honey's antimicrobial activity against *Staphylococcus aureus*, using 56 unpasteurized honeys from different botanical origins. Agar well diffusion method showed to be a rapid and low cost screening method, using less medium and material, to distinguish the samples with and without antibacterial activity. Agar dilution and broth dilution procedures gave similar values. However, the latter proved to be faster and much more informative, providing with minimal antimicrobial and bactericidal concentrations. The highest values of antimicrobial activities were found in multifloral, honeydew and heather honeys. Conversely, lavender samples showed the lowest antimicrobial activity against *Staphylococcus aureus*.

1. Introduction

Honey has been used as a medicine since ancient times, mainly for the treatment of skin wounds, burns, ulcers, ocular infections, sore throat and digital dermatitis, among others (Molan, 1999; The National Honey Board, 2002; Oelschlaegel *et al.*, 2012b). The healing capacity of honey is strongly influenced by both physical and chemical properties of this food (Basualdo *et al.*, 2007), which are also related to botanical source, honey bee's metabolism, as well as environmental, seasonal and climatic conditions. Apart from healing, honey has been also employed as an excellent preservative for other food commodities, due to its antimicrobial activity (Krushna *et al.*, 2007; Malik and Sharma, 2010).

The inappropriate use of antibiotics has led to many forms of bacterial resistance, thereby limiting the use of these agents in strains of microorganisms resistant to antibiotics (Tenover, 1986). Research on potentially antibacterial products, such as honey, is of great interest because they could be successfully used against certain microorganisms' strains.

The antibacterial activity of honey has been extensively studied throughout the last years. Nevertheless, most research was carried out on Australian and New Zealand honeys (Allen *et al.*, 1991; Patton *et al.*, 2006; Irish *et al.*, 2011), existing few studies on European honeys in

general (Fidaleo *et al.*, 2011; Voidarou *et al.*, 2011), and on Spanish samples in particular (Pérez-Martín *et al.*, 2008; Escuredo *et al.*, 2012). The antimicrobial activity of honeys has been attributed to osmolarity, pH, hydrogen peroxide production, flavonoids, phenolic compounds and the presence of other phytochemical components, such as methylglyoxal, leptosin, melanoidins, bee defensing, jelleins, and hydroxyl radicals (Taormina *et al.*, 2001; Lee *et al.*, 2008; Mavric *et al.*, 2008; Sherlock *et al.*, 2010; Kato *et al.*, 2012; Oelschlaegel *et al.*, 2012a; Brudzynski and Sjaarda, 2015). Different processing or storage conditions are able to change the composition of honey, modifying its antimicrobial activity. For example, Chen *et al.* (2012) observed a decrease in antibacterial and antifungal activity in processed honey (heat to 45ºC for 8 h), while Elbanna *et al.* (2014) showed a decrease in antibacterial activity for honeys stored at room temperature for 12 and 24 months and also in autoclaved honeys. The great variability of information about the antimicrobial honey compounds may be attributed to differences in both botanical and geographical origins, and consequently to the chemical composition (Akkol *et al.*, 2007; Feás *et al.*, 2013). In addition, many factors (climate, genetic composition of plants and bee species), are known to affect honey composition, and thereby such properties as antibacterial activity.

Staphylococcus aureus is a Gram-positive bacterium widely distributed throughout the world. Nowadays, this microorganism is one of the main causes of infections related to hospital care (Sydnor and Perl, 2011). This is favoured by the fact that this species is found in both the skin and mucous membranes of humans, which allows its penetration into the patient's bloodstream through surgical wounds, as well as through direct or indirect contact with medical personnel with a contaminated object, or with another patient (Hurtado *et al.*, 2002). Furthermore, this microorganism is also an important foodborne pathogen responsible of several outbreaks (EFSA, 2015). *Staphylococcus aureus* is the bacterium most commonly chosen by researchers to assay antimicrobial activity of honeys. This is because *Staphylococcus aureus* can tolerate honey's high sugar contents and acidity levels, while being sensitive to the antimicrobial action of hydrogen peroxide and the non-peroxide inhibitory action of honey (Dustmann, 1979; Molan and Russell, 1988).

Different protocols have been used to assess honey's antimicrobial activity with very different results. Procedures can be classified as agar diffusion methods, such as agar well diffusion and paper disc diffusion; dilution methods, such as agar dilution and broth dilution; as well as gradient plates, such as wedge system and spiral plating (Estrada *et al.*, 2005; Sherlock *et al.*, 2010; Fidaleo *et al.*, 2011; Voidarou *et al.*, 2011). The use of a great number of non-standardized methods in the determination of honey's antimicrobial activity is a hurdle when assessing and interpreting results. Patton *et al.* (2006) compared several antimicrobial activity assays but using only one honey sample. Allen *et al.* (1991) evaluated a huge amount of samples, but using only one method (agar well diffusion). With this background, it follows that it would be desirable, for the drawing of reliable conclusions, to compare the most used

methods to determine antibacterial activity of honeys using a representative number of samples. Hence, the purpose of this study has been to compare, on more than fifty samples, three different methods (agar well diffusion, agar dilution and broth dilution), widely used to assess honeys' antibacterial activity against *Staphylococcus aureus* in order to propose the most suitable procedure. An additional purpose has been to research possible differences among minimum inhibitory concentrations against *Staphylococcus aureus* of honeys from different botanical origins.

2. Materials and methods

2.1 Samples

This work was carried out on 56 representative, unpasteurized Spanish honeys, harvested in 2011 in Castilla y León (Spain). The sampling area was larger than 94,200 square kilometres with different landscapes (brushwood, steppe grasslands, prairies and mountains). Botanical origins were determined by melissopalynology (Louveaux *et al.*, 1978; Terradillos *et al.*, 1994; Von der Ohe *et al.*, 2004), sensory analysis and such physicochemical parameters as pH, conductivity, colour and sugars profile. Most samples were multifloral honeys. According to their botanical origins, honeys were classified in five groups coded as: A) Multifloral, B) Honeydew, C) Heather (*Calluna vulgaris*, *Erica* sp. and *Erica* sp. /*Calluna vulgaris*), D) Lavender and E) Leguminosae. The samples were stored in darkness at 4ºC until analysis.

Minimum inhibitory concentration (MIC) assay was developed using fresh-daily serial honey dilutions (75%, 37.50%, 18.75%, 9.38%, and 4.69% [w/v]), aseptically prepared in nutrient broth (Oxoid, Basingstoke, UK). Agar well diffusion method (AWD) was carried out with 75% (w/v) honey dilution.

2.2 Bacterial strain

Honeys' antibacterial activities were tested against *Staphylococcus aureus subsp. aureus* CECT 976, using decimal dilutions in RINGER (OXOID).

2.3 Antimicrobial activity

2.3.1 Agar well diffusion

Tubes of 20 ml sterile liquid nutrient agar (OXOID) at 50ºC were inoculated with 500 µl of 7 log CFU/ml *Staphylococcus aureus* (overnight cultures grown at 37ºC on nutrient broth, and plated out in nutrient agar media to take colony count), mixed thoroughly, poured into sterile empty plates and left 1 hour at room temperature until solidification. Then, 8 mm diameter wells were cut into the surface of the agar using the back of a sterile blue tip and 150 µl of 75% (w/v) honey were added to each well. After 24-hours incubation at 37ºC, zones of

inhibition were measured using a Vernier caliper. The diameter of zones, including the diameter of the well, was recorded. Each assay was carried out in triplicate.

2.3.2 Determination of minimum inhibitory concentration (MIC)

MIC is generally defined as the lowest concentration of a given antimicrobial that prevents growth of a microorganism after a specified incubation period (López-Malo *et al.*, 2005). In this study MIC was calculated using agar dilution and broth dilution methods. MIC was determined in this work as the minimal honey concentration where *S. aureus* growth was not visually observed.

Agar dilution method. Four tubes containing 10 ml sterile nutrient broth (OXOID) were used for each honey in order to perform successive serial half-dilutions to obtain final concentrations from 37.50% to 4.69% (w/v). Ten millilitres of each honey dilution (from 75% to 4.69% [w/v]) was added to 10 ml sterile liquid nutrient agar at 50ºC (final concentration from 37.50% to 2.35% [w/v]). This mixture was homogenized in a vortex mixer, poured into plates and then the agar was allowed to solidify. *Staphylococcus aureus* was added at 5 log CFU/ml to each plate in 5 µl spots. A control plate with no added antimicrobial was prepared and inoculated, ensuring adequate growth of *Staphylococcus aureus*. Plates were incubated at 37ºC for 24 h. The MIC was the lowest concentration that completely inhibited growth.

Broth dilution method. Sterile 96 well round bottomed polystyrene microtitre plates (Brand, Wertheim, Germany) were used. A hundred microliters of 5 log CFU/ml *Staphylococcus aureus* was added to 100 µl of test honey, at different concentrations (from 37.5% to 0.6% [w/v]) in each well (three replicates per dilution, seven dilutions tested). Control wells from each honey were performed adding to each honey dilution 100 µl of RINGER instead of culture, in order to observe contamination. Also control wells contained only broth (negative control) or only bacteria and broth (positive control) were made. Plates were incubated at 37ºC during 24 h. Both visible growth and MIC were recorded. Then, 10 µl of each well in which bacterial growth were inhibited, were plated on to nutrient agar (Oxoid) and incubated overnight at 37ºC in order to research if the antibacterial activity of the honey samples was bacteriostatic or bactericidal. Minimum bactericidal concentration (MBC) was determined as the minimal honey concentration where *S. aureus* did not grow in agar plates after 24 h at 37ºC. Plates with visible colony growth were considered to exhibit bacteriostatic activities, while those with no growth were recorded as exhibiting bactericidal activities.

2.4 Statistical analysis

Antimicrobial data were statistically analysed using principal components analysis (PCA) and ANOVA test, where appropriate means were ranked using the least significance difference

test (LSD 0.05). Data analyses were conducted using the statistical software package Statgraphics Centurion XVI (2010).

3. Results and discussion

Table 1 shows the results obtained after applying the three different methods to determine honeys' antibacterial activity against *Staphylococcus aureus*. Most of the samples (78.5%) exhibited antibacterial activity in concentrations lower than 10% (4.69% and 9.38% [w/v]) by both agar dilution and broth dilution methods. Three honeys (41, 50 and 56) showed no antimicrobial activity even in concentrations of 75% (w/v) by agar well diffusion (AWD) method.

Table 1. Antimicrobial activity of honeys: Minimum inhibitory concentration (MIC) expressed in % w/v by agar dilution (AD) and broth dilution (BD), and inhibition diameter (mm) including well (8.0 mm) of honeys at 75% by agar well diffusion (AWD) against *Staphylococcus aureus* (n=3). For broth dilution, minimum bactericidal concentration (MBC) is also given.

No.	Code	AD (MIC)	BD		AWD Ø (mm)	No.	Code[a]	AD (MIC)	BD		AWD Ø (mm)
			MIC	MBC					MIC	MBC	
1	A	9.38	4.69	4.69	14.90±0.79	**29**	B	4.69	4.69	4.69	14.03±0.06
2	A	4.69	4.69	4.69	15.83±1.06	**30**	B	9.38	9.38	9.38	11.70±0.36
3	A	4.69	4.69	4.69	15.80±0.69	**31**	B	18.75	9.38	9.38	12.03±0.35
4	A	4.69	4.69	4.69	15.13±0.23	**32**	B	9.38	9.38	9.38	12.43±0.55
5	A	4.69	4.69	4.69	14.90±0.75	**33**	B	9.38	9.38	9.38	12.30±0.61
6	A	9.38	4.69	4.69	14.23±0.81	**34**	B	4.69	4.69	9.38	13.73±0.55
7	A	9.38	9.38	9.38	13.17±0.57	**35**	B	4.69	4.69	4.69	13.67±0.12
8	A	18.75	18.75	18.75	13.43±0.46	**36**	B	4.69	4.69	4.69	14.83±0.72
9	A	9.38	9.38	9.38	15.27±0.85	**37**	B	4.69	4.69	4.69	13.00±0.30
10	A	18.75	18.75	18.75	12.27±0.81	**38**	B	4.69	4.69	9.38	13.27±1.48
11	A	9.38	9.38	9.38	16.83±0.47	**39**	C	4.69	4.69	4.69	12.53±0.78
12	A	4.69	4.69	4.69	17.57±2.10	**40**	C	9.38	4.69	4.69	13.07±0.68
13	A	9.38	9.38	9.38	16.73±0.49	**41**	C	18.75	18.75	37.5	8.00±0.00
14	A	4.69	4.69	4.69	14.03±1.46	**42**	C	4.69	4.69	4.69	11.80±0.53
15	A	18.75	9.38	9.38	10.20±1.59	**43**	C	4.69	4.69	4.69	10.63±0.93
16	A	18.75	18.75	18.75	11.07 ±0.21	**44**	C	4.69	4.69	4.69	17.07±1.44
17	A	18.75	9.38	18.75	9.40±1.22	**45**	C	4.69	4.69	4.69	15.53±0.85
18	A	4.69	4.69	4.69	12.23±1.19	**46**	C	9.38	9.38	9.38	11.70±0.87
19	B	4.69	4.69	9.38	14.70±1.18	**47**	C	9.38	9.38	9.38	9.50±0.26
20	B	18.75	9.38	18.75	10.43± 0.40	**48**	C	4.69	4.69	4.69	10.60±0.62
21	B	4.69	9.38	9.38	16.23±0.60	**49**	D	4.69	4.69	4.69	12.53±0.45
22	B	9.38	9.38	9.38	13.23±1.57	**50**	D	37.50	37.50	75.00	8.00±0.00
23	B	9.38	9.38	9.38	13.97±1.63	**51**	D	4.69	4.69	4.69	10.53±0.35
24	B	9.38	9.38	9.38	13.63±0.42	**52**	D	18.75	9.38	18.75	11.03 ±0.55
25	B	9.38	9.38	9.38	14.53±0.42	**53**	E	9.38	9.38	9.38	10.50±0.50
26	B	9.38	9.38	9.38	14.10±0.89	**54**	E	18.75	9.38	9.38	11.70±0.75
27	B	9.38	9.38	9.38	11.13±0.75	**55**	E	4.69	9.38	9.38	13.83±0.64
28	B	4.69	9.38	9.38	13.73±1.12	**56**	E	18.75	18.75	18.75	8.00±0.00

A) Multifloral, B) Honeydew, C) Heather (Calluna vulgaris, Erica sp. and Erica sp./Calluna vulgaris), D) Lavender, E) Leguminosae.

In most samples (44 out of 56), agar dilution and broth dilution procedures gave similar results. Even though agar dilution is lower-cost than broth dilution (as less material is needed), the latter is faster to perform and provides the minimal bactericidal concentration, giving additional valuable information.

After comparing agar dilution and broth dilution values with agar well diffusion results some differences were observed. Honeys with high antibacterial activities measured by agar dilution and broth dilution (MIC = 4.69%) showed a wide variation in the antibacterial activity using AWD (diameter from 10.53 mm to 17.57 mm), exhibiting samples 11, 12, 13, 21 and 44 the highest values of antimicrobial activity by AWD procedure. The discrepancies among the methods can be explained by the fact that honeys with potent antibacterial activity due to compounds with relatively high molecular weight, which have limited migration in the agar, may thus be erroneously characterized as having low activity by AWD method (Kwakman and Zaat, 2012).

Agar well diffusion was described as an assay with a relatively low sensitivity (James *et al.*, 1972), because the samples were further diluted as soon as they diffused into the agar (Cooper, 1963). However, this procedure is one of the most employed to determine antibacterial activity of honeys, because it allows the study of a vast number of samples. Our results showed that in order to get proper and accurate data about antibacterial activity of honeys against *Staphylococcus aureus*, agar well diffusion should be combined with other procedure such as broth dilution, in order to know MIC. Other possibility is comparing agar well diffusion with phenol standard solutions, so that antimicrobial activity is expressed as concentration of phenol with equivalent activity (Allen *et al.*, 1991). To know MIC, the use of agar well diffusion with different honey concentrations would also be appropriate. Agar well diffusion could be used as a rapid and inexpensive screening method to differentiate honeys with and without antibacterial activity. Broth dilution would give minimal antimicrobial and bactericidal concentrations of those samples that showed antibacterial activity by agar well dilution.

When comparing our results with those obtained by other researchers, we observed that in some studies, results of honeys' antibacterial activities against *Staphylococcus aureus* were similar to those obtained by us (Sherlock *et al.*, 2010; Voidarou *et al.*, 2011; Escuredo *et al.*, 2012). In other studies, the values were different than ours, which could be due to our honeys themselves or to a loss of antibacterial activity during honey processing and handling (Revathy and Banerji, 1980; Irish *et al.*, 2011).

With regard to botanical origins of the samples, it is relevant to underline that even though the botanical origins of some of our samples were similar, their antibacterial activities were different. Heather honey No. 41 showed a MIC of 18.75%. In contrast, heather honey No. 42 exhibited a MIC of 4.69% (Table 1). This agrees with the fact that the antibacterial activity of

some honeys might not be so related to their botanical source, but to other parameters such as bee metabolism products (Baltrušaitytė *et al.*, 2007), or other factors (environmental, climate, processing) related to the transformation of nectar precursors to antibacterial substances (Manyi-Loh *et al.*, 2011; Chen *et al.*, 2012; Elbanna *et al.*, 2014).

Figure 1 shows the principal components analysis applied to our results, carried out with data of MIC obtained by both agar dilution and broth dilution methods, as well as with the inverse values of the diameters (mm) obtained by AWD method, thus also allowing a better comparison of the three procedures, since the lower the MIC is, the higher the diameter (mm) of the inhibition zones is. Principal components analysis showed that agar dilution and broth dilution methods were correlated. Figure 1 groups the samples into six groups, according to antibacterial activities, gathering in group 1 all honeys with the highest antibacterial activities and in group 6 the samples with the lowest antimicrobial activities (samples No. 41, 50 and 56).

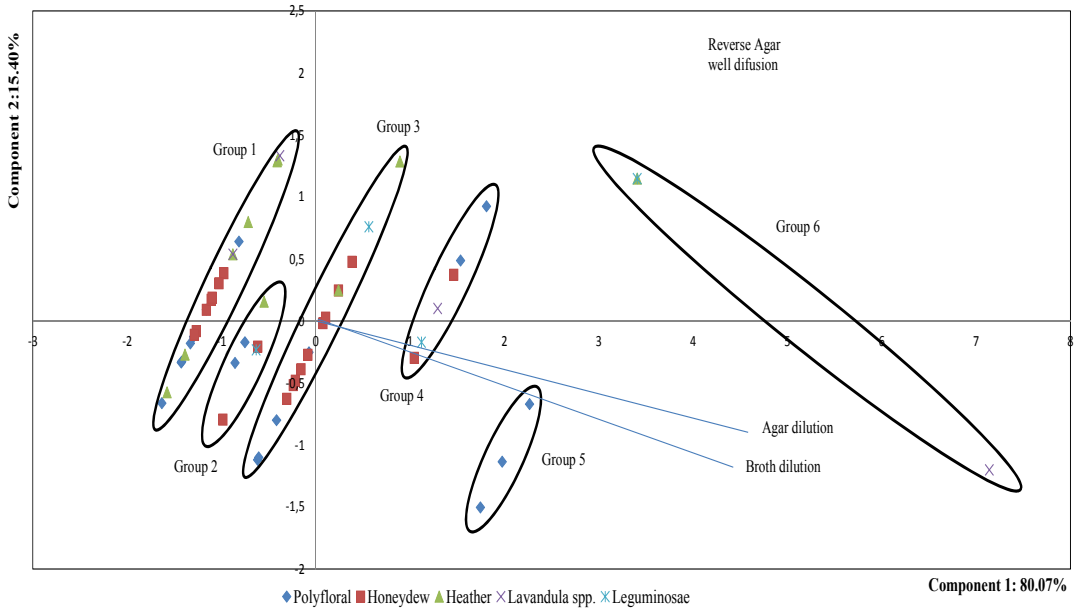

Figure 1. Principal component analysis of three different methods to evaluate antibacterial activities of Spanish honeys against *Staphylococcus aureus* according to the samples' botanical origins.

Table 2 shows that there were significant differences ($p<0.05$) of antibacterial activity among the five established groups of samples (codes A, B, C, D and E) only by agar well diffusion method, where lavender honeys obtained the lowest halo and therefore the lowest antimicrobial activity. Conversely, multifloral, honeydew and heather samples were the most active honeys against *Staphylococcus aureus.* Antibacterial activities of multifloral (A), honeydew (B) and heather (C) samples were not significantly different (Table 2). Nevertheless, by agar dilution and broth dilution methods there were not significant differences ($p>0.05$) between the five botanic origin groups, which highlight the fact that the antibacterial activity was not correlated with the floral source.

Table 2. Antimicrobial activity by the three procedures according to the samples' botanical origins.

Botanical origins	Samples	AD (MIC %w/v)	BD (MIC %w/v)	AWD (mm)
Multifloral	18	10.16±5.85	8.60±5.15	14.05±2.31
Honeydew	20	8.21±4.27	7.74±2.30	13.33±1.37
Heather	10	7.50±4.53	7.03±4.55	12.04±2.70
Lavender	4	16.41±5.54	14.06±5.78	10.52±1.88
Leguminosae	4	12.89±7.02	11.72±4.68	11.01±2.30
p-value (95%)		0.1032	0.1817	0.0055

AD (Agar dilution), BD (Broth dilution), AWD (Agar well diffusion). Values are expressed as Mean ± Standard deviation

Our results contrast with those obtained by Pérez-Martín *et al.* (2008) who, after studying floral and honeydew honeys, described that the former exhibited higher antimicrobial activities against *Staphylococcus aureus* than the latter. Our low values of antibacterial capacity found for lavender honeys were in concordance with the results reported by Alzahrani *et al.* (2012) and Mundo *et al.* (2004) for lavender Saudi Arabian and USA honeys, respectively. However, our results do not agree with those of Henriques *et al.* (2005), where *Lavandula* sp. honeys showed high antimicrobial activity, probably because these honeys came from *Lavandula stoechas*, which has higher concentrations of coumarin, an intermediate cinnamic acid which possesses antibacterial activity (Aljadi and Yusoff, 2003), than other *Lavandula* sp. honeys. In this study two of the lavender samples were "lavandin" honeys (*Lavandula angustifolia* x *latifolia*) and the other two were *Lavandula* sp. (mainly *Lavandula latifolia*), which have lower antimicrobial activity.

REFERENCES

AKKOL, E; YESXILADA, E; GÜVENC, A (2007) Valuation of anti-inflammatory and antinociceptive activities of Erica species native to Turkey. *Journal of Ethnopharmacology* 116(2): 251-257. http://dx.doi.org/10.1016/j.jep.2007.11.023

ALJADI, A; YUSOFF, K M (2003) Isolation and identification of phenolic acids in Malaysian honey with antibacterial properties. *Turkish Journal of Medicine* 33: 229-236.

ALLEN, K L; MOLAN, P C; REID, G M (1991) A survey of the antibacterial activity of some New Zealand Honeys. *Journal of Pharmacy and Pharmacology* 43(12): 817-822. http://dx.doi.org/10.1111/j.2042-7158.1991.tb03186.x

ALZAHRANI, H A; ALSABEHI, R; BOUKRAÂ, L; ABDELLAH, F; BELLIK, Y; BAKHOTMAH, B A (2012) Antibacterial and antioxidant potency of floral honeys form different botanical and geographical origins. *Molecules* 17(9): 10540-10549. http://dx.doi.org/10.3390/molecules170910540

BALTRUŠAITYTĖ, V; RIMANTAS-VENSKUTONIS, P; ČEKSTERYTĖ, V (2007) Antibacterial activity of honey and beebread of different origin against *S. aureus* and *S. epidermidis*. *Food Technology and Biotechnology* 45(2): 201-208.

BASUALDO, C; SGROY, V; FINOLA, M S; MARIOLI, J M (2007) Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Veterinary Microbiology* 124(3-4): 375-381. http://dx.doi.org/10.1016/j.vetmic.2007.04.039

BRUDZYNSKI, K; SJAARDA, C (2015) Antimicrobial peptides, jelleins of the major royal jelly protein 1, are responsible for the cell wall lytic and bactericidal activities of honey. *PLoS ONE* 10(4): e0120238. http://dx.doi.org/10.1371/journal.pone.0120238

CHEN, C; CAMPBELL, L T; BLAIR, S E; CARTER, D A (2012) The effect of standard heat and filtration processing procedures on antimicrobial activity and hydrogen peroxide levels in honey. *Frontiers in microbiology* 3: Article 265. http://dx.doi.org/10.3389/fmicb.2012.00265

COOPER, K E (1963) The theory of antibiotic inhibition zones. In F. Kavanagh (Ed.), *Analytical Microbiology* (pp. 1-86). Academic Press, New York.

DUSTMANN, J H (1979) Antibacterial effect of honey. *Apiacta* 14(1): 7-11.

EFSA (2015) Scientific report of EFSA and ECDC. EU summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2013. *European Food Safety Authority Journal* 13 (2): 4036.

ELBANNA, K; ATTALLA, K.; ELBADRY, M; ABDELTAWAB, A; GAMAL-ELDIN, H; RAMADAN, M F (2014) Impact of floral sources and processing on the antimicrobial activities of different unifloral honeys. *Asian Pacific Journal of Tropical Disease* 4(3): 194-200. http://dx.doi.org/10.1016/S2222-1808(14)60504-1

ESCUREDO, O; SILVA, L R; VALENTÃO, P; SEIJO, M C; ANDRADE, P B (2012) Assessing Rubus honey value: Pollen and phenolic compounds content an antibacterial capacity. *Food Chemistry* 130(3): 671-678. http://dx.doi.org/10.1016/j.foodchem.2011.07.107

ESTRADA, H; GAMBOA, M M; CHAVES, C; ARIAS, M L (2005) Evaluación de la actividad antimicrobiana de la miel de abeja contra *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli, Salmonella enteritidis, Listeria monocytogenes* y *Aspergillus niger*. Evaluación de su carga microbiológica. *Archivos Latinoamericanos de Nutrición* 55(2): 167-171.

FEÁS, X; IGLEISAS, A; RODRIGUES, S; ESTEVINHO, L M (2013) Effect of Erica spp. Honey against microorganisms of clinical importance: study of the factors underlying this biological activity. *Molecules* 18(4): 4233-4246. http://dx.doi.org/10.3390/molecules18044233

FIDALEO, M; ZUORRO, A; LAVECCHIA, R (2011) Antimicrobial activity of some italian honeys against pathogenic bacteria. *Chemical Engineering Transactions* 24: 1015-1020. http://dx.doi.org/10.3303/CET1124170

HENRIQUES, A; BURTON, N F; COOPER, R A (2005) Antibacterial activity of selected Portugueses honeys. *Journal of Apicultural Research* 44 (3): 119-123. http://dx.doi.org/10.1080/00218839.2005.11101161

HURTADO, M P; DE LA PARTE, M A; BRITO, A (2002) *Staphylococcus aureus*: Revisión de los mecanismos de patogenicidad y la fisiopatología de la infección estafilocócica. *Revista de la Sociedad Venezolana de Microbiología* 22(2): 112-118.

IRISH, J; BLAIR, S; CARTER, D A (2011) The antibacterial activity of honey derived from Australian flora. *PloS ONE* 6(3): e18229. http://dx.doi.org/10.1371/journal.pone.0018229

JAMES, O B O; SEGREE, W; VENTURA, A K (1972) Some antibacterial properties of Jamaican honeys. *West Indian Medical Journal* 21(7): 7-17.

KATO, Y; UMEDA, N; MAEDA, A; MATSUMOTO, D; KITAMOTO, N; KIKUZAKI, H (2012) Identification of a novel glycoside, leptosin, as a chemical marker of manuka honey. *Journal of Agricultural and Food Chemistry* 60(13): 3418-3423. http://dx.doi.org/10.1021/jf300068w

KRUSHNA, N S A; KOWSALYA, A; RADHA, S; NARAYANAN, R B (2007) Honey as a natural preservative of milk. *Indian Journal of Experimental Biology* 45(5): 459-464.

KWAKMAN, P H S; ZAAT, S A J (2012) Antibacterial Components of honey. *IUBMB Life* 64(1): 48-55. http://dx.doi.org/10.1002/iub.578

LEE, H; CHUREY, J J; WOROBO, R W (2008) Antimicrobial activity of bacterial isolates from different floral sources of honey. *International Journal of Food Microbiology* 126(1-2): 240-244. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.04.030

LÓPEZ-MALO, A; PALOU, E; PARISH, M E; DAVIDSON, P M (2005) Methods for activity assay and evaluation of results. In: P.M. Davidson, J.N. Sofos, & A.L. Branene, (Eds.), *Antimicrobials in Food* (pp. 659-680). Taylor & Francis Group, Boca Raton, FL, USA.

LOUVEAUX, J; MAURIZIO, A; VORWOHL, G (1978) Methods of Melissopalynology. *Bee World* 59(4): 139-157. http://dx.doi.org/10.1080/0005772X.1978.11097714

MALIK, A H; SHARMA, B D (2010) Comparison of hurdle treatments for buffalo meat. *International Journal of Food Science & Technology* 45 (8): 1552-1563. http://dx.doi.org/10.1111/j.1365-2621.2010.02291.x

MANYI-LOH, C E; CLARKE, A M; NDIP, R N (2011) An overview of honey: Therapeutic properties and contribution in nutrition and human health. *African Journal of Microbiology* 5(8): 844-852. http://dx.doi.org/10.5897/AJMR10.008

MAVRIC, E; WITTMANN, S; BARTH, G; HENLE, T (2008) Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. *Molecular Nutrition & Food Research* 52(4): 483-489. http://dx.doi.org/10.1002/mnfr.200700282

MOLAN, P C (1999) The role of honey in the management of wounds. *Journal of Wound Care* 8(8): 415-418.

MOLAN, P C; RUSSELL, K M (1988) Non-peroxide antibacterial activity in some New Zealand honeys. *Journal of Apicultural Research* 27(1): 62-67. http://dx.doi.org/10.1080/00218839.1988.11100783

MUNDO, M A; PADILLA-ZAKOUR, O I; WOROBO, R W (2004) Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *International Journal of Food Microbiology* 97(1): 1-8. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.025

OELSCHLAEGEL, S; GRUNER, M; WANG, P N; BOETTCHER, A; KOELLING-SPEER, I; SPEER, K (2012a) Classification and characterization of manuka honeys based on phenolic compounds and methylglyoxal. *Journal of Agricultural and Food Chemistry* 60(29): 7229-7237. http://dx.doi.org/10.1021/jf300888q

OELSCHLAEGEL, S; PIEPER, L; STAUFENBIEL, R; GRUNER, M; ZEIPPERT, L; PIEPER, B; KOELLING-SPEER, I; SPEER, K (2012b) Floral markers of cornflower (*Centaurea cyanus*) honey and its peroxide antibacterial activity for an alternative treatment of digital dermatitis. *Journal of Agricultural and Food Chemistry* 60 (47): 11811-11820. http://dx.doi.org/10.1021/jf303699t

PATTON, T; BARRETT, J; BRENNAN, J; MORAN, N (2006) Use of a spectrosphotometric bioassay for determination of microbial sensitivity to manuka honey. *Journal of Microbiological Methods* 64: 84-95. http://dx.doi.org/10.1016/j.mimet.2005.04.007

PÉREZ-MARTÍN, R A; VELA-HORTIGÜELA, L; LORENZO-LOZANO, P; ROJO-CORTINA, M D; DE LORENZO-CARRETERO, C (2008) In vitro antioxidant and antimicrobial activities of Spanish honeys. *International Journal of Food Properties* 11(4): 727-737. http://dx.doi.org/10.1080/10942910701586257

REVATHY, V; BANERJI, S A (1980) A preliminary study of antibacterial properties of Indian honey. *Indian Journal of Biochemistry and Biophysics* 17(242): 62.

SHERLOCK, O; DOLAN, A; ATHMAN, R; POWER, A; GETHIN, G; COWMAN, S; HUMPHREYS, H (2010) Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, Escherichia coli and Pseudomonas aeruginosa. BMC Complementary and Alternative. *Medicine* 10: 47. http://dx.doi.org/10.1186/1472-6882-10-47

SYDNOR, E R M; PERL, T M (2011) Hospital epidemiology and infection control in acute-care settings. *Clinical Microbiology Reviews* 24 (1): 141-173. http://dx.doi.org/10.1128/CMR.00027-10

TAORMINA, P J; NIEMIRA, B A; BEUCHAT, L R (2001) Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. *International Journal of Food Microbiology* 69(3): 217-225. http://dx.doi.org/10.1016/S0168-1605(01)00505-0

TENOVER, F C (1986) Studies of antimicrobial resistance genes using DNA probes. *Antimicrobial Agents Chemotherapy* 29(5): 721-725.

TERRADILLOS, L A; MUNIATEGUI, S; SANCHO, M T; HUIDOBRO, J F; SIMAL-LOZANO, J (1994) An alternative method for analysis of honey sediment. *Bee Science* 3(2): 86-93.

THE NATIONAL HONEY BOARD (2002) Honey-Health and Therapeutic Qualities. 390 Lashley Street Longmont, CO 80501-6045, USA.

VOIDAROU, C; ALEXOPOULOS, A; PLESSAS, S; KARAPANOU, A; MANTZOURANI, I; STAVROPOULOU, E; FOTOU, K; TZORA, A; SKOUFOS, I; BEZIRTZOGLOU, E (2011) Antibacterial activity of different honeys against pathogenic bacteria. *Anaerobe* 17(6): 375-379. http://dx.doi.org/10.1016/j.anaerobe.2011.03.012

VON DER OHE, W; PERESANO-ODDOL, L; PIANA, M L; MORLOT, M; MARTIN, P (2004) Harmonized methods of melissopalinology. *Apidologie* 35(1): S18-S25. http://dx.doi.org/10.1051/apido:2004050

CONCLUSIONS

CONCLUSIONS

ABOUT THE CURRENT EUROPEAN REGULATIONS

1. All the analyzed honeys from Castilla y León (Spain) fulfilled the current European regulations with regard to hydroxymethylfurfural, diastase, moisture, free acidity, electrical conductivity, sum of glucose and fructose and sucrose content.

ABOUT THE ANALYTICAL PROCEDURES

2. Honeys' botanical origins were established on the basis of melissopalinology, sensory and such useful physicochemical analyses as electrical conductivity and pH.
3. The analysis of honeys' total flavonoids in neutral media must be done after removing interferences and carrying out a sample's colour correction.
4. Six minutes was proposed as a suitable time to measure trolox equivalent antioxidant capacity (TEAC) values, because significant linear relationships were found among the TEAC results determined at different times. Therefore, both analysis time and costs would be reduced.
5. Honeys' antibacterial activity against *Staphylococcus aureus* should be measured first by agar well diffusion procedure, in order to know which samples exhibit antimicrobial activity, and then, by broth dilution assay to obtain the minimal antimicrobial and bactericidal concentrations.

ABOUT THE CHARACTERIZATION RESULTS

Physicochemical parameters

6. Chestnut and honeydew honeys were characterized for the highest electrical conductivity and pH mean values, high free acidity, and low lactone acidity as well as lactone/free acidity. Moreover, honeydew honeys had the highest optical rotation and proline averages, whereas chestnut samples possessed high moisture contents and proline concentrations.
7. Heather samples had the highest lactone acidity and lactone/free acidity averages, high free acidity, and the lowest specific rotation mean value.
8. Lavender and clover samples possessed the lowest electrical conductivity and pH mean data. Clover honeys showed the highest moisture as well as free and total acidity mean values, high lactone acidity, lactone/free acidity and proline results. Conversely, lavender samples had the lowest moisture, free and total acidity averages, as well as low lactone acidity and proline contents.

Sugars' profile and sugar-related parameters

9. Fourteen sugars were quantified: two monosaccharides (fructose and glucose), five disaccharides (sucrose, trehalose, maltose, gentiobiose and isomaltose), six trisaccharides (raffinose, erlose, melezitose, maltotriose, panose and isomaltotriose) and one tetrasaccharide (maltotetrose). Fructose was the main sugar followed by glucose in all samples. Maltose and isomaltose were the main disaccharides and erlose and melezitose the main trisaccharides.
10. Honeydew and chestnut honeys had the lowest fructose and glucose, and the highest isomaltose averages. Chestnut samples possessed the lowest sucrose, trehalose and maltotriose mean values and low trisaccharides concentration, whereas honeydew honeys showed the highest disaccharides and trisaccharides averages, and the highest mean concentration of the main trisaccharides considered indicators of the presence of honeydew (erlose, melezitose and raffinose).
11. Heather, lavender and clover honeys possessed the highest monosaccharide averages. Heather honeys presented the lowest disaccharides and trisaccharides mean values, whereas clover and lavender samples had high disaccharide concentrations, showing the highest maltose mean data, high erlose values and the lowest melezitose averages. Conversely, heather samples possessed the lowest maltose and erlose mean concentrations. Moreover, lavender samples were characterized for the highest sucrose and the lowest isomaltose and raffinose mean contents.
12. In respect of crystallization tendencies, lavender honeys showed the fastest granulation tendency, whereas chestnut and honeydew honeys exhibited the slowest one.

Antioxidant-related parameters

13. Regarding the phenolic profiles, fourteen compounds were quantified: six phenolic acids (chlorogenic, caffeic, ellagic, coumaric, sinapic and ferulic) and eight flavonoids (rutin, luteolin, quercetin, naringenin, kaempferol, chrysin, pinocembrin and galangin). Ellagic acid was the most abundant phenolic compound, followed by the flavonoid pinocembrin.
14. The highest ellagic acid average was found in honeydew honeys. Moreover, they also possessed the highest naringenin mean content, high coumaric acid and galangin concentrations, the lowest luteolin average and low quercetin and kaempferol values.
15. Chestnut samples had the highest coumaric acid, ferulic acid, luteolin and galangin mean contents, high ellagic acid content, the lowest quercetin and kaempferol averages and low pinocembrin and chrysin concentrations.
16. Heather honeys were characterized by the highest pinocembrin and chyrsin mean concentrations, high ellagic acid content and low coumaric acid values.

17. Clover samples presented high caffeic acid, quercetin and kaempferol contents, the lowest chlorogenic and coumaric acids averages, as well as the lowest pinocembrin and chrysin mean values. Neither ferulic acid nor galangin were detected in these honeys.
18. Lavender honeys possessed the highest mean concentrations of chlorogenic acid, quercetin and kaempferol, low pinocembrin and chrysin data, and the lowest rutin, ellagic and caffeic acids' averages.
19. Neither luteolin or naringenin for lavender honeys, nor ellagic acid for heather honeys, proved to be suitable markers for their botanical characterization. On the other hand, chestnut honeys possessed the highest average for the sum of all hydroxycinnamic-related compounds (coumaric, caffeic and ferulic acids), offering the possibility that these compounds could be characteristic for this kind of honey.
20. Lavender was the only unifloral honey type that had higher flavonoid than phenolic acids' average.
21. Dark honeys, with the lowest lightness and the highest reddish tones, possessed higher TEAC, total phenolics, phenolic acids and ellagic acid mean values, and lower quercetin and kaempferol averages than light honeys.
22. A significant correlation was found between caffeic and ferulic acids. Pinocembrin was correlated with chrysin, and both flavonoids together with quercetin, were correlated with total flavonoids measured by HPLC.
23. No correlations were found among the results of total flavonoids with total phenolics and TEAC.
24. With regard to the analyses on honeys and extracts, high and moderate significant correlations were found between TEAC values and total phenolic contents, respectively.

Antiomicrobial activity

25. The highest averages of antimicrobial activity against *Staphylococcus aureus* were found in honeydew, multifloral and heather honeys. Conversely, lavender samples showed the lowest one.

ABOUT HONEY CLASSIFICATION ACCORDING TO THEIR BOTANICAL ORIGINS

26. Bioactive compounds and other antioxidant-related parameters proved to be more suitable to classify the samples according to their botanical origins than sugars and other physicochemical features.

CONCLUSIONES

SOBRE LAS ACTUALES REGULACIONES EUROPEAS

1. Todas las mieles producidas en Castilla y León (España) cumplieron con las actuales regulaciones Europeas respecto a los parámetros de hidroximetilfurfural, diastasas, humedad, acidez libre, conductividad eléctrica, suma de glucosa y fructosa, y contenido en sacarosa.

SOBRE LOS PROCEDIMIENTOS ANALÍTICOS

2. El origen botánico de las mieles fue establecido en base de análisis melisopalinológicos, sensoriales y con la ayuda de otros análisis fisicoquímicos tales como la conductividad eléctrica y el pH.
3. El análisis del contenido total de flavonoides en medio neutro debe ser realizado después de eliminar las interferencias, llevando a cabo la corrección de color de las mieles.
4. El tiempo adecuado propuesto para la medida de la capacidad antioxidante equivalente de trolox (TEAC) fue de seis minutos, ya que se encontró una elevada correlación entre los valores de TEAC determinados a distintos tiempos. Con ello, tanto el tiempo de análisis como los costes podrían reducirse.
5. La actividad antibacteriana de la miel frente *Staphylococcus aureus* debería ser medida en primer lugar mediante el procedimiento de difusión en agar, con el objetivo de saber que muestras poseen actividad antimicrobiana, y posteriormente, aplicar el ensayo de dilución en caldo para obtener las concentraciones mínimas antimicrobianas y bactericidas.

SOBRE LOS RESULTADOS DE CARACTERIZACIÓN

Parámetros fisicoquímicos

6. Las mieles de castaño y mielada se caracterizaron por los mayores promedios de conductividad eléctrica y pH, una elevada acidez libre, y bajos valores de acidez lactónica y lactonas/acidez libre. Además, las mieles de mielada poseyeron los valores medios más elevados de rotación específica y de prolina, mientras que las muestras de castaño tuvieron contenidos elevados de humedad y de prolina.
7. Las mieles de brezo mostraron los mayores promedios de acidez lactónica y de lactonas/acidez libre, una elevada acidez libre y el valor medio de rotación específica más bajo.

8. Las muestras de lavanda y trébol poseyeron los valores medios más bajos de conductividad eléctrica y pH. Las mieles de trébol mostraron los promedios de humedad, acidez libre y acidez total más elevados, y unos valores altos de acidez lactónica, lactonas/acidez libre y prolina. Por otro lado, las mieles de lavanda poseyeron los promedios más bajos de humedad, acidez libre y acidez total, además de mostrar valores bajos de acidez lactónica y prolina.

Perfiles de azúcares y parámetros relacionados

9. Se cuantificaron catorce azúcares: dos monosacáridos (fructosa y glucosa), cinco disacáridos (sacarosa, trealosa, maltosa, gentiobiosa e isomaltosa), seis trisacáridos (rafinosa, erlosa, melecitosa, maltotriosa, panosa e isomaltotriosa) y un tetrasacárido (maltotetrosa). En todas las muestras el principal azúcar fue la fructosa, seguido de la glucosa. Los disacáridos más importantes fueron la maltosa y la isomaltosa, mientras que la erlosa y la melecitosa fueron los trisacáridos más abundantes.
10. Las mieles de mielada y castaño tuvieron las concentraciones medias más bajas de fructosa y glucosa, y las más elevadas de isomaltosa. Las muestras de castaño poseyeron los contenidos medios más bajos de sacarosa, trealosa y maltotriosa, y una baja concentración de trisacáridos, mientras que las mieles de mielada mostraron los promedios más elevados de disacáridos, trisacáridos, y de los principales azúcares considerados indicadores de la presencia de mielada.
11. Las mieles de brezo, lavanda y trébol poseyeron los promedios más elevados de monosacáridos. Las mieles de brezo presentaron los menores valores medios de disacáridos y trisacáridos, mientras que las mieles de trébol y lavanda tuvieron una elevada concentración en disacáridos, mostrando los valores medios más elevados de maltosa, elevados contenidos de erlosa, y los promedios más bajos de melecitosa. Por el contrario, las mieles de brezo poseyeron las concentraciones medias más bajas de maltosa y erlosa. Además, las muestras de lavanda se caracterizaron por el valor medio de sacarosa más elevado y los bajos contenidos de isomaltosa y rafinosa.
12. Con respecto a la tendencia de cristalización, las mieles de lavanda mostraron la tendencia a la granulación más elevada, siendo lo contrario en las mieles de castaño y mielada.

Parámetros relacionados con la actividad antioxidante

13. En cuanto al perfil de polifenoles, se identificaron catorce compuestos: seis ácidos fenólicos (clorogénico, cafeico, elágico, cumárico, sinápico y ferúlico) y ocho flavonoides (rutina, luteolina, quercetina, naringenina, canferol, crisina, pinocembrina y galangina). El ácido elágico fue el compuesto más abundante en todas las mieles estudiadas, seguido del flavonoide pinocembrina.

14. El mayor contenido medio en ácido elágico se encontró en las mieles de mielada. Además, estas mieles también poseyeron el mayor promedio de naringenina, elevados valores de ácido cumárico y galangina, el valor medio más bajo de luteolina y una baja concentración de quercetina y canferol.
15. Las muestras de castaño obtuvieron las mayores concentraciones medias de los ácidos cumárico y ferúlico y de los flavonoides luteolina y galangina, un elevado contenido de ácido elágico, los promedios más bajos de quercetina y canferol y una baja concentración de pinocembrina y crisina.
16. Las mieles de brezo se caracterizaron por los valores medios más elevados de pinocembrina y crisina, un contenido elevado de ácido elágico y una concentración baja de ácido cumárico.
17. Las mieles de trébol presentaron un contenido elevado de ácido cafeico, quercetina y canferol, y las concentraciones medias más bajas de los ácidos clorogénico y cumárico, así como de los flavonoides pinocembrina y crisina. Ni el ácido ferúlico ni la naringenina fueron detectados en estas muestras.
18. Las mieles de lavanda poseyeron los promedios más elevados del ácido clorogénico, quercetina y canferol, bajos contenidos de pinocembrina y crisina, y los menores valores medios de los ácidos elágico y cafeico, y del flavonoide rutina.
19. Ni la luteolina ni la naringenina para las mieles de lavanda, ni el ácido elágico para las mieles de brezo, demostraron ser marcadores válidos para su caracterización botánica. Por otro lado, las mieles de castaño poseyeron los valores más elevados de la suma de los compuestos hidroxicinámicos (los ácidos cumárico, cafeico y ferúlico), pudiendo ser compuestos característicos de este tipo de miel.
20. La miel de lavanda fue el único tipo de miel con un mayor promedio en flavonoides que en ácidos fenólicos.
21. Las mieles oscuras, con la menor luminosidad y las mayores tonalidades rojizas, poseyeron mayores valores medios de TEAC, fenoles totales, ácidos fenólicos y de ácido elágico, y menores promedios de quercetina y canferol que las mieles claras.
22. Se encontró una correlación significativa entre el ácido cafeico y el ácido ferúlico. Además, los valores de pinocembrina se correlacionaron con los de crisina, y ambos flavonoides junto con la quercetina, se correlacionaron con el contenido total de flavonoides medido por HPLC.
23. Los flavonoides totales no estuvieron correlacionados ni con los fenoles totales ni con los valores de TEAC.

24. Con respecto a los análisis realizados en la miel y en los extractos, se encontró una elevada y una moderada correlación significativa entre los valores de TEAC y los valores de fenoles totales, respectivamente.

Actividad antimicrobiana

25. Los valores medios más elevados de actividad antimicrobiana frente *Staphylococcus aureus* fueron encontrados en las mieles de mielada, milflores y brezo, mientras que los más bajos fueron observados en las mieles de lavanda.

SOBRE LA CLASIFICACIÓN DE LAS MIELES SEGÚN SUS ORÍGENES BOTÁNICOS

26. Los compuestos bioactivos y otros parámetros relacionados con la actividad antioxidante demostraron ser más adecuados que los azúcares y los parámetros fisicoquímicos para la clasificación de las mieles por su origen botánico.

FUTURE PROSPECTS

FUTURE PROSPECTS

The future prospects linked to this PhD Thesis have the purposes of first, continuing to characterize the honeys from Castilla y León; second, developing new foods with beehive products; and finally, setting up new tools for honeys' multi-component analysis. Within these general prospects, the research lines on a short-term basis are:

- Study of the natural volatile and semivolatile profiles of honeys from Castilla y León. This will help have more data for their characterization, which is key to obtain a Protected Designation of Origin (PDO) that would increase the value of Castilla y León honeys.
- Development of new food commodities made with beehive products among their ingredients, which would boost the beekeeping sector. Up to now the research group "Quality, Characterization and Aging of Honey" of the University of Burgos has already designed a food product with honey and small amounts of soft propolis extract, verifying its interesting functional properties (Annex 2, Article 2), some of which having been assessed with the procedure set up for honeys in this PhD Thesis. Thus, it is interesting to continue to working on this research line.
- Honeys' characterization by setting up voltammetric techniques with different working electrodes. In this regard, electronic tongues have recently proved to be simple, fast and low-cost alternatives to traditional analytical methods. These systems combine electrochemical techniques with multivariate analysis, with the purposes of classifying samples and/or quantifying their physicochemical properties. A collaborative study about a prediction model to determine antioxidant activity of Spanish honeys by using different potentiometric tongues (Juan-Borrás *et al.*, LWT - Food Science and Technology, *submitted*) was made in a research stay in the Universitat Politécnica of Valencia. Voltamperometric tongues seem to be better tools than potentiometric ones, because they provide with more information, so that they are potentially more suitable to characterize honeys.

PERSPECTIVAS FUTURAS

Las perspectivas futuras ligadas a esta Tesis Doctoral tienen por objeto continuar con la caracterización de las mieles de Castilla y León, desarrollar nuevos alimentos a partir de productos de la colmena y poner a punto nuevas herramientas de análisis multicomponente en mieles. Dentro de estas perspectivas generales, a corto plazo las líneas de investigación planteadas son las siguientes:

- Estudio del perfil de sustancias naturales volátiles y semivolátiles en las mieles castellano-leonesas. Con ello se dispondría de un mayor número de datos para su caracterización, lo que es importante a la hora de obtener una Denominación de Origen Protegida que valorizaría las mieles de Castilla y León.
- Desarrollo de nuevos alimentos entre cuyos ingredientes se encuentren los productos de la colmena. Esto impulsaría la apicultura, siendo importante para el sector. El grupo de investigación "Calidad, tipificación y envejecimiento de la miel" de la Universidad de Burgos ya ha diseñado un alimento a base de miel y pequeñas concentraciones de extracto blando de propóleos (Anexo 2, Artículo 2), comprobando que presenta interesantes propiedades funcionales, algunas de las cuales se han evaluado con los métodos puestos a punto para las mieles analizadas en esta Tesis Doctoral. Por ello, resulta de interés continuar con esta línea de trabajo.
- Contribuir a la caracterización de las mieles mediante la puesta a punto de técnicas voltamperométricas con diferentes electrodos de trabajo. En este sentido hay que indicar que las lenguas electrónicas han demostrado recientemente ser una alternativa simple, rápida y económica a los métodos tradicionales de análisis. Estos sistemas combinan técnicas electroquímicas con análisis multivariantes con el objetivo de clasificar muestras o cuantificar sus propiedades fisicoquímicas. En una estancia de investigación realizada en la Universidad Politécnica de Valencia se colaboró en un trabajo consistente en establecer un modelo de predicción para la determinación de la actividad antioxidante de mieles españolas mediante el uso de distintas lenguas potenciométricas (Juan-Borrás *et al*., LWT - Food Science and Technology, *enviado*). Las lenguas voltamperométricas parecen ser herramientas más potentes que las potenciométricas, proporcionando en un solo barrido mucha más información, por lo que son potencialmente más adecuadas para la caracterización de mieles.

ANNEXES

ANNEX 1

TABLES

TABLES

TABLE 1

Literature data of sugars and other physicochemical parameters

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CHESTNUT	HMF (mg/kg)	2.00±1.60	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	180
		4.50±1.80	*Castanea sativa*	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	13
		1.25±0.85	*Castanea sativa*	Golob and Plestenjak, 1999	Slovenia	1996	14
		2.65±2.12	*Castanea sativa*	Devillers *et al.*, 2004	France	-	62
		1.94±0.16	*Castanea sativa*	Marini *et al.*, 2004	Italy	-	13
		28.60±1.90	*Castanea sativa*	Küçük *et al.*, 2007	Turkey	-	1
		4.80±4.53	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2003	7
		3.50±2.85	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2004	7
		29.50±31.90	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2005	14
		4.64±2.39	*Castanea sativa*	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	11
		0.74±0.07	*Castanea sativa*	Truzzi *et al.*, 2012	Italy	2009	6
		9.28±7.13	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	Diastase (º Schade)	23.90±5.00	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	180
		32.30±8.91	*Castanea sativa*	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	13
		17.45±2.54	*Castanea sativa*	Golob and Plestenjak, 1999	Slovenia	1996	14
		23.29±3.75	*Castanea sativa*	Devillers *et al.*, 2004	France	-	62
		24.47±0.88	*Castanea sativa*	Marini *et al.*, 2004	Italy	-	13
		24.30±5.70	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	298
		17.70±1.40	*Castanea sativa*	Küçük *et al.*, 2007	Turkey	-	1
		29.90±3.64	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2003	7
		20.70±4.25	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2004	7
		24.50	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2005	14
		27.30±0.90	*Castanea sativa*	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	11
		9.12±2.99	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	Moisture (%)	17.4±0.9	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	180
		16.3±0.7	*Castanea sativa*	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	13
		15.4±0.7	*Castanea sativa*	Golob and Plestenjak, 1999	Slovenia	1996	14
		18.8±0.9	*Castanea sativa*	Devillers *et al.*, 2004	France	-	62
		16.9±0.6	*Castanea sativa*	Marini *et al.*, 2004	Italy	-	13
		17.5±1.2	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	210

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CHESTNUT	Moisture (%)	18.0±1.0	*Castanea sativa*	Escuredo *et al.*, 2013	Northwest of Spain	-	34
		18.5±0.0	*Castanea sativa*	Fallico *et al.*, 2004	Italy	2001	1
		19.7±1.7	*Castanea sativa*	Küçük *et al.*, 2007	Turkey	-	1
		16.9±1.0	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2003	7
		16.6±1.8	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2004	7
		16.6±1.4	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2005	14
		15.9±0.9	*Castanea sativa*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	29
		16.1±0.9	*Castanea sativa*	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	11
		17.4±0.1	*Castanea sativa*	Truzzi *et al.*, 2012	Italy	2009	6
		16.5±1.0	*Castanea sativa*	Scandurra *et al.*, 2013	Italy	-	5
		17.2±0.0	*Castanea sativa*	Anjos *et al.*, 2015	Portugal and Spain	-	1
		19.7±1.3	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	Electrical conductivity (mS/cm)	1.410±0.240	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	180
		1.540±0.320	*Castanea sativa*	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	13
		1.483±0.345	*Castanea sativa*	Golob and Plestenjak, 1999	Slovenia	1996	14
		1.308±0.363	*Castanea sativa*	Devillers *et al.*, 2004	France	-	62
		1.480±0.200	*Castanea sativa*	Marini *et al.*, 2004	Italy	-	13
		1.380±0.270	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	406
		1.128±0.001	*Castanea sativa*	Fallico *et al.*, 2004	Italy	2001	1
		1.270±0.317	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2003	7
		1.040±0.300	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2004	7
		1.505	*Castanea sativa*	Kropf *et al.*, 2010	Slovenia	2004-2006	37
		1.610±0.240	*Castanea sativa*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	29
		1.060±0.160	*Castanea sativa*	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	11
		1.188±0.008	*Castanea sativa*	Truzzi *et al.*, 2012	Italy	2009	6
		1.500±0.041	*Castanea sativa*	Scandurra *et al.*, 2013	Italy	-	5
		0.640±0.110	*Castanea sativa*	Anjos *et al.*, 2015	Portugal and Spain	-	1
		1.500±0.310	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CHESTNUT	pH	5.50±0.40	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	180
		4.90±0.19	*Castanea sativa*	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	13
		5.39±0.57	*Castanea sativa*	Golob and Plestenjak, 1999	Slovenia	1996	14
		5.28±0.46	*Castanea sativa*	Devillers *et al.*, 2004	France	-	62
		5.22±0.26	*Castanea sativa*	Marini *et al.*, 2004	Italy	-	13
		5.30±0.50	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	365
		5.90±0.01	*Castanea sativa*	Fallico *et al.*, 2004	Italy	2001	1
		5.43	*Castanea sativa*	Kropf *et al.*, 2010	Slovenia	2004-2006	37
		5.51±0.42	*Castanea sativa*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	29
		4.62±0.34	*Castanea sativa*	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	11
		5.42±0.02	*Castanea sativa*	Truzzi *et al.*, 2012	Italy	2009	6
		4.30±0.80	*Castanea sativa*	Scandurra *et al.*, 2013	Italy	-	5
		3.90	*Castanea sativa*	Anjos *et al.*, 2015	Portugal and Spain	-	1
	Free acidity (meq/kg)	12.2±2.5	*Castanea sativa*	Devillers *et al.*, 2004	France	-	62
		14.2±1.1	*Castanea sativa*	Marini *et al.*, 2004	Italy	-	13
		13.0±3.5	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	263
		9.7±0.3	*Castanea sativa*	Fallico *et al.*, 2004	Italy	2001	1
		12.6	*Castanea sativa*	Kropf *et al.*, 2010	Slovenia	2004-2006	37
		13.3±4.7	*Castanea sativa*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	29
		45.0±13.8	*Castanea sativa*	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	11
		12.0±0.7	*Castanea sativa*	Truzzi *et al.*, 2012	Italy	2009	6
		27.0±5.0	*Castanea sativa*	Scandurra *et al.*, 2013	Italy	-	5
		33.6±0.4	*Castanea sativa*	Anjos *et al.*, 2015	Portugal and Spain	-	1
	Lactones (meq/kg)	1.9±0.5	*Castanea sativa*	Marini *et al.*, 2004	Italy	-	13
		3.1±2.4	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	233
		1.7±0.3	*Castanea sativa*	Fallico *et al.*, 2004	Italy	2001	1
		2.4	*Castanea sativa*	Kropf *et al.*, 2010	Slovenia	2004-2006	37
		2.5±1.6	*Castanea sativa*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	29
		3.3±0.7	*Castanea sativa*	Truzzi *et al.*, 2012	Italy	2009	6
		5.1±0.3	*Castanea sativa*	Scandurra *et al.*, 2013	Italy	-	5

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CHESTNUT	Total acidity (meq/kg)	13.8±3.8	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	180
		17.5±4.9	*Castanea sativa*	Golob and Plestenjak, 1999	Slovenia	1996	14
		16.2±1.0	*Castanea sativa*	Marini *et al.*, 2004	Italy	-	13
		16.1±4.1	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	233
		11.4±0.7	*Castanea sativa*	Fallico *et al.*, 2004	Italy	2001	1
		36.7±1.9	*Castanea sativa*	Küçük *et al.*, 2007	Turkey	-	1
		12.4±4.7	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2003	7
		11.6±5.3	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2004	7
		14.9	*Castanea sativa*	Kropf *et al.*, 2010	Slovenia	2004-2006	37
		15.8±5.7	*Castanea sativa*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	29
		15.2±0.8	*Castanea sativa*	Truzzi *et al.*, 2012	Italy	2009	6
		32.1±6.5	*Castanea sativa*	Scandurra *et al.*, 2013	Italy	-	5
	Proline (mg/100 g)	60.39±3.69	*Castanea sativa*	Pirini *et al.*, 1992	Italy	-	6
		55.40±13.90	*Castanea sativa*	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	13
		59.23±9.53	*Castanea sativa*	Cotte *et al.*, 2004	France	-	38
		58.80±16.70	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	69
		56.87±10.56	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2003	7
		54.90	*Castanea sativa*	Kropf *et al.*, 2010	Slovenia	2004-2006	37
		55.80±10.80	*Castanea sativa*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	29
		61.90±8.00	*Castanea sativa*	Truzzi *et al.*, 2012	Italy	2009	6
		64.40±15.50	Chestnut	Czipa *et al.*, 2012	Hungary	-	5
		80.00±17.80	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
		94.63±0.94	Chestnut	Kivrak, 2015	Turkey	2013	3
	Specific rotation (°)	-17.00±3.50	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	180
		-16.11±0.73	*Castanea sativa*	Marini *et al.*, 2004	Italy	-	13
		-16.70±3.40	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	240
		-2.70±0.73	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2003	7
		-3.00±0.19	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2004	7
		-1.70	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2005	14
		-15.37	*Castanea sativa*	Kropf *et al.*, 2010	Slovenia	2004-2006	37
		-17.40±6.90	*Castanea sativa*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	29

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CHESTNUT	Specific rotation (º)	-21.00±3.00	*Castanea sativa*	Primorac *et al.*, 2011	Croatia	Different production seasons	17
		-2.73±1.55	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	Fructose (%)	41.90±2.10	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	85
		41.50±2.35	*Castanea sativa*	Golob and Plestenjak, 1999	Slovenia	1996	14
		40.72±2.28	Chestnut	Cotte *et al.*, 2003	France	-	38
		37.39±1.37	*Castanea sativa*	Devillers *et al.*, 2004	France	-	62
		36.80±0.90	*Castanea sativa*	Fallico *et al.*, 2004	Italy	2001	1
		40.17±0.12	*Castanea sativa*	Marini *et al.*, 2004	Italy	-	13
		40.80±2.60	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	276
		31.67±1.59	*Castanea sativa*	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	11
		42.00±2.80	*Castanea sativa*	Primorac *et al.*, 2011	Croatia	Different production seasons	17
		38.00±3.20	*Castanea sativa*	Escuredo *et al.*, 2013	Northwest of Spain	-	34
		38.44±2.72	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	Glucose (%)	26.40±1.40	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	85
		29.36±2.15	*Castanea sativa*	Golob and Plestenjak, 1999	Slovenia	1996	14
		26.52±2.07	Chestnut	Cotte *et al.*, 2003	France	-	38
		31.60±1.89	*Castanea sativa*	Devillers *et al.*, 2004	France	-	62
		25.10±0.80	*Castanea sativa*	Fallico *et al.*, 2004	Italy	2001	1
		25.88±0.20	*Castanea sativa*	Marini *et al.*, 2004	Italy	-	13
		27.90±2.50	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	273
		26.75±2.01	*Castanea sativa*	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	11
		27.40±2.40	*Castanea sativa*	Primorac *et al.*, 2011	Croatia	Different production seasons	17
		25.40±2.60	*Castanea sativa*	Escuredo *et al.*, 2013	Northwest of Spain	-	34
		19.35±3.00	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	Sucrose (%)	0.10±0.10	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	85
		0.77±0.23	*Castanea sativa*	Golob and Plestenjak, 1999	Slovenia	1996	6
		0.16±0.22	Chestnut	Cotte *et al.*, 2003	France	-	38
		0.25±0.28	*Castanea sativa*	Devillers *et al.*, 2004	France	-	62
		0.20±0.30	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	228

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CHESTNUT	Sucrose (%)	2.87±0.06	*Castanea sativa*	Küçük *et al.*, 2007	Turkey	-	1
		3.10±0.70	*Castanea sativa*	Šarić *et al.*, 2008	Croatia	2003	7
		1.10±1.42	*Castanea sativa*	Šarić *et al.*, 2008	Croatia	2004	7
		2.20±0.50	*Castanea sativa*	Primorac *et al.*, 2011	Croatia	Different production seasons	17
		0.27±0.50	*Castanea sativa*	Escuredo *et al.*, 2013	Northwest of Spain	-	34
		1.02±0.58	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	Trehalose (%)	2.05±0.66	Chestnut	Cotte *et al.*, 2003	France	-	38
		0.14±0.21	*Castanea sativa*	Escuredo *et al.*, 2013	Northwest of Spain	-	34
		0.09±0.01	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	Maltose (%)	0.80±0.50	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	85
		1.48±0.55	Chestnut	Cotte *et al.*, 2003	France	-	38
		2.40±1.10	*Castanea sativa*	Primorac *et al.*, 2011	Croatia	Different production seasons	17
		1.80±1.30	*Castanea sativa*	Escuredo *et al.*, 2013	Northwest of Spain	-	34
		0.53±0.12	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	Gentiobiose (%)	0.22±0.47	Chestnut	Cotte *et al.*, 2003	France	-	38
	Isomaltose (%)	2.10±0.80	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	85
		1.80±0.81	Chestnut	Cotte *et al.*, 2003	France	-	38
	Raffinose (%)	0.04±0.11	Chestnut	Cotte *et al.*, 2003	France	-	38
		0.22±0.25	*Castanea sativa*	Devillers *et al.*, 2004	France	-	62
		0.00	*Castanea sativa*	Primorac *et al.*, 2011	Croatia	Different production seasons	17
	Erlose (%)	0.24±0.20	Chestnut	Cotte *et al.*, 2003	France	-	38
		0.05±0.08	*Castanea sativa*	Devillers *et al.*, 2004	France	-	62
	Melezitose (%)	1.39±1.13	*Castanea sativa*	Golob and Plestenjak, 1999	Slovenia	1996	5
		0.42±0.31	*Castanea sativa*	Devillers *et al.*, 2004	France	-	62
		0.22±0.43	Chestnut	Cotte *et al.*, 2003	France	-	38
		0.08±0.13	*Castanea sativa*	Escuredo *et al.*, 2013	Northwest of Spain	-	34
		0.56±0.22	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CLOVER	Maltotriose (%)	0.19±0.13	Chestnut	Cotte *et al.*, 2003	France	-	38
	Panose (%)	0.21±0.11	Chestnut	Cotte *et al.*, 2003	France	-	38
	Total sugars (%)	74.40±3.70	*Castanea sativa*	Primorac *et al.*, 2011	Croatia	Different production seasons	17
		65.70±6.00	*Castanea sativa*	Escuredo *et al.*, 2013	Northwest of Spain	-	34
	Fructose+glucose	68.20±2.70	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	85
		70.85±3.94	*Castanea sativa*	Golob and Plestenjak, 1999	Slovenia	1996	14
		68.70±2.50	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	273
		66.80±6.20	*Castanea sativa*	Küçük *et al.*, 2007	Turkey	-	1
		73.10±2.49	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2003	7
		71.70±4.05	*Aesculus hippocastanum*	Šarić *et al.*, 2008	Croatia	2004	7
		69.50±4.20	*Castanea sativa*	Primorac *et al.*, 2011	Croatia	Different production seasons	17
		57.79	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	Fructose/glucose ratio	1.59±0.11	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	85
		1.42±0.10	*Castanea sativa*	Golob and Plestenjak, 1999	Slovenia	1996	14
		1.54±0.11	Chestnut	Cotte *et al.*, 2003	France	-	38
		1.48±0.19	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	273
		1.50±0.10	*Castanea sativa*	Primorac *et al.*, 2011	Croatia	Different production seasons	17
		1.98	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	Glucose/moisture ratio	1.51±0.13	*Castanea sativa*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	85
		1.62±0.13	*Castanea sativa*	Persano-Oddo and Piro, 2004	Europe	1970-2002	173
	HMF (mg/kg)	1.90	*Trifolium repens*	Abell *et al.*, 1996	Canada	-	1
		6.70±8.40	*Trifolium* sp.	Malacalza *et al.*, 2005	Argentina	-	3
		7.50±9.00	*Melilotus albus*	Malacalza *et al.*, 2005	Argentina	-	3
		4.21	Clover	Langford *et al.*, 2012	New Zealand	-	1
		1.23±0.18	*Trifolium alexandrinum*	Kamboj *et al.*, 2013	India	2009-2012	20
		35.13±0.00	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2006	1
		2.30±0.00	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2007	1
		10.92±7.95	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CLOVER	Diastase (º Schade)	6.80	*Medicago sativa*	Abu-Tarboush *et al.*, 1993	Saudi Arabia	-	1
		16.50	*Trifolium repens*	Abell *et al.*, 1996	Canada	-	1
		32.50	*Trifolium* sp.	Singh and Bath, 1997	India	1994-1995	1
		26.08±2.87	*Trifolium alexandrinum*	Kamboj *et al.*, 2013	India	2009-2012	20
		10.90±0.00	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2006	1
		7.90±1.30	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Moisture (%)	15.5	*Medicago sativa*	Abu-Tarboush *et al.*, 1993	Saudi Arabia	-	1
		16.7	*Trifolium repens*	Abell *et al.*, 1996	Canada	-	1
		18.7	*Trifolium* sp.	Singh and Bath, 1997	India	1994-1995	1
		18.7±0.5	*Trifolium alexandrinum*	Nanda *et al.*, 2003	India	-	30
		18.1±1.3	*Trifolium* sp.	Malacalza *et al.*, 2005	Argentina	-	3
		16.4±1.6	*Melilotus* sp.	Malacalza *et al.*, 2005	Argentina	-	3
		17.9±0.2	*Trifolium* sp.	Vanhanen *et al.*, 2011	New Zealand	2007	1
		17.0	Clover	Langford *et al.*, 2012	New Zealand	-	1
		17.5±0.3	*Trifolium alexandrinum*	Kamboj *et al.*, 2013	India	2009-2012	20
		17.0±0.0	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2006	1
		20.0±0.0	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2007	1
		19.5±2.4	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Electrical conductivity (mS/cm)	0.820	*Trifolium* sp.	Singh and Bath, 1997	India	1994-1995	1
		0.260±0.060	*Trifolium* sp.	Malacalza *et al.*, 2005	Argentina	-	3
		0.270±0.110	*Melilotus* sp.	Malacalza *et al.*, 2005	Argentina	-	3
		0.260±0.020	*Trifolium* sp.	Vanhanen *et al.*, 2011	New Zealand	2007	1
		0.320±0.360	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	pH	4.77	*Medicago sativa*	Abu-Tarboush *et al.*, 1993	Saudi Arabia	-	1
		3.67±0.02	*Trifolium* sp.	Vanhanen *et al.*, 2011	New Zealand	2007	1
		4.16±0.23	*Trifolium alexandrinum*	Kamboj *et al.*, 2013	India	2009-2012	20
		3.61±0.01	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2006	1
		3.74±0.01	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2007	1
	Free acidity (meq/kg)	26.5	*Trifolium* sp.	Singh and Bath, 1997	India	1994-1995	1
		21.9±0.4	*Trifolium alexandrinum*	Nanda *et al.*, 2003	India	-	30
		22.8±3.7	*Trifolium* sp.	Malacalza *et al.*, 2005	Argentina	-	3
		18.8±7.8	*Melilotus* sp.	Malacalza *et al.*, 2005	Argentina	-	3

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CLOVER	Lactones (meq/kg)	15.0	*Trifolium* sp.	Singh and Bath, 1997	India	1994-1995	1
		17.6±0.3	*Trifolium alexandrinum*	Nanda *et al.*, 2003	India	-	30
		21.0±13.9	*Trifolium* sp.	Malacalza *et al.*, 2005	Argentina	-	3
		21.8±13.4	*Melilotus* sp.	Malacalza *et al.*, 2005	Argentina	-	3
	Total acidity (meq/kg)	18.1	*Medicago sativa*	Abu-Tarboush *et al.*, 1993	Saudi Arabia	-	1
		41.5	*Trifolium* sp.	Singh and Bath, 1997	India	1994-1995	1
		39.5±0.6	*Trifolium alexandrinum*	Nanda *et al.*, 2003	India	-	30
		43.9±16.9	*Trifolium* sp.	Malacalza *et al.*, 2005	Argentina	-	3
		40.6±20.6	*Melilotus* sp.	Malacalza *et al.*, 2005	Argentina	-	3
		33.9±0.1	*Trifolium alexandrinum*	Kamboj *et al.*, 2013	India	2009-2012	20
		19.0±0.0	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2006	1
		17.5±0.0	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2007	1
	Proline (mg/100 g)	72.40±32.40	*Trifolium* sp.	Malacalza *et al.*, 2005	Argentina	-	3
		68.20±39.80	*Melilotus* sp.	Malacalza *et al.*, 2005	Argentina	-	3
		29.40±0.08	*Trifolium alexandrinum*	Kamboj *et al.*, 2013	India	2009-2012	20
		51.00 ± 6.40	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
		118.24±0.82	Clover	Kivrak, 2015	Turkey	2013	4
	Specific rotation (°)	-9.30	*Trifolium alexandrinum*	Nanda *et al.*, 2003	India	-	30
		-2.57±0.45	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Fructose (%)	40.00±0.70	*Trifolium repens*	Abell *et al.*, 1996	Canada	-	1
		38.40±4.30	*Medicago sativa*	Shin and Ustunol, 2005	USA	-	3
		38.45	*Melilotus* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		39.56	*Trifolium* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		37.70	Clover	Langford *et al.*, 2012	New Zealand	-	1
		37.79±1.01	*Trifolium alexandrinum*	Kamboj *et al.*, 2013	India	2009-2012	20
		42.47±2.00	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Glucose (%)	38.20±0.70	*Trifolium repens*	Abell *et al.*, 1996	Canada	-	1
		35.10±6.10	*Medicago sativa*	Shin and Ustunol, 2005	USA	-	3
		34.67	*Melilotus* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		29.10	*Trifolium* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		32.30	Clover	Langford *et al.*, 2012	New Zealand	-	1
		35.99±0.78	*Trifolium alexandrinum*	Kamboj *et al.*, 2013	India	2009-2012	20
		17.40±3.26	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CLOVER	Sucrose (%)	1.89	*Medicago sativa*	Abu-Tarboush *et al.*, 1993	Saudi Arabia	-	1
		2.70±0.30	*Medicago sativa*	Shin and Ustunol, 2005	USA	-	3
		0.81	*Melilotus* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		2.10	*Trifolium* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		0.85±0.01	*Trifolium alexandrinum*	Kamboj *et al.*, 2013	India	2009-2012	20
		ND	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Trehalose (%)	0.00	*Melilotus* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		0.00	*Trifolium* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		ND	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Maltose (%)	10.20±1.80	*Medicago sativa*	Shin and Ustunol, 2005	USA	-	3
		1.65	*Melilotus* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		1.45	*Trifolium* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		ND	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Isomaltose (%)	0.00	*Melilotus* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		1.61	*Trifolium* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
	Raffinose (%)	0.00	*Melilotus* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		0.00	*Trifolium* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
	Erlose (%)	0.04	*Melilotus* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		0.95	*Trifolium* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
	Melezitose (%)	0.00	*Melilotus* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		0.00	*Trifolium* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		0.55±0.01	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Total sugars (%)	76.78	*Melilotus* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		73.05	*Trifolium* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
	Fructose+glucose	63.80±0.02	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2006	1
HEATHER	Fructose+glucose	79.40	*Medicago sativa*	Abu-Tarboush *et al.*, 1993	Saudi Arabia	-	1
		73.12	*Melilotus* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		70.05	*Trifolium* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		64.20±0.02	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2007	1
		59.87	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	Fructose/glucose ratio	1.31	*Medicago sativa*	Abu-Tarboush *et al.*, 1993	Saudi Arabia	-	1
		1.11	*Melilotus* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		1.16	*Trifolium* sp.	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	1
		1.05±0.01	*Trifolium alexandrinum*	Kamboj *et al.*, 2013	India	2009-2012	20
		2.44	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	HMF (mg/kg)	8.70±2.05	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		11.60±4.60	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	31
		4.30±3.20	*E. verticillata, E. carnea*	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	20
		15.92±8.82	Ericaceae	Andrade *et al.*, 1999	Portugal	1991-1993	60
		21.32±2.40	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		5.16±2.65	*Erica cinerea*	Devillers *et al.*, 2004	France	-	43
		11.22±0.67	*Erica* sp.	Marini *et al.*, 2004	Italy	-	12
		2.95	*Calluna vulgaris*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	15
		3.72	*Erica* sp.	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	11
		7.00±6.80	*Erica* sp.	Pires *et al.*, 2009	Portugal	-	23
		11.93±5.30	*Calluna vulgaris*	Smanalieva and Senge, 2009	Germany	2003-2005	10
		6.50±6.80	*Erica* sp.	Feás *et al.*, 2010	Portugal	-	45
		4.80±4.10	*Calluna vulgaris*	Waś *et al.*, 2011	Poland	2008-2010	26
		0.46±0.00	Heather	Castro-Vázquez *et al.*, 2012	Spain	-	1
		10.00±3.02	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30
		12.16±5.60	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
		62.24 ± 29.27	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Diastase (º Schade)	51.89±14.66	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		10.80±5.50	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	31
		27.60±5.33	*E. verticillata, E. carnea*	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	20
		23.61±6.71	Ericaceae	Andrade *et al.*, 1999	Portugal	1991-1993	60
		8.33±3.40	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		14.73±3.00	*Erica cinerea*	Devillers *et al.*, 2004	France	-	43
		9.24±0.85	*Erica* sp.	Marini *et al.*, 2004	Italy	-	12
		23.40±6.30	*Calluna vulgaris*	Persano-Oddo and Piro, 2004	Europe	1970-2002	40

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	Diastase (º Schade)	8.70±3.50	*Erica arborea*	Persano-Oddo and Piro, 2004	Europe	1970-2002	-
		46.00	*Calluna vulgaris*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	15
		43.40	*Erica* sp.	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	11
		17.87±5.30	*Erica* sp.	Pires *et al.*, 2009	Portugal	-	23
		18.00±6.00	*Erica* sp.	Feás *et al.*, 2010	Portugal	-	45
		32.70±9.40	*Calluna vulgaris*	Waś *et al.*, 2011	Poland	2008-2010	26
		24.04±0.01	Heather	Castro-Vázquez *et al.*, 2012	Spain	-	1
		14.50±6.10	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
		6.30±4.13	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Moisture (%)	17.4±2.1	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		18.0±1.2	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	31
		18.8±2.2	*E. verticillata, E. carnea*	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	20
		18.2±0.2	Ericaceae	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		17.8±1.2	Ericaceae	Andrade *et al.*, 1999	Portugal	1991-1993	60
		17.7±1.7	Heather	Popek, 2002	Poland	-	8
		17.6±1.1	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		18.2±0.5	*Erica cinerea*	Devillers *et al.*, 2004	France	-	43
		17.2±0.6	*Erica* sp.	Marini *et al.*, 2004	Italy	-	12
		18.5±1.5	*Calluna vulgaris*	Persano-Oddo and Piro, 2004	Europe	1970-2002	200
		17.7±1.0	*Erica* sp.	Escuredo *et al.*, 2013	Northwest of Spain	-	10
		17.6±1.1	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		18.9	*Calluna vulgaris*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	15
		18.2	*Erica* sp.	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	11
		17.6±0.4	*Erica* sp.	Pires *et al.*, 2009	Portugal	-	23
		18.7±1.3	*Calluna vulgaris*	Smanalieva and Senge, 2009	Germany	2003-2005	10
		17.6±0.4	*Erica* sp.	Feás *et al.*, 2010	Portugal	-	45
		19.1±1.5	*Calluna vulgaris*	Waś *et al.*, 2011	Poland	2008-2010	26
		18.6±1.3	*Erica* sp.	Alves *et al.*, 2013	Portugal	-	14
		20.5±0.4	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30
		17.2±0.5	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
		18.1±1.4	*Calluna vulgaris*	Kuś *et al.*, 2014	Poland	2009-2010	3

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	Moisture (%)	18.9±0.6	*Erica* sp.	Anjos *et al.*, 2015	Portugal and Spain	-	7
		20.9±2.1	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Electrical conductivity (mS/cm)	0.813±0.161	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		0.670±0.100	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	31
		0.670±0.160	*E. verticillata, E. carnea*	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	20
		0.976±0.029	Ericaceae	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		0.523±0.091	Ericaceae	Andrade *et al.*, 1999	Portugal	1991-1993	60
		0.609±0.153	Heather	Popek, 2002	Poland	-	8
		0.780±0.101	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		0.604±0.066	*Erica cinerea*	Devillers *et al.*, 2004	France	-	43
		0.740±0.100	*Erica* sp.	Marini *et al.*, 2004	Italy	-	12
		0.730±0.120	*Calluna vulgaris*	Persano-Oddo and Piro, 2004	Europe	1970-2002	189
		0.700±0.090	*Erica arborea*	Persano-Oddo and Piro, 2004	Europe	1970-2002	-
		0.710±0.080	*Erica* sp.	Pires *et al.*, 2009	Portugal	-	23
		0.800±0.100	*Calluna vulgaris*	Smanalieva and Senge, 2009	Germany	2003-2005	10
		0.700±0.080	*Erica* sp.	Feás *et al.*, 2010	Portugal	-	45
		0.620±0.100	*Calluna vulgaris*	Waś *et al.*, 2011	Poland	2008-2010	26
		0.620±0.110	*Erica* sp.	Alves *et al.*, 2013	Portugal	-	14
		0.660±0.070	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30
		0.610±0.140	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
		0.641±0.186	*Erica* sp.	Anjos *et al.*, 2015	Portugal and Spain	-	7
		0.800±0.250	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	pH	4.07±0.19	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		4.00±0.10	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	31
		4.20±0.27	*E. verticillata, E. carnea*	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	20
		4.45±0.04	Ericaceae	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		4.12±0.18	Ericaceae	Andrade *et al.*, 1999	Portugal	1991-1993	60
		3.95±0.08	Heather	Popek, 2002	Poland	-	8
		4.33±0.22	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		4.06±0.15	*Erica cinerea*	Devillers *et al.*, 2004	France	-	43

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	pH	3.98±0.32	*Erica* sp.	Marini *et al.*, 2004	Italy	-	12
		4.20±0.20	*Calluna vulgaris*	Persano-Oddo and Piro, 2004	Europe	1970-2002	35
		3.91±0.16	*Erica* sp.	Pires *et al.*, 2009	Portugal	-	23
		4.54±0.33	*Calluna vulgaris*	Smanalieva and Senge, 2009	Germany	2003-2005	10
		4.83±0.01	Heather	Castro-Vázquez *et al.*, 2012	Spain	-	1
		3.90±0.20	*Erica* sp.	Feás *et al.*, 2010	Portugal	-	45
		4.37±0.17	*Calluna vulgaris*	Waś *et al.*, 2011	Poland	2008-2010	26
		4.14±0.18	*Erica* sp.	Alves *et al.*, 2013	Portugal	-	14
		4.27±0.07	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30
		4.06±0.21	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
		3.91±0.22	*Erica* sp.	Anjos *et al.*, 2015	Portugal and Spain	-	7
	Free acidity (meq/kg)	42.3±6.2	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		28.3±5.9	Ericaceae	Andrade *et al.*, 1999	Portugal	1991-1993	60
		25.8±6.1	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		19.0±1.7	*Erica cinerea*	Devillers *et al.*, 2004	France	-	43
		35.2±1.2	*Erica* sp.	Marini *et al.*, 2004	Italy	-	12
		32.1±5.6	*Calluna vulgaris*	Persano-Oddo and Piro, 2004	Europe	1970-2002	40
		34.7±5.0	*Erica arborea*	Persano-Oddo and Piro, 2004	Europe	1970-2002	-
		33.2	*Calluna vulgaris*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	15
		35.7	*Erica* sp.	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	11
		30.9±5.6	*Erica* sp.	Pires *et al.*, 2009	Portugal	-	23
		18.5±3.0	*Calluna vulgaris*	Smanalieva and Senge, 2009	Germany	2003-2005	10
		30.8±5.9	*Erica* sp.	Feás *et al.*, 2010	Portugal	-	45
		22.1±5.4	*Calluna vulgaris*	Waś *et al.*, 2011	Poland	2008-2010	26
		28.3±0.1	Heather	Castro-Vázquez *et al.*, 2012	Spain	-	1
		38.6±8.8	*Erica* sp.	Alves *et al.*, 2013	Portugal	-	14
		16.9±2.2	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30
		33.2±4.6	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Spain	2005-2009	9
		30.3±6.1	*Erica* sp.	Anjos *et al.*, 2015	Portugal and Spain	-	7

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	Lactones (meq/kg)	3.3±2.2	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		6.1±3.8	Ericaceae	Andrade *et al.*, 1999	Portugal	1991-1993	60
		2.9±0.4	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		6.0±0.9	*Erica* sp.	Marini *et al.*, 2004	Italy	-	12
		3.2±0.1	Heather	Castro-Vázquez *et al.*, 2012	Spain	-	1
		2.5±3.6	*Erica* sp.	Alves *et al.*, 2013	Portugal	-	14
		10.5±0.3	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30
	Total acidity (meq/kg)	45.5±6.9	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		42.1±2.7	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	31
		34.4±6.6	Ericaceae	Andrade *et al.*, 1999	Portugal	1991-1993	60
		28.7±3.6	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		40.3±1.2	*Erica* sp.	Marini *et al.*, 2004	Italy	-	12
		41.0±8.7	*Erica* sp.	Alves *et al.*, 2013	Portugal	-	14
		27.4±2.1	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30
	Proline (mg/100 g)	53.60±33.20	*E. verticillata, E. carnea*	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	20
		22.00±2.20	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		64.60±19.60	*Calluna vulgaris*	Persano-Oddo and Piro, 2004	Europe	1970-2002	144
		98.60	*Erica* sp.	Bernal *et al.* 2005	Soria (Spain)	-	1
		108.40	*Calluna vulgaris*	Bernal *et al.* 2005	Soria (Spain)	-	1
		42.50±0.60	*Erica arborea*	Ouchemoukh *et al.*, 2007	Algeria	2002	11
		55.30±12.50	*Calluna vulgaris*	Waś *et al.*, 2011	Poland	2008-2010	26
		104.44±1.44	*Calluna vulgaris*	Aazza *et al.*, 2013	Portugal	2011	1
		74.10±17.10	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Spain	2005-2009	9
		84.50±4.20	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
		97.90±0.72	Heather	Kivrak, 2015	Turkey	2013	4
	Specific rotation (º)	-14.50±1.50	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	31
		-13.40±0.64	*Erica* sp.	Marini *et al.*, 2004	Italy	-	12
		-13.90±1.60	*Erica arborea*	Persano-Oddo and Piro, 2004	Europe	1970-2002	-
		-2.63±1.35	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	Fructose (%)	43.30±2.01	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		38.00±1.20	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	13
		37.60±1.20	Ericaceae	Mateo and Boch-Reig, 1997	Spain	-	13
		37.40±0.32	Ericaceae	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		38.30±1.01	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		40.17±1.21	*Erica cinerea*	Devillers *et al.*, 2004	France	-	43
		39.47±0.19	*Erica* sp.	Marini *et al.*, 2004	Italy	-	12
		40.80±2.00	*Calluna vulgaris*	Persano-Oddo and Piro, 2004	Europe	1970-2002	109
		38.40±1.30	*Erica arborea*	Persano-Oddo and Piro, 2004	Europe	1970-2002	-
		36.60±4.60	*Calluna vulgaris*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		42.94±3.95	*Erica* sp.	Martins *et al.*, 2008	Portugal	1991	20
		45.07±3.77	*Erica* sp.	Martins *et al.*, 2008	Portugal	1992	20
		44.97±3.78	*Erica* sp.	Martins *et al.*, 2008	Portugal	1993	20
		41.12±1.09	*Calluna vulgaris*	Smanalieva and Senge, 2009	Germany	2003-2005	10
		38.27±1.15	*Erica arborea*	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	4
		37.80	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
		39.30±1.80	*Calluna vulgaris*	Waś *et al.*, 2011	Poland	2008-2010	26
		41.80±3.50	*Erica* sp.	Escuredo *et al.*, 2013	Northwest of Spain	-	10
		39.00±1.12	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30
		41.29±1.21	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
		45.11±0.24	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Glucose (%)	31.00±2.13	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		34.80±1.30	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	13
		29.50±1.40	Ericaceae	Mateo and Boch-Reig, 1997	Spain	-	13
		29.50±0.43	Ericaceae	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		30.60±0.14	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		35.75±1.40	*Erica cinerea*	Devillers *et al.*, 2004	France	-	43
		31.69±0.18	*Erica* sp.	Marini *et al.*, 2004	Italy	-	12
		32.50±1.60	*Calluna vulgaris*	Persano-Oddo and Piro, 2004	Europe	1970-2002	109
		34.70±1.20	*Erica arborea*	Persano-Oddo and Piro, 2004	Europe	1970-2002	-

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	Glucose (%)	31.40±5.80	*Calluna vulgaris*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		28.48±4.15	*Erica* sp.	Martins *et al.*, 2008	Portugal	1991	20
		32.41±3.43	*Erica* sp.	Martins *et al.*, 2008	Portugal	1992	20
		32.65±4.34	*Erica* sp.	Martins *et al.*, 2008	Portugal	1993	20
		31.19±2.16	*Calluna vulgaris*	Smanalieva and Senge, 2009	Germany	2003-2005	10
		33.89±1.23	*Erica arborea*	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	4
		31.15	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
		29.90±2.00	*Calluna vulgaris*	Waś *et al.*, 2011	Poland	2008-2010	26
		28.10±4.00	*Erica* sp.	Escuredo *et al.*, 2013	Northwest of Spain	-	10
		33.07±2.26	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30
		33.50±1.18	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
		25.00±0.32	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Sucrose (%)	0.16±0.08	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		0.30±0.30	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	13
		0.06±0.05	Ericaceae	Mateo and Boch-Reig, 1997	Spain	-	13
		0.06±0.02	Ericaceae	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		1.12±0.48	Ericaceae	Andrade *et al.*, 1999	Portugal	1991-1993	60
		0.99±0.27	Heather	Popek, 2002	Poland	-	8
		4.12±0.90	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		0.12±0.12	*Erica cinerea*	Devillers *et al.*, 2004	France	-	43
		1.40±1.10	*Calluna vulgaris*	Persano-Oddo and Piro, 2004	Europe	1970-2002	52
		3.85±0.38	*Erica* sp.	Pires *et al.*, 2009	Portugal	-	23
		2.05	*Calluna vulgaris*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	15
		1.80	*Erica* sp.	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	11
		0.21±0.07	*Calluna vulgaris*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.09±0.12	*Calluna vulgaris*	Smanalieva and Senge, 2009	Germany	2003-2005	10
		1.02±0.44	*Erica arborea*	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	4
		3.80±0.40	*Erica* sp.	Feás *et al.*, 2010	Portugal	-	45
		0.14	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
		1.21±1.20	*Erica* sp.	Escuredo *et al.*, 2013	Northwest of Spain	-	10
		0.02±0.01	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	Sucrose (%)	0.51±0.37	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
		ND	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Trehalose (%)	1.32±0.42	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		ND	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		0.38±0.20	*Calluna vulgaris*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.04±0.03	*Erica* sp.	Martins *et al.*, 2008	Portugal	1991	20
		0.04±0.04	*Erica* sp.	Martins *et al.*, 2008	Portugal	1992	20
		0.06±0.03	*Erica* sp.	Martins *et al.*, 2008	Portugal	1993	20
		1.12±0.36	*Calluna vulgaris*	Smanalieva and Senge, 2009	Germany	2003-2005	10
		0.00	*Erica arborea*	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	4
		0.41	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
		1.30±0.40	*Calluna vulgaris*	Waś *et al.*, 2011	Poland	2008-2010	26
		0.05±0.17	*Erica* sp.	Escuredo *et al.*, 2013	Northwest Spain	-	10
		0.76±0.07	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30
		1.81±0.29	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
		0.04±0.00	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Maltose (%)	10.60±2.02	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		1.00±0.30	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	13
		3.70±0.60	Ericaceae	Mateo and Boch-Reig, 1997	Spain	-	13
		3.60±0.15	Ericaceae	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		5.26±0.20	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		0.80±0.31	*Calluna vulgaris*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		3.16±1.18	*Erica* sp.	Martins *et al.*, 2008	Portugal	1991	20
		3.35±1.38	*Erica* sp.	Martins *et al.*, 2008	Portugal	1992	20
		1.19±0.22	*Erica* sp.	Martins *et al.*, 2008	Portugal	1993	20
		1.80±0.17	*Calluna vulgaris*	Smanalieva and Senge, 2009	Germany	2003-2005	10
		1.59±0.54	*Erica arborea*	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	4
		1.07	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
		1.80±0.40	*Calluna vulgaris*	Waś *et al.*, 2011	Poland	2008-2010	26
		1.90±1.00	*Erica* sp.	Escuredo *et al.*, 2013	Northwest of Spain	-	10
		2.43±0.22	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	Maltose (%)	4.54±0.70	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
		0.31 ± 0.03	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Gentiobiose (%)	ND	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		0.22±0.16	*Calluna vulgaris*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.01	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
	Isomaltose (%)	3.60±0.71	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		0.30±0.30	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	13
		0.93±0.30	Ericaceae	Mateo and Boch-Reig, 1997	Spain	-	13
		1.00±0.07	Ericaceae	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		0.38±0.25	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		1.49±0.52	*Calluna vulgaris*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		3.03±1.35	*Erica* sp.	Martins *et al.*, 2008	Portugal	1991	20
		4.22±1.43	*Erica* sp.	Martins *et al.*, 2008	Portugal	1992	20
		2.20±0.77	*Erica* sp.	Martins *et al.*, 2008	Portugal	1993	20
		0.29±0.05	*Calluna vulgaris*	Smanalieva and Senge, 2009	Germany	2003-2005	10
		0.41±0.08	*Erica arborea*	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	4
		1.18	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
		0.79±0.08	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30
		0.64±0.20	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
	Raffinose (%)	0.12±0.25	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		0.16±0.27	Ericaceae	Mateo and Boch-Reig, 1997	Spain	-	13
		ND	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		0.00	*Erica cinerea*	Devillers *et al.*, 2004	France	-	43
		2.03±0.83	*Erica* sp.	Martins *et al.*, 2008	Portugal	1991	20
		0.16±0.41	*Erica* sp.	Martins *et al.*, 2008	Portugal	1992	20
		0.21±0.66	*Erica* sp.	Martins *et al.*, 2008	Portugal	1993	20
		0.01±0.02	*Erica arborea*	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	4
	Erlose (%)	0.13±0.22	Ericaceae	Mateo and Boch-Reig, 1997	Spain	-	13
		ND	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		0.00	*Erica cinerea*	Devillers *et al.*, 2004	France	-	43
		1.96±0.43	*Calluna vulgaris*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	Erlose (%)	0.00	*Calluna vulgaris*	Smanalieva and Senge, 2009	Germany	2003-2005	10
		0.08±0.14	*Erica arborea*	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	4
		0.22	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
		0.33±0.24	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30
	Melezitose (%)	0.13±0.15	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		ND	*E. arborea, E. australis*	Terrab *et al.*, 2003	Morocco	-	3
		0.00	*Erica cinerea*	Devillers *et al.*, 2004	France	-	43
		0.20±0.11	*Calluna vulgaris*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.59±0.42	*Erica* sp.	Martins *et al.*, 2008	Portugal	1991	20
		0.08±0.21	*Erica* sp.	Martins *et al.*, 2008	Portugal	1992	20
		0.16±0.33	*Erica* sp.	Martins *et al.*, 2008	Portugal	1993	20
		0.01±0.02	*Erica arborea*	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	4
		0.09	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
		0.04±0.12	*Erica* sp.	Escuredo *et al.*, 2013	Northwest of Spain	-	10
		0.41±0.22	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
		0.55±0.01	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Maltotriose (%)	0.21±0.11	*Calluna vulgaris*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.01	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
	Panose (%)	0.37±0.17	*Calluna vulgaris*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.12	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
	Isomaltotriose (%)	0.28±0.13	*Calluna vulgaris*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.02	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
	Total sugars (%)	72.88±1.09	Heather	Popek, 2002	Poland	-	8
		75.74±1.43	*Erica arborea*	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	4
		80.57	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
		83.82±1.64	*Calluna vulgaris*	Waś *et al.*, 2011	Poland	2008-2010	26
		73.10±5.40	*Erica* sp.	Escuredo *et al.*, 2013	Northwest of Spain	-	10
	Fructose+glucose	72.80±1.50	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	13
		72.30±1.95	Ericaceae	Andrade *et al.*, 1999	Portugal	1991-1993	60
		71.74±1.19	Heather	Popek, 2002	Poland	-	8
		73.40±3.10	*Calluna vulgaris*	Persano-Oddo and Piro, 2004	Europe	1970-2002	109

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	Fructose+glucose	73.10±1.60	*Erica arborea*	Persano-Oddo and Piro, 2004	Europe	1970-2002	-
		65.54	*Calluna vulgaris*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	15
		65.86	*Erica* sp.	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	11
		72.16±2.43	*Erica* sp.	Pires *et al.*, 2009	Portugal	-	23
		72.24±2.04	*Erica* sp.	Feás *et al.*, 2010	Portugal	-	45
		72.15±0.98	*Erica arborea*	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	4
		68.96	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
		69.30±3.30	*Calluna vulgaris*	Waś *et al.*, 2011	Poland	2008-2010	26
		74.79±1.57	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
		70.11	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Total disaccharides	10.38	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
		1.23	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
	Fructose/glucose ratio	1.40±0.07	*Calluna vulgaris*	Serra-Bonvehí and Granados-Tarrés, 1993	Soria, La Rioja (Spain)	1985-1986	23
		1.10±0.06	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	13
		1.28±0.07	Ericaceae	Mateo and Boch-Reig, 1997	Spain	-	13
		1.27±0.02	Ericaceae	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		1.26±0.07	*Calluna vulgaris*	Persano-Oddo and Piro, 2004	Europe	1970-2002	109
		1.11±0.06	*Erica arborea*	Persano-Oddo and Piro, 2004	Europe	1970-2002	-
		1.32	*Calluna vulgaris*	Smanalieva and Senge, 2009	Germany	2003-2005	10
		1.13±0.07	*Erica arborea*	Ouchemoukh *et al.*, 2010	Algeria	2004-2006	4
		1.20	Ericaceae	De la Fuente *et al.*, 2011	Spain	Through 3 years	13
		1.32±0.10	*Calluna vulgaris*	Waś *et al.*, 2011	Poland	2008-2010	26
		1.23±0.06	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
		1.80	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Glucose/moisture ratio	1.49±0.23	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	13
		1.82±0.11	*Erica arborea*	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	13
		1.62±0.08	Ericaceae	Mateo and Boch-Reig, 1997	Spain	-	13
		1.62±0.02	Ericaceae	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		1.76±0.16	*Calluna vulgaris*	Persano-Oddo and Piro, 2004	Europe	1970-2002	105
		1.89±0.18	*Erica arborea*	Persano-Oddo and Piro, 2004	Europe	1970-2002	-

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	Glucose/moisture ratio	1.94±0.11	*Erica arborea*	Bentabol-Manzanares *et al.*, 2014	Tenerife (Spain)	2005-2009	9
	Maltose/ isomaltose ratio	0.57±0.22	*Calluna vulgaris*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
HONEYDEW	HMF (mg/kg)	2.00±1.60	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	52
		2.20±1.20	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		2.70±2.88	*Pinus* sp.	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	48
		2.10±1.41	*Abies* sp.	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	16
		1.46±0.44	*Abies* sp.	Golob and Plestenjak, 1999	Slovenia	1996	5
		30.10±37.50	Honeydew	Celechovská and Vorlová, 2001	Czech Republic	1999	10
		31.66±19.73	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2002	Morocco	-	3
		3.49±2.14	*Abies* sp.	Devillers *et al.*, 2004	France	-	57
		1.72±0.06	Honeydew	Marini *et al.*, 2004	Italy	-	11
		1.54	*Quercus* sp.	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	9
		7.57±0.54	*Pinus* sp.	Turhan *et al.*, 2008	Turkey	-	4
		10.84±6.48	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		1.87±0.34	Honeydew	Chakir *et al.*, 2011	Morocco	2007	2
		3.22±4.83	*Quercus* sp.	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	11
		3.00±3.00	*Quercus* sp.	Escuredo *et al.*, 2012	Galicia (Spain)	2008-2009	22
		9.79±0.03	*Pinus* sp.	İnan *et al.*, 2012	Turkey	-	1
		11.42±5.01	Honeydew	Kasperová *et al.*, 2012	Slovakia	2006-2008	7
		22.60±5.50	*Pinus* sp., *Abies* sp.	Oroian, 2012	Romania	2011	3
		0.87±0.06	Honeydew	Truzzi *et al.*, 2012	Italy	2009	2
		0.10±0.20	*Quercus* sp.	Rodríguez-Flores *et al.*, 2015	Spain	2009-2011	32
		0.61±1.55	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		3.57±2.06	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Diastase (º Schade)	22.60±6.70	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	52
		31.90±9.30	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		28.40±2.20	*Pinus* sp.	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	48
		18.50±5.46	*Abies* sp.	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	16
		16.55±3.25	*Abies* sp.	Golob and Plestenjak, 1999	Slovenia	1996	5

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	Diastase (º Schade)	11.20±2.60	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2002	Morocco	-	3
		24.15±3.78	*Abies* sp.	Devillers *et al.*, 2004	France	-	57
		39.20±9.50	*Quercus* sp.	Iglesias *et al.*, 2004	Central Spain	2000-2001	19
		23.96±0.65	Honeydew	Marini *et al.*, 2004	Italy	-	11
		22.60±5.60	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	264
		38.52	*Quercus* sp.	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	9
		16.74±6.64	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		19.10±6.80	Honeydew	Chakir *et al.*, 2011	Morocco	2007	2
		18.80±5.90	Honeydew	Escuredo *et al.*, 2012	Galicia (Spain)	2008-2009	22
		28.40±9.40	*Abies alba*	Rybak-Chmielewska *et al.*, 2013	Poland	2006-2007	27
		10.50±2.22	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		11.60±1.31	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
		23.90±4.20	*Quercus* sp.	Rodríguez-Flores *et al.*, 2015	Northwest Spain	2009-2011	32
	Moisture (%)	16.1±1.0	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	52
		16.0±0.8	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		16.6±1.1	*Pinus* sp.	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	48
		15.2±1.5	*Abies* sp.	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	16
		16.6±2.1	*Abies* sp.	Golob and Plestenjak, 1999	Slovenia	1996	5
		15.8±0.1	*Quercus* sp.	Mateo and Boch-Reig, 1998	Spain	1980-1987	16
		15.6±1.7	Honeydew	Celechovská and Vorlová, 2001	Czech Republic	1999	10
		16.1±1.7	Honeydew	Popek, 2002	Poland	-	10
		20.3±3.7	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2002	Morocco	-	3
		17.6±0.6	*Abies* sp.	Devillers *et al.*, 2004	France	-	57
		15.2±0.2	Honeydew	Marini *et al.*, 2004	Italy	-	11
		16.1±1.2	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	598
		16.7	*Quercus* sp.	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	9
		15.8±0.9	*Quercus* sp.	Iglesias *et al.*, 2004	Central Spain	2000-2001	19
		15.5±0.8	*Quercus* sp.	Pérez *et al.*, 2007	Madrid (Spain)	2002	5
		15.5±0.8	*Quercus* sp.	Pérez-Martín *et al.*, 2008	Spain	-	21
		17.4±1.4	Honeydew	Marghitas *et al.*, 2009	Romania	2005-2006	7
		17.0±0.8	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	Moisture (%)	14.6±0.4	Honeydew	Chakir *et al.*, 2011	Morocco	2007	2
		15.3±0.4	*Quercus* sp.	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	5
		15.2±0.9	*Abies alba*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	30
		16.7±0.6	Honeydew	Escuredo *et al.*, 2012	Galicia (Spain)	2008-2009	22
		17.9±0.8	Honeydew	Kasperová *et al.*, 2012	Slovakia	2006-2008	7
		17.1±0.4	*Pinus* sp., *Abies* sp.	Oroian, 2012	Romania	2011	3
		16.8±0.1	Honeydew	Truzzi *et al.*, 2012	Italy	2009	2
		16.9±0.9	*Quercus* sp.	Escuredo *et al.*, 2013	Northwest of Spain	-	13
		16.8±0.8	*Abies alba*	Rybak-Chmielewska *et al.*, 2013	Poland	2006-2007	27
		17.3±1.9	*Abies* sp.	Karabagias *et al.*, 2014	Greece	2011	31
		14.7±2.4	*Pinus* sp.	Karabagias *et al.*, 2014	Greece	2011	39
		17.9±0.1	*Quercus rotundifolia*	Anjos *et al.*, 2015	Portugal and Spain	-	1
		17.4±0.9	*Quercus* sp.	Rodríguez-Flores *et al.*, 2015	Spain	2009-2011	32
		17.1±1.3	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		17.2± 1.0	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Electrical conductivity (mS/cm)	1.500±0.220	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	52
		1.640±0.270	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		1.260±0.120	*Pinus* sp.	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	48
		1.400±0.190	*Abies* sp.	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	16
		0.986±0.027	*Quercus* sp.	Mateo and Boch-Reig, 1998	Spain	1980-1987	16
		1.150±0.146	*Abies* sp.	Golob and Plestenjak, 1999	Slovenia	1996	5
		1.078±0.132	Honeydew	Celechovská and Vorlová, 2001	Czech Republic	1999	10
		0.972±0.600	Honeydew	Popek, 2002	Poland	-	10
		1.734±0.300	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2002	Morocco	-	3
		1.069±0.122	*Abies* sp.	Devillers *et al.*, 2004	France	-	57
		1.502±0.092	Honeydew	Marini *et al.*, 2004	Italy	-	11
		1.200±0.220	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	648
		1.315±0.143	*Quercus* sp.	Iglesias *et al.*, 2004	Central Spain	2000-2001	19
		1.079	*Quercus* sp.	Soria *et al.*, 2005	Madrid (Spain)	-	4
		1.044±0.010	*Quercus* sp.	Pérez *et al.*, 2007	Madrid (Spain)	2002	5
		1.006	*Quercus* sp.	Vela *et al.*, 2007	Spain	-	17

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	Electrical conductivity (mS/cm)	0.900±0.190	*Quercus* sp.	González-Lorente *et al.*, 2008	Spain	-	8
		1.020±0.190	*Quercus* sp.	Pérez-Martín *et al.*, 2008	Spain	-	22
		1.210±0.330	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		1.320±0.180	*Abies alba*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	30
		1.120±0.622	Honeydew	Chakir *et al.*, 2011	Morocco	2007	2
		0.600±0.270	*Quercus* sp.	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	5
		0.830±0.200	Honeydew	Escuredo *et al.*, 2012	Galicia (Spain)	2008-2009	22
		1.122±0.111	Honeydew	Kasperová *et al.*, 2012	Slovakia	2006-2008	7
		0.833±0.050	*Pinus* sp., *Abies* sp.	Oroian, 2012	Romania	2011	3
		1.446±0.004	Honeydew	Truzzi *et al.*, 2012	Italy	2009	2
		1.140±0.100	*Abies alba*	Rybak-Chmielewska *et al.*, 2013	Poland	2006-2007	27
		1.502±0.428	*Abies* sp.	Karabagias *et al.*, 2014	Greece	2011	31
		1.103±0.273	*Pinus* sp.	Karabagias *et al.*, 2014	Greece	2011	39
		1.114±0.001	*Quercus rotundifolia*	Anjos *et al.*, 2015	Portugal and Spain	-	1
		1.090±0.160	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		0.990±0.320	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
		1.000±0.100	*Quercus* sp.	Rodríguez-Flores *et al.*, 2015	Spain	2009-2011	32
	pH	5.30±0.20	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	52
		5.00±0.40	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		4.80±0.21	*Pinus* sp.	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	48
		5.00±0.26	*Abies* sp.	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	16
		4.61±0.04	*Quercus* sp.	Mateo and Boch-Reig, 1998	Spain	1980-1987	16
		4.58±0.16	*Abies* sp.	Golob and Plestenjak, 1999	Slovenia	1996	5
		4.53±0.16	Honeydew	Celechovská and Vorlová, 2001	Czech Republic	1999	10
		4.24±0.26	Honeydew	Popek, 2002	Poland	-	10
		4.28±0.39	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2002	Morocco	-	3
		5.15±0.29	*Abies* sp.	Devillers *et al.*, 2004	France	-	57
		4.70±0.20	*Quercus* sp.	Iglesias *et al.*, 2004	Central Spain	2000-2001	19
		5.30±0.17	Honeydew	Marini *et al.*, 2004	Italy	-	11
		5.10±0.30	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	254
		4.87	*Quercus* sp.	Soria *et al.*, 2005	Madrid (Spain)	-	4

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	pH	4.96±0.14	*Quercus* sp.	Pérez *et al.*, 2007	Madrid (Spain)	2002	5
		4.64	*Quercus* sp.	Vela *et al.*, 2007	Spain	-	17
		4.47±0.25	*Quercus* sp.	González-Lorente *et al.*, 2008	Spain	-	8
		4.65±0.32	*Quercus* sp.	Pérez-Martín *et al.*, 2008	Spain	-	22
		4.21±0.01	*Pinus* sp.	Turhan *et al.*, 2008	Turkey	-	4
		4.58±0.69	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		5.14±0.25	*Abies alba*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	30
		4.93±0.21	Honeydew	Chakir *et al.*, 2011	Morocco	2007	2
		4.66±0.29	*Quercus* sp.	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	5
		4.30±0.40	Honeydew	Escuredo *et al.*, 2012	Galicia (Spain)	2008-2009	22
		4.54±0.01	*Pinus* sp.	İnan *et al.*, 2012	Turkey	-	1
		4.66±0.37	Honeydew	Kasperová *et al.*, 2012	Slovakia	2006-2008	7
		4.40±0.20	*Pinus* sp., *Abies* sp.	Oroian, 2012	Romania	2011	3
		4.72±0.01	Honeydew	Truzzi *et al.*, 2012	Italy	2009	2
		4.63±0.18	*Abies alba*	Rybak-Chmielewska *et al.*, 2013	Poland	2006-2007	27
		4.9±0.21	*Abies* sp.	Karabagias *et al.*, 2014	Greece	2011	31
		4.76±0.26	*Pinus* sp.	Karabagias *et al.*, 2014	Greece	2011	39
		4.40±0.00	*Quercus rotundifolia*	Anjos *et al.*, 2015	Portugal and Spain	-	1
		4.40±0.20	*Quercus* sp.	Rodríguez-Flores *et al.*, 2015	Spain	2009-2011	32
	Free acidity (meq/kg)	32.7±10.6	Honeydew	Celechovská and Vorlová, 2001	Czech Republic	1999	10
		88.6±23.4	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2002	Morocco	-	3
		24.2±3.5	*Abies* sp.	Devillers *et al.*, 2004	France	-	57
		37.9±5.2	*Quercus* sp.	Iglesias *et al.*, 2004	Central Spain	2000-2001	19
		26.4±0.5	Honeydew	Marini *et al.*, 2004	Italy	-	11
		26.0±5.6	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	205
		29.3	*Quercus* sp.	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	9
		39.0	*Quercus* sp.	Soria *et al.*, 2005	Madrid (Spain)	-	4
		35.9±4.0	*Quercus* sp.	Pérez *et al.*, 2007	Madrid (Spain)	2002	5
		43.2	*Quercus* sp.	Vela *et al.*, 2007	Spain	-	17
		34.7±10.6	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		20.1±4.1	*Abies alba*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	30
		21.4±5.4	Honeydew	Chakir *et al.*, 2011	Morocco	2007	2

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	Free acidity (meq/kg)	29.7±0.6	*Quercus* sp.	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	5
		32.0±7.1	Honeydew	Kasperová *et al.*, 2012	Slovakia	2006-2008	7
		38.0±0.6	Honeydew	Truzzi *et al.*, 2012	Italy	2009	2
		27.6±4.1	*Abies alba*	Rybak-Chmielewska *et al.*, 2013	Poland	2006-2007	27
		27.7±4.1	*Abies* sp.	Karabagias *et al.*, 2014	Greece	2011	31
		30.1±6.8	*Pinus* sp.	Karabagias *et al.*, 2014	Greece	2011	39
		45.4±0.4	*Quercus rotundifolia*	Anjos *et al.*, 2015	Portugal and Spain	-	1
	Lactones (meq/kg)	8.1±4.9	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2002	Morocco	-	3
		2.0±0.1	Honeydew	Marini *et al.*, 2004	Italy	-	11
		2.8±2.0	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	143
		2.2±1.7	*Quercus* sp.	Iglesias *et al.*, 2004	Central Spain	2000-2001	19
		4.9	*Quercus* sp.	Vela *et al.*, 2007	Spain	-	17
		2.3±1.8	*Abies alba*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	30
		4.7±0.2	Honeydew	Chakir *et al.*, 2011	Morocco	2007	2
		3.0±0.9	Honeydew	Truzzi *et al.*, 2012	Italy	2009	2
		5.2±0.7	*Abies* sp.	Karabagias *et al.*, 2014	Greece	2011	31
		4.3±0.8	*Pinus* sp.	Karabagias *et al.*, 2014	Greece	2011	39
	Total acidity (meq/kg)	25.4±5.8	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	52
		40.6±6.6	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		29.8±6.2	*Abies* sp.	Golob and Plestenjak, 1999	Slovenia	1996	5
		96.7±24.0	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2001, 2002, 2003	Morocco	-	3
		40.1±5.2	*Quercus* sp.	Iglesias *et al.*, 2004	Central Spain	2000-2001	19
		28.5±0.4	Honeydew	Marini *et al.*, 2004	Italy	-	11
		28.4±6.1	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	143
		37.4±4.8	*Quercus* sp.	Pérez *et al.*, 2007	Madrid (Spain)	2002	5
		48.1	*Quercus* sp.	Vela *et al.*, 2007	Spain	-	17
		50.8±9.7	*Quercus* sp.	González-Lorente *et al.*, 2008	Spain	-	8
		48.5±12.3	*Quercus* sp.	Pérez-Martín *et al.*, 2008	Spain	-	22
		43.2±2.7	*Pinus* sp.	Turhan *et al.*, 2008	Turkey	-	4
		22.4±4.0	*Abies alba*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	30
		26.1±5.3	Honeydew	Chakir *et al.*, 2011	Morocco	2007	2

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	Total acidity (meq/kg)	40.9±0.5	Honeydew	Truzzi *et al.*, 2012	Italy	2009	2
		32.8±3.6	*Abies* sp.	Karabagias *et al.*, 2014	Greece	2011	31
		34.4±6.1	*Pinus* sp.	Karabagias *et al.*, 2014	Greece	2011	39
	Lactones/free acidity	4.6±1.3	Honeydew	Chakir *et al.*, 2011	Morocco	2007	2
		0.2±0.1	*Abies* sp.	Karabagias *et al.*, 2014	Greece	2011	31
		0.2±0.1	*Pinus* sp.	Karabagias *et al.*, 2014	Greece	2011	39
	Proline (mg/100 g)	51.40±26.00	*Pinus* sp.	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	48
		39.00±19.40	*Abies* sp.	Thrasyvoulou and Manikis, 1995	Greece	1989-1993	16
		227.20±80.35	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2001, 2002, 2003	Morocco	-	3
		56.45±2.33	Honeydew	Dinkov, 2003	Bulgaria	2000	9
		46.80±12.70	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	305
		45.62± 8.66	*Abies* sp.	Cotte *et al.*, 2004	France	-	37
		90.46±24.68	*Quercus* sp.	Iglesias *et al.*, 2004	Central Spain	2000-2001	19
		63.30	*Quercus* sp.	Bernal *et al.* 2005	Soria (Spain)	-	1
		84.41±19.42	*Quercus pyrenaica*	Iglesias *et al.*, 2006	Madrid (Spain)	-	5
		72.68±11.97	*Quercus* sp.	Pérez *et al.*, 2007	Madrid (Spain)	2002	5
		106.90±28.40	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		42.40±6.50	*Abies alba*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	30
		108.90±13.70	Honeydew	Czipa *et al.*, 2012	International	-	4
		64.90±22.10	*Abies alba*	Rybak-Chmielewska *et al.*, 2013	Poland	2006-2007	27
		20.73±6.04	*Pinus* sp.	Silici and Karaman, 2014	Turkey	-	12
		40.40±9.90	*Pinus* sp.	Silici and Karaman, 2014	Turkey	-	12
		61.40±1.40	*Metcalfa* sp.	Truzzi *et al.*, 2014	Italy	2009	2
		47.40±11.50	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		43.70±19.00	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
		94.36±0.17	Honeydew	Kivrak, 2015	Turkey	-	7
	Specific rotation (º)	14.00±5.00	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	52
		17.00±7.40	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		15.91±0.55	Honeydew	Marini *et al.*, 2004	Italy	-	11
		13.90±5.70	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	71
		2.40±1.13	-	Šarić *et al.*, 2008	Croatia	2004	5

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	Specific rotation (º)	12.60±6.80	*Abies alba*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	30
		0.74±0.25	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		1.38±1.40	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Fructose (%)	31.80±2.80	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	29
		31.90±3.30	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		34.30±1.10	*Quercus* sp.	Mateo and Boch-Reig, 1997	Spain	-	16
		34.30±0.27	*Quercus* sp.	Mateo and Boch-Reig, 1998	Spain	1980-1987	16
		34.20±2.87	*Abies* sp.	Golob and Plestenjak, 1999	Slovenia	1996	5
		32.02±3.74	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2001, 2002, 2003	Morocco	-	3
		33.37±1.42	*Abies* sp.	Devillers *et al.*, 2004	France	-	57
		32.80±2.50	*Quercus* sp.	Iglesias *et al.*, 2004	Central Spain	2000-2001	19
		35.10±3.60	Honeydew	Marini *et al.*, 2004	Italy	-	11
		32.50±1.90	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	362
		38.10±5.00	*Quercus* sp.	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		38.25±1.63	*Quercus ilex*	De la Fuente *et al.*, 2007	Spain	-	2
		38.72±2.66	Honeydew	Marghitas *et al.*, 2009	Romania	2005-2006	7
		39.64±1.71	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		42.42±0.87	Honeydew	Chakir *et al.*, 2011	Morocco	2007	2
		30.35±2.89	*Quercus* sp.	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	5
		36.60±2.30	*Pinus* sp., *Abies* sp.	Oroian, 2012	Romania	2011	3
		35.60±4.30	*Quercus* sp.	Escuredo *et al.*, 2013, 2014	Northwest of Spain	2008-2010	13
		34.20±1.30	*Abies alba*	Rybak-Chmielewska *et al.*, 2013	Poland	2006-2007	27
		43.28±2.18	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		39.80±2.84	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Glucose (%)	24.00±2.20	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	29
		23.70±2.70	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		25.80±1.50	*Quercus* sp.	Mateo and Boch-Reig, 1997	Spain	-	16
		25.80±0.38	*Quercus* sp.	Mateo and Boch-Reig, 1998	Spain	1980-1987	16
		27.71±2.56	*Abies* sp.	Golob and Plestenjak, 1999	Slovenia	1996	5

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	Glucose (%)	26.91±3.44	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2001, 2002, 2003	Morocco	-	3
		25.63±1.59	*Abies* sp.	Devillers *et al.*, 2004	France	-	57
		27.60±1.70	Honeydew	Marini *et al.*, 2004	Italy	-	11
		26.20±2.60	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	362
		33.60±4.90	*Quercus* sp.	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		27.22±1.60	*Quercus pyrenaica*	Iglesias *et al.*, 2006	Madrid (Spain)	-	5
		26.30±1.98	*Quercus ilex*	De la Fuente *et al.*, 2007	Spain	-	2
		36.06±2.13	Honeydew	Marghitas *et al.*, 2009	Romania	2005-2006	7
		31.19±2.33	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		32.27±0.96	Honeydew	Chakir *et al.*, 2011	Morocco	2007	2
		20.22±2.10	*Quercus* sp.	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	5
		34.90±2.50	*Pinus* sp., *Abies* sp.	Oroian, 2012	Romania	2011	3
		26.50±3.50	*Quercus* sp.	Escuredo *et al.*, 2013, 2014	Northwest of Spain	2008-2010	13
		27.80±1.30	*Abies alba*	Rybak-Chmielewska *et al.*, 2013	Poland	2006-2007	27
		21.73±2.40	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		23.67±2.21	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Sucrose (%)	0.40±0.40	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	29
		0.10±0.10	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		0.21±0.17	*Quercus* sp.	Mateo and Boch-Reig, 1997	Spain	-	16
		0.21±0.04	*Quercus* sp.	Mateo and Boch-Reig, 1998	Spain	1980-1987	16
		0.73	*Abies* sp.	Golob and Plestenjak, 1999	Slovenia	1996	1
		2.54±1.93	Honeydew	Celechovská and Vorlová, 2001	Czech Republic	1999	10
		3.89±0.33	Honeydew	Popek, 2002	Poland	-	10
		0.00	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2001, 2002, 2003	Morocco	-	3
		1.35±0.61	*Abies* sp.	Devillers *et al.*, 2004	France	-	57
		0.80±0.90	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	310
		3.36	*Quercus* sp.	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	9
		0.35±0.16	*Quercus* sp.	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.79±0.52	*Quercus ilex*	De la Fuente *et al.*, 2007	Spain	-	2
		0.16±0.10	Honeydew	Marghitas *et al.*, 2009	Romania	2005-2006	7

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	Sucrose (%)	0.50±0.56	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		1.59±0.85	Honeydew	Chakir *et al.*, 2011	Morocco	2007	2
		0.23±0.22	*Quercus* sp.	Escuredo *et al.*, 2013, 2014	Northwest of Spain	2008-2010	13
		ND	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		ND	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Trehalose (%)	0.05±0.03	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2001, 2002, 2003	Morocco	-	3
		0.73±0.29	*Quercus* sp.	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.50±0.14	*Quercus ilex*	De la Fuente *et al.*, 2007	Spain	-	2
		1.51±1.12	Honeydew	Marghitas *et al.*, 2009	Romania	2005-2006	7
		1.89±0.42	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		0.18±0.29	*Quercus* sp.	Escuredo *et al.*, 2013, 2014	Galicia (Spain)	-	13
		2.70±1.10	*Abies alba*	Rybak-Chmielewska *et al.*, 2013	Poland	2006-2007	27
		0.42±0.12	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		0.23±0.10	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Maltose (%)	0.90±0.30	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	29
		1.40±0.40	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		4.90±0.80	*Quercus* sp.	Mateo and Boch-Reig, 1997	Spain	-	16
		4.90±0.20	*Quercus* sp.	Mateo and Boch-Reig, 1998	Spain	1980-1987	16
		2.82±1.54	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2001, 2002, 2003	Morocco	-	3
		1.33±0.52	*Quercus* sp.	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		2.25±0.23	*Quercus ilex*	De la Fuente *et al.*, 2007	Spain	-	2
		3.30±1.70	Honeydew	Marghitas *et al.*, 2009	Romania	2005-2006	7
		5.52±0.89	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		3.20±0.60	*Abies alba*	Rybak-Chmielewska *et al.*, 2013	Poland	2006-2007	27
		2.20±1.50	*Quercus* sp.	Escuredo *et al.*, 2014	Northwest of Spain	2008-2010	13
		0.19±0.44	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		0.54±0.64	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Gentiobiose (%)	0.03±0.04	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2001, 2002, 2003	Morocco	-	3
		0.20±0.09	*Quercus* sp.	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.05±0.02	*Quercus ilex*	De la Fuente *et al.*, 2007	Spain	-	2

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	Isomaltose (%)	2.50±0.90	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	29
		2.00±0.60	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		1.80±0.93	*Quercus* sp.	Mateo and Boch-Reig, 1997	Spain	-	16
		1.80±0.23	*Quercus* sp.	Mateo and Boch-Reig, 1998	Spain	1980-1987	16
		0.22±0.17	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2001, 2002, 2003	Morocco	-	3
		1.64±0.39	*Quercus* sp.	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		1.11±0.33	*Quercus ilex*	De la Fuente *et al.*, 2007	Spain	-	2
	Isomaltose (%)	1.11±0.40	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
	Raffinose (%)	0.58±0.35	*Quercus* sp.	Mateo and Boch-Reig, 1997	Spain	-	16
		ND	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2003	Morocco	-	3
		1.57±0.47	*Abies* sp.	Devillers *et al.*, 2004	France	-	57
		0.11±0.06	*Quercus ilex*	De la Fuente *et al.*, 2007	Spain	-	2
	Erlose (%)	ND	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2001, 2002, 2003	Morocco	-	3
		0.82±0.20	*Abies* sp.	Devillers *et al.*, 2004	France	-	57
		1.08±0.47	*Quercus* sp.	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.85±0.22	*Quercus ilex*	De la Fuente *et al.*, 2007	Spain	-	2
	Melezitose (%)	0.80±0.69	*Quercus* sp.	Mateo and Boch-Reig, 1997	Spain	-	16
		15.08±6.98	*Abies* sp.	Golob and Plestenjak, 1999	Slovenia	1996	5
		0.83±0.67	*Quercus* sp., *Cedrus atlantica*	Terrab *et al.*, 2001, 2002, 2003	Morocco	-	3
		2.22±0.48	*Abies* sp.	Devillers *et al.*, 2004	France	-	57
		2.47±0.60	*Quercus* sp.	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.30±0.06	*Quercus ilex*	De la Fuente *et al.*, 2007	Spain	-	2
		1.34±1.24	Honeydew	Marghitas *et al.*, 2009	Romania	2005-2006	7
		0.75±0.39	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		3.20±1.30	*Abies alba*	Rybak-Chmielewska *et al.*, 2013	Poland	2006-2007	27
		0.21±0.22	*Quercus* sp.	Escuredo *et al.*, 2014	Northwest of Spain	2008-2010	13
		0.94±0.07	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		0.62±0.34	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Maltotriose (%)	0.32±0.13	*Quercus* sp.	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.13±0.01	*Quercus ilex*	De la Fuente *et al.*, 2007	Spain	-	2

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	Panose (%)	0.42±0.12	*Quercus* sp.	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.16±0.05	*Quercus ilex*	De la Fuente *et al.*, 2007	Spain	-	2
	Isomaltotriose (%)	0.21±0.13	*Quercus* sp.	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
	Total sugars (%)	73.19±2.99	Honeydew	Popek, 2002	Poland	-	10
		80.60±2.80	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		75.20±1.70	*Abies alba*	Rybak-Chmielewska *et al.*, 2013	Poland	2006-2007	27
		64.90±8.10	*Quercus* sp.	Escuredo *et al.*, 2014	Northwest of Spain	2008-2010	13
	Fructose+glucose	55.80±4.60	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	29
		55.60±4.60	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		61.92±4.81	*Abies* sp.	Golob and Plestenjak, 1999	Slovenia	1996	5
		69.07±2.73	Honeydew	Popek, 2002	Poland	-	10
		58.70±3.80	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	362
		67.41	*Quercus* sp.	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	9
		58.66±6.35	*Quercus* sp.	Pérez *et al.*, 2007	Madrid (Spain)	2002	5
		64.68	*Quercus* sp.	Vela *et al.*, 2007	Spain	-	17
		70.83±2.32	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		71.60±2.10	*Pinus* sp., *Abies* sp.	Oroian, 2012	Romania	2011	3
		62.00±1.90	*Abies alba*	Rybak-Chmielewska *et al.*, 2013	Poland	2006-2007	27
		65.01	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		63.47	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Fructose/glucose ratio	1.33±0.09	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	29
		1.36±0.19	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		1.33±0.06	*Quercus* sp.	Mateo and Boch-Reig, 1997	Spain	-	16
		1.33±0.02	*Quercus* sp.	Mateo and Boch-Reig, 1998	Spain	1980-1987	16
		1.24±0.09	*Abies* sp.	Golob and Plestenjak, 1999	Slovenia	1996	5
		1.25±0.12	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	362
		1.08±0.06	Honeydew	Marghitas *et al.*, 2009	Romania	2005-2006	7
		1.28±0.13	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
		1.10±0.00	*Pinus* sp., *Abies* sp.	Oroian, 2012	Romania	2011	3

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	Fructose/glucose ratio	1.20±0.10	*Abies alba*	Rybak-Chmielewska *et al.*, 2013	Poland	2006-2007	27
		1.99	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		1.68	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Glucose/moisture ratio	1.50±0.12	*Abies* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	29
		1.48±0.15	*Metcalfa* sp.	Persano-Oddo *et al.*, 1995	Italy	Period of 10 years before 1995	78
		1.63±0.10	*Quercus* sp.	Mateo and Boch-Reig, 1997	Spain	-	16
		1.63±0.25	*Quercus* sp.	Mateo and Boch-Reig, 1998	Spain	1980-1987	16
		1.61±0.17	Honeydew	Persano-Oddo and Piro, 2004	Europe	1970-2002	336
		1.84±0.16	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
	Maltose/ isomaltose ratio	1.06±0.82	*Quercus* sp.	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	15
		7.04±2.41	Honeydew	Bentabol-Manzanares *et al.*, 2011	Tenerife (Spain)	2005-2008	24
LAVENDER	HMF (mg/kg)	8.12±2.40	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		0.98±0.02	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		4.79±0.12	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		3.21±1.49	*Lavandula* sp.	Devillers *et al.*, 2004	France	-	57
		3.09	*Lavandula stoechas*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	7
		1.65	*Lavandula latifolia*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	15
		17.85±4.27	*Lavandula stoechas*	Chakir *et al.*, 2011	Morocco	2007	2
		1.14±0.20	*Lavandula stoechas*	Gomes *et al.*, 2011	Portugal	2009	73
		0.52±3.27	Lavender	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	9
		4.50±4.97	*Lavandula latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		2.28±2.69	*Lavandula angustifolia x latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		24.42±6.78	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	Diastase (º Schade)	20.90±6.60	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		25.40±0.63	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		10.80±0.27	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		14.51±1.96	*Lavandula* sp.	Devillers *et al.*, 2004	France	-	57
		14.10±2.40	*Lavandula* sp.	Persano-Oddo and Piro, 2004	Europe	1970-2002	64
		40.54	*Lavandula stoechas*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	7
		32.23	*Lavandula latifolia*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	15

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
LAVENDER	Diastase (º Schade)	15.65±0.05	*Lavandula stoechas*	Chakir *et al.*, 2011	Morocco	2007	2
		15.35±0.61	*Lavandula stoechas*	Gomes *et al.*, 2011	Portugal	2009	73
		8.44±2.85	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	Moisture (%)	17.0±0.6	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		17.2±0.4	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		16.0±0.4	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		16.6±0.1	*Lavandula latifolia*	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		16.7±0.5	*Lavandula* sp.	Devillers *et al.*, 2004	France	-	57
		16.7±0.7	*Lavandula* sp.	Persano-Oddo and Piro, 2004	Europe	1970-2002	96
		16.9	*Lavandula stoechas*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	7
		17.5	*Lavandula latifolia*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	15
		16.9±0.6	*Lavandula stoechas*	Chakir *et al.*, 2011	Morocco	2007	2
		17.2±0.6	*Lavandula stoechas*	Gomes *et al.*, 2011	Portugal	2009	73
		15.9±1.6	Lavender	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	9
		16.6±1.6	*Lavandula latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		15.4±0.7	*Lavandula angustifolia x latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		18.7±1.5	*Lavandula* sp.	Anjos *et al.*, 2015	Portugal and Spain	-	12
		17.2±0.9	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	Electrical conductivity (mS/cm)	0.309±0.090	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		0.184±0.005	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		0.166±0.004	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		0.269±0.029	*Lavandula latifolia*	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		0.221±0.053	*Lavandula* sp.	Devillers *et al.*, 2004	France	-	57
		0.210±0.050	*Lavandula* sp.	Persano-Oddo and Piro, 2004	Europe	1970-2002	231
		0.433±0.114	*Lavandula stoechas*	Chakir *et al.*, 2011	Morocco	2007	2
		0.260±0.090	*Lavandula stoechas*	Gomes *et al.*, 2011	Portugal	2009	73
		0.300±0.070	Lavender	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	9
		0.220±0.070	*Lavandula latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		0.290±0.050	*Lavandula angustifolia x latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		0.326±0.121	*Lavandula* sp.	Anjos *et al.*, 2015	Portugal and Spain	-	12

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
LAVENDER	Electrical conductivity (mS/cm)	0.320±0.120	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	pH	3.55±0.18	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		3.54	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		3.74	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		3.97±0.05	*Lavandula latifolia*	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		3.70±0.08	*Lavandula* sp.	Devillers *et al.*, 2004	France	-	57
		3.80±0.10	*Lavandula* sp.	Persano-Oddo and Piro, 2004	Europe	1970-2002	89
		3.98±0.08	*Lavandula stoechas*	Chakir *et al.*, 2011	Morocco	2007	2
		3.74±0.13	*Lavandula stoechas*	Gomes *et al.*, 2011	Portugal	2009	73
		4.21±0.30	Lavender	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	9
		3.88±0.25	*Lavandula latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		4.02±0.13	*Lavandula angustifolia x latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		3.81±0.33	*Lavandula* sp.	Anjos *et al.*, 2015	Portugal and Spain	-	12
	Free acidity (meq/kg)	38.8±8.4	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		26.2±0.7	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		14.0±0.4	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		14.9±1.4	*Lavandula* sp.	Devillers *et al.*, 2004	France	-	57
		17.3±4.0	*Lavandula* sp.	Persano-Oddo and Piro, 2004	Europe	1970-2002	90
		23.4	*Lavandula stoechas*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	7
		22.9	*Lavandula latifolia*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	15
		30.7±3.0	*Lavandula stoechas*	Chakir *et al.*, 2011	Morocco	2007	2
		40.3±0.7	*Lavandula stoechas*	Gomes *et al.*, 2011	Portugal	2009	73
		23.4±3.7	Lavender	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	9
		19.3±4.2	*Lavandula latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		20.7±3.3	*Lavandula angustifolia x latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		23.7±7.7	*Lavandula* sp.	Anjos *et al.*, 2015	Portugal and Spain	-	12
	Lactones (meq/kg)	2.8±2.8	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		3.9±0.1	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		1.3±0.0	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
LAVENDER	Lactones (meq/kg)	9.7±2.5	*Lavandula* sp.	Persano-Oddo and Piro, 2004	Europe	1970-2002	84
		12.8±0.0	*Lavandula stoechas*	Chakir *et al.*, 2011	Morocco	2007	2
	Total acidity (meq/kg)	41.6±9.0	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		30.1±0.8	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		15.3±0.4	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		26.3±2.9	*Lavandula* sp.	Persano-Oddo and Piro, 2004	Europe	1970-2002	84
		43.5±3.1	*Lavandula stoechas*	Chakir *et al.*, 2011	Morocco	2007	2
	Lactones/free acidity	2.41±0.23	*Lavandula stoechas*	Chakir *et al.*, 2011	Morocco	2007	2
	Proline (mg/100 g)	55.60±29.70	Lavender	Hermosin *et al.*, 2003	Spain	-	4
		55.31±14.04	*Lavandula angustifolia, Lavandula angustifolia x latifolia*	Cotte *et al.*, 2004	France	-	53
		75.90	*Lavandula latifolia*	Bernal *et al.* 2005	Soria (Spain)	-	1
		78.40	*Lavandula stoechas*	Bernal *et al.* 2005	Soria (Spain)	-	1
		54.18	Lavender	Alzaharani *et al.*, 2012	Saudi Arabia	-	1
		53.70±10.80	Lavender	Czipa *et al.*, 2012	International	-	3
		47.05±1.44	*Lavandula stoechas*	Aazza *et al.*, 2013	Portugal	2011	2
		61.50±12.60	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
		111.75±1.91	Lavender	Kivrak, 2015	Turkey	2013	4
	Specific rotation (º)	-1.97±0.05	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		-3.14±0.08	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		-8.30±3.80	*Lavandula* sp.	Persano-Oddo and Piro, 2004	Europe	1970-2002	4
		-2.44±1.10	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	Fructose (%)	38.20±0.93	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		37.9±0.95	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		40.5±1.01	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		37.60±0.90	*Lavandula latifolia*	Mateo and Boch-Reig, 1997	Spain	-	15
		37.40±0.23	*Lavandula latifolia*	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		38.46±1.30	*Lavandula* sp.	Cotte *et al.*, 2003	France	-	53
		35.51±1.09	*Lavandula* sp.	Devillers *et al.*, 2004	France	-	57
		36.00±1.90	*Lavandula* sp.	Persano-Oddo and Piro, 2004	Europe	1970-2002	219
		37.40±3.20	*Lavandula latifolia*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	17

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
LAVENDER	Fructose (%)	37.00±8.50	*Lavandula stoechas*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	8
		40.95±5.10	*Lavandula* sp.	Martins *et al.*, 2008	Portugal	1991	20
		41.19±0.85	*Lavandula stoechas*	Chakir *et al.*, 2011	Morocco	2007	2
		37.72±2.85	Lavender	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	9
		32.65±1.28	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	Glucose (%)	31.20±1.96	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		32.10±0.80	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		28.80±0.72	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		30.80±2.20	*Lavandula latifolia*	Mateo and Boch-Reig, 1997	Spain	-	15
		30.50±0.73	*Lavandula latifolia*	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		32.33±1.31	*Lavandula* sp.	Cotte *et al.*, 2003	France	-	53
		31.37±1.83	*Lavandula* sp.	Devillers *et al.*, 2004	France	-	57
		30.60±1.90	*Lavandula* sp.	Persano-Oddo and Piro, 2004	Europe	1970-2002	218
		36.10±3.90	*Lavandula latifolia*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	17
		35.60±6.90	*Lavandula stoechas*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	8
		28.73±2.82	*Lavandula* sp.	Martins *et al.*, 2008	Portugal	1991	20
		27.79±0.71	*Lavandula stoechas*	Chakir *et al.*, 2011	Morocco	2007	2
		29.53±1.78	Lavender	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	9
		22.19±6.79	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	Sucrose (%)	0.26±0.26	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		1.09±0.03	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		0.35±0.01	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		0.82±1.10	*Lavandula latifolia*	Mateo and Boch-Reig, 1997	Spain	-	15
		0.88±0.33	*Lavandula latifolia*	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		4.68±2.82	*Lavandula* sp.	Cotte *et al.*, 2003	France	-	53
		2.69±0.85	*Lavandula* sp.	Devillers *et al.*, 2004	France	-	57
		5.70±3.30	*Lavandula* sp.	Persano-Oddo and Piro, 2004	Europe	1970-2002	218
		1.94	*Lavandula stoechas*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	7
		2.08	*Lavandula latifolia*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	15
		0.47±0.12	*Lavandula latifolia*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	17

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
LAVENDER	Sucrose (%)	0.24±0.09	*Lavandula stoechas*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	8
		1.06±0.97	*Lavandula* sp.	Martins *et al.*, 2008	Portugal	1991	20
		8.01±0.22	*Lavandula stoechas*	Gomes *et al.*, 2011	Portugal	2009	73
		0.61±0.36	*Lavandula stoechas*	Chakir *et al.*, 2011	Morocco	2007	2
		ND	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	Trehalose (%)	0.69±0.09	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		0.79±0.25	*Lavandula* sp.	Cotte *et al.*, 2003	France	-	53
		0.14±0.06	*Lavandula latifolia*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	17
		0.12±0.05	*Lavandula stoechas*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	8
		0.33±0.19	*Lavandula* sp.	Martins *et al.*, 2008	Portugal	1991	20
		ND	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	Maltose (%)	5.10±0.98	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		8.12±0.20	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		6.69±0.17	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		4.40±0.60	*Lavandula latifolia*	Mateo and Boch-Reig, 1997	Spain	-	15
		4.40±0.17	*Lavandula latifolia*	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		2.56±0.58	*Lavandula* sp.	Cotte *et al.*, 2003	France	-	53
		0.90±0.31	*Lavandula latifolia*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	17
		1.12±0.36	*Lavandula stoechas*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	8
		3.82±1.67	*Lavandula* sp.	Martins *et al.*, 2008	Portugal	1991	20
		0.36±0.03	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	Gentiobiose (%)	0.03±0.02	*Lavandula* sp.	Cotte *et al.*, 2003	France	-	53
		0.08±0.03	*Lavandula latifolia*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	17
		0.14±0.10	*Lavandula stoechas*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	8
	Isomaltose (%)	0.96±0.27	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		0.97±0.41	*Lavandula latifolia*	Mateo and Boch-Reig, 1997	Spain	-	15
		1.09±0.11	*Lavandula latifolia*	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		0.47±0.17	*Lavandula* sp.	Cotte *et al.*, 2003	France	-	53
		1.14±0.35	*Lavandula latifolia*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	17
		1.44 0.45	*Lavandula stoechas*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	8
		0.34±0.43	*Lavandula* sp.	Martins *et al.*, 2008	Portugal	1991	20

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
LAVENDER	Raffinose (%)	tr	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		0.26±0.32	*Lavandula latifolia*	Mateo and Boch-Reig, 1997	Spain	-	15
		0.05±0.04	*Lavandula* sp.	Cotte *et al.*, 2003	France	-	53
		0.00	*Lavandula* sp.	Devillers *et al.*, 2004	France	-	57
	Erlose (%)	0.18±0.08	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		0.60±0.01	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		1.76±0.04	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		0.39±0.37	*Lavandula latifolia*	Mateo and Boch-Reig, 1997	Spain	-	15
		1.40±0.59	*Lavandula* sp.	Cotte *et al.*, 2003	France	-	53
		0.92±0.56	*Lavandula* sp.	Devillers *et al.*, 2004	France	-	57
		0.67±0.27	*Lavandula latifolia*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	17
		1.12±0.36	*Lavandula stoechas*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	8
	Melezitose (%)	0.18±0.08	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		0.25±0.01	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		ND	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		0.08±0.07	*Lavandula* sp.	Cotte *et al.*, 2003	France	-	53
		0.00	*Lavandula* sp.	Devillers *et al.*, 2004	France	-	57
		0.18±0.09	*Lavandula latifolia*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	17
		0.25±0.13	*Lavandula stoechas*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	8
		0.52±0.01	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	Maltotriose (%)	0.21±0.11	*Lavandula* sp.	Cotte *et al.*, 2003	France	-	53
		0.20±0.11	*Lavandula latifolia*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	17
		0.66±0.32	*Lavandula stoechas*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	8
	Panose (%)	0.12±0.08	*Lavandula* sp.	Cotte *et al.*, 2003	France	-	53
		0.27±0.12	*Lavandula latifolia*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	17
		0.41±0.18	*Lavandula stoechas*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	8
	Isomaltotriose (%)	0.24±0.14	*Lavandula latifolia*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	17
		0.36±0.13	*Lavandula stoechas*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	8

HONEY	PARAMETER	DATA	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
LAVENDER	Fructose+glucose	66.60±2.90	*Lavandula* sp.	Persano-Oddo and Piro, 2004	Europe	1970-2002	218
		71.04	*Lavandula stoechas*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	7
		71.12	*Lavandula latifolia*	Nozal-Nalda *et al.*, 2005	Soria (Spain)	2001-2003	15
		67.80±0.53	*Lavandula stoechas*	Gomes *et al.*, 2011	Portugal	2009	73
		54.84	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	Fructose/glucose ratio	1.23±0.10	*Lavandula stoechas*	Serra-Bonvehí and Ventura-Coll, 1993	Ávila, Segovia, Madrid, Badajoz (Spain)	1988-1989	26
		1.18±0.03	*Lavandula latifolia*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		1.23±0.00	*Lavandula latifolia*	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		1.18±0.07	*Lavandula* sp.	Persano-Oddo and Piro, 2004	Europe	1970-2002	218
		1.41±0.04	*Lavandula stoechas*	Pérez-Arquillué *et al.*, 1995	Spain	-	1
		1.22±0.08	*Lavandula latifolia*	Mateo and Boch-Reig, 1997	Spain	-	15
		1.19±0.05	*Lavandula* sp.	Cotte *et al.*, 2003	France	-	53
		1.47	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	Glucose/moisture ratio	1.86±0.15	*Lavandula latifolia*	Mateo and Boch-Reig, 1997	Spain	-	15
		1.83±0.05	*Lavandula latifolia*	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		1.88±0.09	*Lavandula* sp.	Persano-Oddo and Piro, 2004	Europe	1970-2002	59
	Maltose/ isomaltose ratio	0.89±1.17	*Lavandula stoechas*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	8
		0.53±0.17	*Lavandula latifolia*	Nozal *et al.*, 2005	Soria (Spain)	2001-2003	17

REFERENCES

AAZZA, S; LYOUSSI, B; ANTUNES, D; MIGUEL, M G (2013) physicochemical characterization and antioxidant activity of commercial Portuguese honeys. *Journal of Food Science* 78(8): C1159-C1165. http://dx.doi.org/10.1111/1750-3841.12201

ABELL, D C; FRIEBE, H; SCHWEGER, C; KWOK, A S K; SPORNS, P (1996) Comparison of processed unifloral clover and canola honey. *Apidologie* 27(6): 451-460. http://dx.doi.org/10.1051/apido:19960603

ABU-TARBOUSH, H M; AL-KAHTANI, H A; EL-SARRAGE, M S (1993) Floral-type identification and quality evaluation of some honey types. *Food Chemistry* 46(1): 13-17. http://dx.doi.org/10.1016/0308-8146(93)90068-q

ALVES, A; RAMOS, A; GONÇALVES, M M; BERNARDO, M; MENDES, B (2013) Antioxidant activity, quality parameters and mineral content of Portuguese monofloral honeys. *Journal of Food Composition and Analysis* 30(2): 130-138. http://dx.doi.org/10.1016/j.jfca.2013.02.009

ALZAHRANI, H A; ALSABEHI, R; BOUKRAÂ, L; ABDELLAH, F; BELLIK, Y; BAKHOTMAH, B A (2012) Antibacterial and antioxidant potency of floral honeys from different botanical and geographical origins. *Molecules* 17: 10540-10549. http://dx.doi.org/10.3390/molecules170910540

ANDRADE, P B; AMARAL, M T; ISABEL, P; CARVALHO, J C M F; SCABRA, R M; PROENÇA DA CUNHA, A (1999) Physicochemical attributes and pollen spectrum of Portuguese Heather honeys. *Food Chemistry* 66(4): 503-510. http://dx.doi.org/10.1016/S0308-8146(99)00100-4

ANJOS, O; CAMPOS, M G; RUIZ, P C; ANTUNES, P (2015) Application of FTIR-ATR spectroscopy to the quantification of sugar in honey. *Food Chemistry* 169: 218-223. http://dx.doi.org/10.1016/j.foodchem.2014.07.138

BENTABOL, A; HERNÁNDEZ-GARCÍA, Z; RODRÍGUEZ-GALDÓN, B; RODRÍGUEZ-RODRÍGUEZ, E; DÍAZ-ROMERO, C (2011) Differentiation of blossom and honeydew honeys using multivariate analysis on the physicochemical parameters and sugar composition. *Food Chemistry* 126(2): 664-672. http://dx.doi.org/10.1016/j.foodchem.2010.11.003

BENTABOL, A; HERNÁNDEZ-GARCÍA, Z; RODRÍGUEZ-GALDÓN, B; RODRÍGUEZ-RODRÍGUEZ, E; DÍAZ-ROMERO, C (2014) Physicochemical characteristics of minor monofloral honeys from Tenerife, Spain. *LWT - Food Science and Technology* 55(2): 572-578. http://dx.doi.org/10.1016/j.lwt.2013.09.024

BERNAL, J L; NOZAL, M J; TORIBIO, L; DIEGO, J C; RUIZ, A (2005) A comparative study of several HPLC methods for determining free amino acid profiles in honey. *Journal of Separation Science* 28: 1039-1047.

BERTONCELJ, J; GOLOB, T; KROPF, U; KOROŠEC, M (2011) Characterisation of Slovenian honeys on the basis of sensory and physicochemical analysis with a chemometric approach. *International Journal of Food Science & Technology* 46(8): 1661-1671. http://dx.doi.org/10.1111/j.1365-2621.2011.02664.x

CAN, Z; YILDIZ, O; SAHIN, H; TURUMTAY, E A; SILICI, S; KOLAYLI, S (2015) An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles. *Food Chemistry* 180: 133-141. http://dx.doi.org/10.1016/j.foodchem.2015.02.024

CASTRO-VÁZQUEZ, L; ALAÑON, M E; GONZÁLEZ-VIÑAS, M A; PÉREZ-COELLO, M S (2012) Changes in the volatile fractions and sensory properties of Heather honey during storage under different temperatures. *European Food Research Technology* 235(2): 185-193. http://dx.doi.org/10.1007/s00217-012-1756-1

CASTRO-VÁZQUEZ, L; LEÓN-RUIZ, V; ALAÑÓN, M E; PÉREZ-COELLO, M S; GONZÁLEZ-PORTO, A V (2014) Floral origin markers for authenticating Lavandin honey (*Lavandula angustifolia x latifolia*). Discrimination from Lavender honey (*Lavandula latifolia*). *Food Control* 37: 362-370. http://dx.doi.org/10.1016/j.foodcont.2013.09.003

CELECHOVSKÁ, O; VORLOVÁ, L (2001) Groups of honey-physicochemical properties and heavy metals. *Acta Veterinaria Brno* 70: 91-95.

CHAKIR, A; ROMANE, A; MARCAZZAN, G L; FERRAZZI, P (2011) Physicochemical properties of some honeys produced from different plants in Morocco. *Arabian Journal of Chemistry*. In press. http://dx.doi.org/10.1016/j.arabjc.2011.10.013

COTTE, J .; CASABIANCA, H; CHARDON, S; LHERITIER, J; GRENIER-LOUSTALOT, M F (2003) Application of carbohydrate analysis to verify honey authenticity. *Journal of Chromatography A* 1021(1/2): 145-155. http://dx.doi.org/10.1016/j.chroma.2003.09.005

COTTE, J F; CASABIANCA, H; CHARDON, S; LHERITIER, J; GRENIER-LOUSTALOT, M F (2004) Chromatographic analysis of sugars applied to the characterization of monofloral honey. *Analytical and Bioanalytical Chemistry* 380(4): 698-705. http://dx.doi.org/ 10.1007/s00216-004-2764-1

CZIPA, N; BORBÉLY, M; GYŐRI, Z (2012) Proline content of different honey types. *Acta Alimentaria* 41(1): 26-32. http://dx.doi.org/10.1556/AAlim.2011.0002

DE LA FUENTE, E; VALENCIA-BARRERA, R M; MARTÍNEZ-CASTRO, I; SANZ, J (2007) Occurrence of 2-hydroxy-5-methyl-3-hexanone and 3-hydroxy-5-methyl-2-hexanone as indicators of botanic origin in eucalyptus honeys. *Food Chemistry* 103(4): 1176-1180. http://dx.doi.org/10.1016/j.foodchem.2006.10.020

DE LA FUENTE, E; RUIZ-MATUTE, A I; VALENCIA-BARRERA, R M; SANZ, J; MARTÍNEZ CASTRO, I (2011) Carbohydrate composition of Spanish unifloral honeys. *Food Chemistry* 129(4): 1483-1489. http://dx.doi.org/10.1016/j.foodchem.2011.05.121

DEVILLERS, J; MORLOT, M; PHAM-DELÈGUE, M H; DORÉ, J C (2004) Classification of monofloral honeys based on their quality control data. *Food Chemistry* 86(2): 305-312. http://dx.doi.org/10.1016/j.foodchem.2003.09.029

DINKOV, D (2003) A scientific note on the specific optical rotation of three honey types from Bulgaria. *Apidologie* 34(4): 319-320. http://dx.doi.org/10.1051/apido:2003017

ESCUREDO, O; FERNÁNDEZ-GONZÁLEZ, M; SEIJO, M C (2012) Differentiation of blossom honey and honeydew honey from North-West Spain. *Agriculture* 2(1): 25-37. http://dx.doi.org/10.3390/agriculture2010025

ESCUREDO, O; MÍGUEZ, M; FERNÁNDEZ-GONZÁLEZ, M; SEIJO, M C (2013) Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. *Food Chemistry* 138(2/3): 851-856. http://dx.doi.org/10.1016/j.foodchem.2012.11.015

ESCUREDO, O; DOBRE, I; FERNÁNDEZ-GONZÁLEZ, M; SEIJO, M C (2014) Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. *Food Chemistry* 149: 84-90. http://dx.doi.org/10.1016/j.foodchem.2013.10.097

FALLICO, B; ZAPPALÀ, M; ARENA, E; VERZERA, A (2004) Effects of conditioning on HMF content in unifloral honeys. *Food Chemistry* 85(2): 305-313. http://dx.doi.org/10.1016/j.foodchem.2003.07.010

FEÁS, X; PIRES, J; IGLESIAS, A; ESTEVINHO, M L (2010) Characterization of artisanal honey produced on the Northwest of Portugal by melissopalynological and physico-chemical data. *Food and Chemical Toxicology* 48(12): 3462-3470. http://dx.doi.org/10.1016/j.fct.2010.09.024

GOLOB, T; PLESTENJAK, A (1999) Quality of Slovene Honey. *Food Technology and Biotechnology* 37(3): 195-201.

GOMES, T; FEÁS, X; IGLESIAS, A; ESTEVINHO, L M (2011) Study of organic honey from the Northeast of Portugal. *Molecules* 16(12): 5374-5386. http://dx.doi.org/10.3390/molecules16075374

GONZÁLEZ-LORENTE, M; DE LORENZO-CARRETERO, C; PÉREZ-MARTÍN, R A (2008) Sensory attributes and antioxidant capacity of Spanish honeys. *Journal of Sensory Studies* 23(3): 293-302. http://dx.doi.org/10.1111/j.1745-459X.2008.00156.x

HERMOSÍN, I; CHICÓN, R M; CABEZUDO, D (2003) Free amino acid composition and botanical origin of honey. *Food Chemistry* 83(2): 263-268. http://dx.doi.org/10.1016/S0308-8146(03)00089-X

IGLESIAS, M T; DE LORENZO, C; POLO, M D C; MARTÍN-ÁLVAREZ, P J; PUEYO, E (2004) Usefulness of amino acid composition to discriminate between honeydew and floral honeys. application to honeys from a small geographic area. *Journal of Agricultural and Food Chemistry* 52(1): 84-89. http://dx.doi.org/10.1021/jf030454q

IGLESIAS, M T; MARTÍN-ÁLVAREZ, P J; POLO, M C; DE LORENZO, C; GONZÁLEZ, M; PUEYO, E (2006) Changes in the free amino acid contents of honeys during storage at ambient temperature. *Journal of Agricultural and Food Chemistry* 54(24): 9099-9104. http://dx.doi.org/10.1021/jf061712x

İNAN, Ö; ÖZCA, M M; ARSLAN, D; ÜNVER, A (2012) Some physico-chemical and sensory properties of heat treated commercial pine and blossom honey. *Journal of Apicultural Research* 51(4): 347-352. http://dx.doi.org/10.3896/IBRA.1.51.4.09

KAMBOJ, R; BERA, M B; NANDA, V (2013) Evaluation of physico-chemical properties, trace metal content and antioxidant activity of Indian honeys. *International Journal of Food Science & Technology* 48(3): 578-587. http://dx.doi.org/10.1111/ijfs.12002

KARABAGIAS, I K; BADEKA, A V.; KONTAKOS, S; KARABOURNIOTI, S; KONTOMINAS, M G (2014) Botanical discrimination of Greek unifloral honeys with physico-chemical and chemometric analyses. *Food Chemistry* 165: 181-190. http://dx.doi.org/10.1016/j.foodchem.2014.05.033

KASPEROVÁ, J; NAGY, J; POPELKA, P; DIČÁKOVÁ, Z; NAGYOVÁ, A; MAĽA, P (2012) Physico-chemical indicators and identification of selected Slovak honeys based on colour measurement. *Acta Veterinaria Brno* 81(1): 57-61. http://dx.doi.org/10.2754/avb201281010057

KIVRAK, İ (2015) Free amino acid profiles of 17 Turkish unifloral honeys. *Journal of Liquid Chromatography & Related Technologies* 38(8): 855-862. http://dx.doi.org/10.1080/10826076.2014.976712

KROPF, U; KOROŠEC, M; BERTONCELJ, J; OGRINC, N; NEČEMER, M; KUMP, P; GOLOB, T (2010) Determination of the geographical origin of Slovenian black locust, lime and chestnut honey. *Food Chemistry* 121(3): 839-846.http://dx.doi.org/10.1016/j.foodchem.2009.12.094

KÜÇÜK, M; KOLAYLI, S; KARAOĞLU, Ş; ULUSOY, E; BALTACI, C; CANDAN, F (2007) Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chemistry* 100(2): 526-534. http://dx.doi.org/10.1016/j.foodchem.2005.10.010

KUŚ, P M; CONGIU, F; TEPER, D; SROKA, Z; JERKOVIĆ, I; TUBEROSO, C I G (2014a) Antioxidant activity, color characteristics, total phenol content and general HPLC fingerprints of six Polish unifloral honey types. *LWT-Food Science and Technology* 55(1): 124-130. http://dx.doi.org/10.1016/j.lwt.2013.09.016

LANGFORD, V; GRAY, J; FOULKES, B; BRAY, P; MCEWAN, M J (2012) Application of selected ion flow tube-mass spectrometry to the characterization of monofloral New Zealand honeys. *Journal of Agricultural and Food Chemistry* 60(27): 6806-6815. http://dx.doi.org/10.1021/jf3025002

LEÓN-RUIZ, V; VERA, S; GONZÁLEZ-PORTO, A V.; ANDRÉS, M P S (2011) Vitamin C and sugar levels as simple markers for discriminating Spanish honey sources. *Journal of Food Science* 76(3): C356-C361. http://dx.doi.org/10.1111/j.1750-3841.2011.02041.x

MALACALZA, N H; CACCAVARI, M A; FAGÚNDEZ, G; LUPANO, C E (2005) Unifloral honeys of the province of Buenos Aires, Argentine. *Journal of the Science of Food and Agriculture* 85(8): 1389-1396. http://dx.doi.org/10.1002/jsfa.2105

MARINI, F; MAGRÌ, A L; BALESTRIERI, F; FABRETTI, F; MARINI, D (2004) Supervised pattern recognition applied to the discrimination of the floral origin of six types of Italian honey samples. *Analytica Chimica Acta* 515(1): 117-125. http://dx.doi.org/10.1016/j.aca.2004.01.013

MARTINS, R C; LOPES, V V.; VALENTÃO, P; CARVALHO, J C M F; ISABEL, P; AMARAL, M T; BATISTA, M T; ANDRADE, P B; SILVA, B M (2008) Relevant principal component analysis applied to the characterisation of Portuguese heather honey. *Natural Product Research* 22(17): 1560-1582. http://dx.doi.org/10.1080/14786410701825004

MATEO, R; BOSCH-REIG, F (1997) Sugar profiles of Spanish unifloral honeys. *Food Chemistry* 60(1): 33-41. http://dx.doi.org/10.1016/S0308-8146(96)00297-X

MATEO, R; BOSCH-REIG, F (1998) Classification of Spanish unifloral honeys by discriminant analysis of electrical conductivity, color, water content, sugars, and pH. *Journal of Agricultural and Food Chemistry* 46(2): 393-400. http://dx.doi.org/10.1021/jf970574w

MĂRGHITAŞ, L A; DEZMIREAN, D; MOISE, A; BOBIS, O; LASLO, L; BOGDANOV, S (2009) Physico-chemical and bioactive properties of different floral origin honeys from Romania. *Food Chemistry* 112(4): 863-867. http://dx.doi.org/10.1016/j.foodchem.2008.06.055

MOISE, A; MĂRGHITAŞ, A L; DEZMIREAN, D; BOBIS, O (2013) Nutraceutical properties of Romanian heather honey. *Nutrition & Food Science* 43(3): 218-227. http://dx.doi.org/10.1108/00346651311327864

NANDA, V; SARKAR, B C; SHARMA, H K; BAWA, A S (2003) Physico-chemical properties and estimation of mineral content in honey produced from different plants in Northern India. *Journal of Food Composition and Analysis* 16(5): 613-619. http://dx.doi.org/10.1016/S0889-1575(03)00062-0

NOZAL, M J; BERNAL, J L; TORIBIO, L; ALAMO, M; DIEGO, J C; TAPIA, J (2005) The use of carbohydrate profiles and chemometrics in the characterization of natural honeys of identical geographical origin. *Journal of Agricultural and Food Chemistry* 53(8): 3095-3100. http://dx.doi.org/10.1021/jf0489724

NOZAL-NALDA, M J; BERNAL-YAGÜE, J L; DIEGO-CALVA, J C; MARTÍN-GÓMEZ, M T (2005) Classifying honeys from the Soria Province of Spain via multivariate analysis. *Analytical and Bioanalytical Chemistry* 382(2): 311-319. http://dx.doi.org/10.1007/s00216-005-3161-0

OROIAN, M (2012) Physicochemical and Rheological Properties of Romanian Honeys. *Food Biophysics* 7(4): 296-307. http://dx.doi.org/10.1007/s11483-012-9268-x

OUCHEMOUKH, S; LOUAILECHE, H; SCHWEITZER, P (2007) Physicochemical characteristics and pollen spectrum of some Algerian honeys. *Food Control* 18(1): 52-58. http://dx.doi.org/10.1016/j.foodcont.2005.08.007

OUCHEMOUKH, S; SCHWEITZER, P; BACHIR-BEY, M; DJOUDAD-KADJI, H; LOUAILECHE, H (2010) HPLC sugar profiles of Algerian honeys. *Food Chemistry* 121(2): 561-568. http://dx.doi.org/10.1016/j.foodchem.2009.12.047

ÖZCAN, M M; ÖLMEZ, Ç (2014) Some qualitative properties of different monofloral honeys. *Food Chemistry* 163: 212-218. http://dx.doi.org/10.1016/j.foodchem.2014.04.072

PÉREZ, R A; IGLESIAS, M T; PUEYO, E; GONZALEZ, M; DE LORENZO, C (2007) Amino acid composition and antioxidant capacity of Spanish honeys. *Journal of Agricultural and Food Chemistry* 55(2): 360-365. http://dx.doi.org/10.1021/jf062055b

PÉREZ-ARQUILLUÉ, C; CONCHELLO, P; ARIÑO, A; JUAN, T; HERRERA, A (1995) Physicochemical attributes and pollen spectrum of some unifloral Spanish honeys. *Food Chemistry* 54(2): 167-172. http://dx.doi.org/10.1016/0308-8146(95)00022-B

PÉREZ-MARTÍN, R A; VELA-HORTIGÜELA, L; LORENZO-LOZANO, P; ROJO-CORTINA, M D; LORENZO-CARRETERO, C (2008) In vitro antioxidant and antimicrobial activities of Spanish honeys. *International Journal of Food Properties* 11(4): 727-737. http://dx.doi.org/10.1080/10942910701586257

PERSANO-ODDO, L; PIAZZA, M G; SABATINI, A G; ACCORTI, M (1995) Characterization of unifloral honeys. *Apidologie* 26(6): 453-465. http://dx.doi.org/10.1051/apido:19950602

PERSANO-ODDO, L; PIRO, R (2004) Main European unifloral honeys: descriptive sheets. *Apidologie* 35(Suppl. 1): S38-S81. http://dx.doi.org/10.1051/apido:2004049

PIRES, J; ESTEVINHO, M L; FEÁS, X; CANTALAPIEDRA, J; IGLESIAS, A (2009) Pollen spectrum and physico-chemical attributes of Heather (*Erica* sp.) honeys of north Portugal. *Journal of the Science of Food and Agriculture* 89(11): 1862-1870. http://dx.doi.org/10.1002/jsfa.3663

PIRINI, A; CONTE, L; FRANCIOSO, O; LERCKER, G (1992) Capillary gas chromatographic determination of free amino acids in honey as a means of discrimination between botanical sources. *Journal of High Resolution Chromatography* 15(3): 165-170. http://dx.doi.org/10.1002/jhrc.1240150306

POPEK, S (2002) A procedure to identify a honey type. *Food Chemistry* 79(3): 401-406. http://dx.doi.org/10.1016/S0308-8146(02)00391-6

PRIMORAC, L; FLANJAK, I; KENJERIC, D; BUBALO, D; TOPOLNJAK, Z (2011) Specific rotation and carbohydrate profile of Croatian unifloral honeys. *Czech Journal of Food Science* 29(5): 515-519.

RODRÍGUEZ-FLORES, M S; ESCUREDO, O; SEIJO, M C (2015) Assessment of physicochemical and antioxidant characteristics of *Quercus pyrenaica* honeydew honeys. *Food Chemistry* 166: 101-106. http://dx.doi.org/10.1016/j.foodchem.2014.06.005

RYBAK-CHMIELEWSKA, H; SZCZĘSNA, T; WAŚ, E; JAŚKIEWICZ, K; TEPER, D (2013) Characteristics of Polish Unifloral Honeys IV. Honeydew Honey, Mainly *Abies Alba* L. *Journal of Apicultural Science* 57(1): 51-59. http://dx.doi.org/10.2478/jas-2013-0006

ŠARIĆ, G; MATKOVIĆ, D; HRUŠKAR, M; VAHČIĆ, N (2008) Characterisation and classification of Croatian honey by physicochemical parameters. *Food Technology and Biotechnology* 46(4): 355-367.

SCANDURRA, G; TRIPODI, G; VERZERA, A (2013) Impedance spectroscopy for rapid determination of honey floral origin. *Journal of Food Engineering* 119(4): 738-743. http://dx.doi.org/10.1016/j.jfoodeng.2013.06.042

SERRA-BONVEHÍ, J S; GRANADOS-TARRÉS, E (1993) Physicochemical properties, composition and pollen spectrum of ling Heather (*Calluna vulgaris* (L) Hull) honey produced in Spain. *Apidologie* 24(6): 586-596. http://dx.doi.org/10.1051/apido:19930606

SERRA-BONVEHÍ, J S; VENTURA-COLL, F (1993) Physico-chemical properties, composition and pollen spectrum of french lavender (*Lavandula stoechas* L.) honey produced in Spain. *Lebensmittel-Wissenschaft Und-Technologie* 196(6): 511-517.

SHIN, H-S; USTUNOL, Z (2005) Carbohydrate composition of honey from different floral sources and their influence on growth of selected intestinal bacteria: An in vitro comparison. *Food Research International* 38(6): 721-728. http://dx.doi.org/10.1016/j.foodres.2005.01.007

SILICI, S; KARAMAN, K (2014) Chemometric approaches for the characterization of Turkish rhododendron and honeydew honeys depending on amino acid composition. *Journal of Liquid Chromatography & Related Technologies* 37(6): 864-877. http://dx.doi.org/10.1080/10826076.2012.758149

SINGH, N; BATH, P K (1997) Quality evaluation of different types of Indian honey. *Food Chemistry* 58(1): 129-133. http://dx.doi.org/10.1016/S0308-8146(96)00231-2

SMANALIEVA, J; SENGE, B (2009) Analytical and rheological investigations into selected unifloral German honey. *European Food Research and Technology* 229(1): 107-113. http://dx.doi.org/10.1007/s00217-009-1031-2

SORIA, A C; GONZÁLEZ, M; DE LORENZO, C; MARTÍNEZ-CASTRO, I; SANZ, J (2005) Estimation of the honeydew ratio in honey samples from their physicochemical data and from their volatile composition obtained by SPME and GC-MS. *Journal of the Science of Food and Agriculture* 85(5): 817-824. http://dx.doi.org/10.1002/jsfa.1890

TERRAB, A; VEGA-PÉREZ, J M; DÍEZ, M J; HEREDIA, F J (2001) Characterisation of northwest Moroccan honeys by gas chromatographic-mass spectrometric analysis of their sugar components. *Journal of the Science of Food and Agriculture* 82(2): 179-185. http://dx.doi.org/10.1002/jsfa.1011

TERRAB, A; DÍEZ, M J; HEREDIA, F J (2002) Characterisation of Moroccan unifloral honeys by their physicochemical characteristics. *Food Chemistry* 79(3): 373-379. http://dx.doi.org/10.1016/S0308-8146(02)00189-9

TERRAB, A; GONZÁLEZ, A G; DÍEZ, M J; HEREDIA, F J (2003) Characterisation of Moroccan unifloral honeys using multivariate analysis. *European Food Research and Technology* 218(1): 88-95. http://dx.doi.org/10.1007/s00217-003-0797-x

THRASYVOULOU, A; MANIKIS, J (1995) Some physicochemical and microscopic characteristics of Greek unifloral honeys. *Apidologie* 26(6): 441-452. http://dx.doi.org/10.1051/apido:19950601

TRUZZI, C; ANNIBALDI, A; ILLUMINATI, S; FINALE, C; ROSSETTI, M; SCARPONI, G (2012) Determination of very low levels of 5-(Hydroxymethyl)-2-furaldehyde (HMF) in natural honey: comparison between the HPLC technique and the spectrophotometric White method. *Journal of Food Science* 77(7): C784-C790. http://dx.doi.org/10.1111/j.1750-3841.2012.02782.x

TRUZZI, C; ANNIBALDI, A; ILLUMINATI, S; FINALE, C; SCARPONI, G (2014) Determination of proline in honey: Comparison between official methods, optimization and validation of the analytical methodology. *Food Chemistry* 150: 477-481. http://dx.doi.org/10.1016/j.foodchem.2013.11.003

TURHAN, I; TETIK, N; KARHAN, M; GUREL, F; TAVUKCUOGLU, H R (2008) Quality of honeys influenced by thermal treatment. *LWT-Food Science and Technology* 41(8): 1396-1399. http://dx.doi.org/10.1016/j.lwt.2007.09.008

VANHANEN, L P; EMMERTZ, A; SAVAGE, G P (2011) Mineral analysis of mono-floral New Zealand honey. *Food Chemistry* 128(1): 236-240. http://dx.doi.org/10.1016/j.foodchem.2011.02.064

VELA, L; DE LORENZO, C; PÉREZ, R A (2007) Antioxidant capacity of Spanish honeys and its correlation with polyphenol content and other physicochemical properties. *Journal of the Science of Food and Agriculture* 87(6): 1069-1075. http://dx.doi.org/10.1002/jsfa.2813

WAŚ, E; RYBAK-CHMIELEWSKA, H; SZCZESNA, T; KACHANIUK, K; TEPER, D (2011) Characteristics of Polish unifloral honeys. III. Heather honey (*Calluna vulgaris* L.). *Journal of Apicultural Science* 55(1): 129-137.

TABLE 2

Pearson's correlation matrix of sugars and other physicochemical parameters

	HMF	Diastase	Moisture	Conductivity	pH	Free acidity	Lactones	Total acidity	Lactones/ free acidity
HMF		-0.3821*	-0.2845*	-0.4288*	-0.3893*	-0.0753	0.1114	-0.0413	0.1046
Diastase	-0.3821*		-0.1037	0.2648	0.3291*	0.0253	-0.3158*	-0.0513	-0.2976*
Moisture (W)	-0.2845*	-0.1037		0.1499	-0.0355	0.1367	0.3089*	0.1949	0.3041*
Conductivity	-0.4288*	0.2648	0.1499		**0.8484***	0.4101*	-0.115	0.3386*	-0.2228
pH	-0.3893*	0.3291*	-0.0355	**0.8484***		0.1005	-0.4612*	-0.0185	-0.4983*
Free acidity	-0.0753	0.0253	0.1367	0.4101*	0.1005		0.3599*	**0.9759***	0.0438
Lactones	0.1114	-0.3158*	0.3089*	-0.115	-0.4612*	0.3599*		0.5548*	**0.9326***
Total acidity	-0.0413	-0.0513	0.1949	0.3386*	-0.0185	**0.9759***	0.5548*		0.2573
Lactones/free acidity	0.1046	-0.2976*	0.3041*	-0.2228	-0.4983*	0.0438	**0.9326***	0.2573	
Proline	-0.2206	0.3997*	-0.0257	0.3362*	0.1616	0.6743*	0.1569	0.6376*	-0.0162
Specific rotation	-0.0233	0.2489	-0.3059*	0.4031*	0.4680*	0.2402	-0.5013*	0.0965	-0.5979*
Fructose (F)	0.0960	-0.2085	0.2021	-0.4421*	-0.4353*	-0.3918*	0.2691*	-0.2857*	0.3857*
Glucose (G)	0.2108	-0.4017*	0.1109	**-0.7374***	**-0.7235***	-0.3279*	0.3322*	-0.2141	0.4451*
Sucrose	0.3638*	-0.2427	-0.2906*	-0.3300*	-0.2004	-0.3025*	-0.2136	-0.3195*	-0.2028
Trehalose	0.1589	-0.2426	-0.2086	-0.0632	-0.1209	0.0166	0.1823	0.0571	0.1182
Maltose (M)	0.1177	0.1373	-0.3169*	-0.0777	-0.0974	0.1810	-0.0547	0.1475	-0.1352
Gentiobiose	-0.1623	0.4406*	-0.1718	0.6189*	0.6278*	0.2120	-0.205	0.1400	-0.2788*
Isomaltose (I)	-0.2561	0.4412*	-0.2604	0.6474*	0.6721*	0.3133*	-0.3846*	0.1882	-0.4841*
Raffinose	-0.014	0.1915	-0.3115*	0.3469*	0.3544*	0.1974	-0.1498	0.1417	-0.2666
Erlose	0.2459	-0.0828	-0.4948*	-0.1231	0.0984	-0.0861	-0.4689*	-0.1864	-0.4823*
Melezitose	-0.2104	0.0762	-0.1891	0.3868*	0.3277*	0.1508	-0.148	0.0989	-0.2129
Maltotriose	0.1148	0.0511	-0.4513*	-0.2137	-0.1396	0.0408	-0.1452	0.0017	-0.2199
Panose	-0.104	0.4074*	-0.3829*	0.4183*	0.4817*	0.3322*	-0.3932*	0.2033	-0.5064*
Isomatotriose	-0.2611	0.3783*	-0.1509	0.4956*	0.4372*	0.3058*	-0.1433	0.2377	-0.2207
Maltotetraose	-0.1238	0.1993	-0.2069	-0.0665	-0.0837	0.0260	0.0339	0.0314	-0.0182
Total sugars	0.2510	-0.3159*	-0.0618	-0.6828*	-0.6336*	-0.3762*	0.2019	-0.2879*	0.2959*
Fructose+glucose	0.1693	-0.3343*	0.1589	-0.6411*	-0.6297*	-0.3750*	0.3210*	-0.2587	0.4417*
Total disaccharides	0.1657	0.1636	-0.4952*	0.0584	0.1160	0.1158	-0.2988*	0.0321	-0.3975*
Total oligosaccharides	0.0047	0.0909	-0.5067*	0.2149	0.3210*	0.1039	-0.4220*	-0.0067	-0.4987*
Total DS+OS	0.1327	0.1644	-0.5870*	0.1292	0.2157	0.1316	-0.3991*	0.0228	-0.5068*
F/G	-0.2437	0.4280*	0.0296	**0.7470***	**0.7131***	0.1207	-0.2472	0.0495	-0.3035*
G/W	0.3428*	-0.3041*	-0.3874*	**-0.7649***	-0.6578*	-0.3837*	0.1452	-0.3081*	0.2549
(G-W)/F	0.3167*	-0.3793*	-0.2736*	**-0.8000***	**-0.7151***	-0.3203*	0.2001	-0.2386	0.3018*
M/I	0.1742	-0.3916*	0.1637	-0.5329*	-0.6682*	-0.1306	0.5046*	0.0018	0.5477*

**** Correlation is significant at the p<0.05; In bold type correlations with r>0.7***

	Proline	Specific rotation	Fructose	Glucose	Sucrose	Trehalose	Maltose	Gentiobiose	Isomaltose
HMF	-0.2206	-0.0233	0.0960	0.2108	0.3638*	0.1589	0.1177	-0.1623	-0.2561
Diastase	0.3997*	0.2489	-0.2085	-0.4017*	-0.2427	-0.2426	0.1373	0.4406*	0.4412*
Moisture (W)	-0.0257	-0.3059*	0.2021	0.1109	-0.2906*	-0.2086	-0.3169*	-0.1718	-0.2604
Conductivity	0.3362*	0.4031*	-0.4421*	**-0.7374***	-0.3300*	-0.0632	-0.0777	0.6189*	0.6474*
pH	0.1616	0.4680*	-0.4353*	**-0.7235***	-0.2004	-0.1209	-0.0974	0.6278*	0.6721*
Free acidity	0.6743*	0.2402	-0.3918*	-0.3279*	-0.3025*	0.0166	0.1810	0.2120	0.3133*
Lactones	0.1569	-0.5013*	0.2691*	0.3322*	-0.2136	0.1823	-0.0547	-0.205	-0.3846*
Total acidity	0.6376*	0.0965	-0.2857*	-0.2141	-0.3195*	0.0571	0.1475	0.1400	0.1882
Lactones/free acidity	-0.0162	-0.5979*	0.3857*	0.4451*	-0.2028	0.1182	-0.1352	-0.2788*	-0.4841*
Proline		0.3000*	-0.3689*	-0.3777*	-0.3605*	-0.2573	0.2685*	0.2355	0.3713*
Specific rotation	0.3000*		**-0.7628***	**-0.7468***	0.0822	-0.1321	0.1466	0.5303*	**0.7275***
Fructose (F)	-0.3689*	**-0.7628***		**0.7971***	-0.0338	0.1228	-0.1375	-0.4184*	-0.6566*
Glucose (G)	-0.3777*	**-0.7468***	**0.7971***		0.0610	0.1022	-0.1246	-0.6926*	**-0.8197***
Sucrose	-0.3605*	0.0822	-0.0338	0.0610		-0.0922	0.1914	-0.3057*	-0.2672
Trehalose	-0.2573	-0.1321	0.1228	0.1022	-0.0922		-0.161	0.2576	0.0106
Maltose (M)	0.2685*	0.1466	-0.1375	-0.1246	0.1914	-0.161		-0.1719	0.0519
Gentiobiose	0.2355	0.5303*	-0.4184*	-0.6926*	-0.3057*	0.2576	-0.1719		**0.8154***
Isomaltose (I)	0.3713*	**0.7275***	-0.6566*	**-0.8197***	-0.2672	0.0106	0.0519	**0.8154***	
Raffinose	0.1490	0.4173*	-0.3095*	-0.3963*	-0.0365	0.4192*	0.1049	0.3634*	0.3518*
Erlose	-0.0531	0.4441*	-0.3318*	-0.1599	0.4933*	-0.1357	0.1993	-0.1243	0.0727
Melezitose	0.1175	0.4147*	-0.2381	-0.3900*	-0.0783	0.3746*	-0.1188	0.4340*	0.4198*
Maltotriose	0.1573	0.1559	-0.0779	-0.0519	0.1608	0.3090*	0.2318	0.0110	0.1312
Panose	0.3889*	0.6777*	**-0.7200***	**-0.7210***	-0.0897	0.0261	0.1530	0.5866*	**0.8357***
Isomatotriose	0.2805*	0.3470*	-0.4529*	-0.5256*	-0.1777	0.0279	-0.1766	0.5923*	**0.7386***
Maltotetraose	0.2024	-0.1997	0.2624	0.0908	-0.0931	0.2299	0.0906	0.0277	-0.0047
Total sugars	-0.3786*	-0.6661*	**0.8730***	**0.8973***	0.2289	0.1304	0.1305	-0.6153*	**-0.7260***
Fructose+glucose	-0.3940*	**-0.7945***	**0.9337***	**0.9605***	0.0206	0.1172	-0.1373	-0.6036*	**-0.7888***
Total disaccharides	0.1641	0.4523*	-0.3891*	-0.4098*	0.5146*	-0.1079	**0.8353***	0.0974	0.3348*
Total oligosaccharides	0.1268	0.6023*	-0.4367*	-0.4375*	0.2263	0.1918	0.0922	0.2861*	0.4342*
Total DS+OS	0.1786	0.5900*	-0.4761*	-0.4928*	0.4940*	-0.0112	0.6957*	0.1875	0.4322*
F/G	0.2066	0.4293*	-0.2904*	**-0.8059***	-0.1362	-0.0156	0.0366	0.6940*	0.6517*
G/W	-0.3355*	-0.5417*	0.6464*	**0.8721***	0.2008	0.2005	0.0452	-0.5545*	-0.6359*
(G-W)/F	-0.3273*	-0.5460*	0.5681*	**0.9057***	0.1928	0.1396	0.0104	-0.6435*	-0.6870*
M/I	-0.2803*	-0.6438*	0.4761*	0.6582*	0.3738*	-0.1042	0.1940	**-0.7740***	**-0.8109***

**** Correlation is significant at the p<0.05; In bold type correlations with r>0.7***

	Raffinose	Erlose	Melezitose	Maltotriose	Panose	Isomatotriose	Maltotetraose	Total sugar	F+G
HMF	-0.014	0.2459	-0.2104	0.1148	-0.104	-0.2611	-0.1238	0.251	0.1693
Diastase	0.1915	-0.0828	0.0762	0.0511	0.4074*	0.3783*	0.1993	-0.3159*	-0.3343*
Moisture (W)	-0.3115*	-0.4948*	-0.1891	-0.4513*	-0.3829*	-0.1509	-0.2069	-0.0618	0.1589
Conductivity	0.3469*	-0.1231	0.3868*	-0.2137	0.4183*	0.4956*	-0.0665	-0.6828*	-0.6411*
pH	0.3544*	0.0984	0.3277*	-0.1396	0.4817*	0.4372*	-0.0837	-0.6336*	-0.6297*
Free acidity	0.1974	-0.0861	0.1508	0.0408	0.3322*	0.3058*	0.026	-0.3762*	-0.3750*
Lactones	-0.1498	-0.4689*	-0.148	-0.1452	-0.3932*	-0.1433	0.0339	0.2019	0.3210*
Total acidity	0.1417	-0.1864	0.0989	0.0017	0.2033	0.2377	0.0314	-0.2879*	-0.2587
Lactones/free acidity	-0.2666	-0.4823*	-0.2129	-0.2199	-0.5064*	-0.2207	-0.0182	0.2959*	0.4417*
Proline	0.1490	-0.0531	0.1175	0.1573	0.3889*	0.2805*	0.2024	-0.3786*	-0.3940*
Specific rotation	0.4173*	0.4441*	0.4147*	0.1559	0.6777*	0.3470*	-0.1997	-0.6661*	**-0.7945***
Fructose (F)	-0.3095*	-0.3318*	-0.2381	-0.0779	**-0.7200***	-0.4529*	0.2624	**0.8730***	**0.9337***
Glucose (G)	-0.3963*	-0.1599	-0.3900*	-0.0519	**-0.7210***	-0.5256*	0.0908	**0.8973***	**0.9605***
Sucrose	-0.0365	0.4933*	-0.0783	0.1608	-0.0897	-0.1777	-0.0931	0.2289	0.0206
Trehalose	0.4192*	-0.1357	0.3746*	0.3090*	0.0261	0.0279	0.2299	0.1304	0.1172
Maltose (M)	0.1049	0.1993	-0.1188	0.2318	0.1530	-0.1766	0.0906	0.1305	-0.1373
Gentiobiose	0.3634*	-0.1243	0.4340*	0.0110	0.5866*	0.5923*	0.0277	-0.6153*	-0.6036*
Isomaltose (I)	0.3518*	0.0727	0.4198*	0.1312	**0.8357***	**0.7386***	-0.0047	**-0.7260***	**-0.7888***
Raffinose		0.2926*	0.4635*	0.4111*	0.3974*	0.1499	0.3158*	-0.2619	-0.3777*
Erlose	0.2926*		0.1953	0.2600	0.3095*	-0.1268	-0.171	-0.0307	-0.2477
Melezitose	0.4635*	0.1953		0.3360*	0.3673*	0.2549	0.1424	-0.2457	-0.3411*
Maltotriose	0.4111*	0.2600	0.3360*		0.3888*	0.1319	0.6873*	0.1108	-0.0667
Panose	0.3974*	0.3095*	0.3673*	0.3888*		0.6751*	0.0658	-0.6384*	**-0.7595***
Isomatotriose	0.1499	-0.1268	0.2549	0.1319	0.6751*		0.1409	-0.5298*	-0.5205*
Maltotetraose	0.3158*	-0.171	0.1424	0.6873*	0.0658	0.1409		0.2304	0.1748
Total sugars	-0.2619	-0.0307	-0.2457	0.1108	-0.6384*	-0.5298*	0.2304		**0.9345***
Fructose+glucose	-0.3777*	-0.2477	-0.3411*	-0.0667	-0.7595*	-0.5205*	0.1748	0.9345*	
Total disaccharides	0.2301	0.4085*	0.0732	0.3143*	0.4230*	0.0995	0.0290	-0.0978	-0.4224*
Total oligosaccharides	0.5948*	**0.7406***	**0.7631***	0.5241*	0.5854*	0.2193	0.1280	-0.2466	-0.4608*
Total DS+OS	0.4116*	0.6089*	0.3526*	0.4508*	0.5603*	0.1634	0.0723	-0.1725	-0.5117*
F/G	0.3317*	-0.084	0.3986*	-0.0097	0.4283*	0.3973*	0.0976	-0.5795*	-0.6118*
G/W	-0.207	0.0943	-0.2672	0.1861	-0.4791*	-0.4168*	0.2005	0.8669*	**0.8151***
(G-W)/F	-0.2750*	0.0940	-0.3398*	0.1187	-0.5021*	-0.4398*	0.0918	0.8206*	**0.7990***
M/I	-0.3677*	-0.1068	-0.3701*	-0.1098	-0.6707*	-0.4529*	-0.0092	0.6265*	0.6098*

**** Correlation is significant at the p<0.05; In bold type correlations with r>0.7***

	Total disaccharides	Total oligosaccharides	Total DS+TS	F/G	G/W	(G-W)/F	M/I
HMF	0.1657	0.0047	0.1327	-0.2437	0.3428*	0.3167*	0.1742
Diastase	0.1636	0.0909	0.1644	0.4280*	-0.3041*	-0.3793*	-0.3916*
Moisture (W)	-0.4952*	-0.5067*	-0.5870*	0.0296	-0.3874*	-0.2736*	0.1637
Conductivity	0.0584	0.2149	0.1292	**0.7470***	**-0.7649***	**-0.8000***	-0.5329*
pH	0.1160	0.3210*	0.2157	**0.7131***	-0.6578*	**-0.7151***	-0.6682*
Free acidity	0.1158	0.1039	0.1316	0.1207	-0.3837*	-0.3203*	-0.1306
Lactones	-0.2988*	-0.4220*	-0.3991*	-0.2472	0.1452	0.2001	0.5046*
Total acidity	0.0321	-0.0067	0.0228	0.0495	-0.3081*	-0.2386	0.0018
Lactones/free acidity	-0.3975*	-0.4987*	-0.5068*	-0.3035*	0.2549	0.3018*	0.5477*
Proline	0.1641	0.1268	0.1786	0.2066	-0.3355*	-0.3273*	-0.2803*
Specific rotation	0.4523*	0.6023*	0.5900*	0.4293*	-0.5417*	-0.5460*	-0.6438*
Fructose (F)	-0.3891*	-0.4367*	-0.4761*	-0.2904*	0.6464*	0.5681*	0.4761*
Glucose (G)	-0.4098*	-0.4375*	-0.4928*	**-0.8059***	**0.8721***	**0.9057***	0.6582*
Sucrose	0.5146*	0.2263	0.4940*	-0.1362	0.2008	0.1928	0.3738*
Trehalose	-0.1079	0.1918	-0.0112	-0.0156	0.2005	0.1396	-0.1042
Maltose (M)	**0.8353***	0.0922	0.6957*	0.0366	0.0452	0.0104	0.1940
Gentiobiose	0.0974	0.2861*	0.1875	0.6940*	-0.5545*	-0.6435*	**-0.7740***
Isomaltose (I)	0.3348*	0.4342*	0.4322*	0.6517*	-0.6359*	-0.6870*	**-0.8109***
Raffinose	0.2301	0.5948*	0.4116*	0.3317*	-0.207	-0.2750*	-0.3677*
Erlose	0.4085*	**0.7406***	0.6089*	-0.084	0.0943	0.0940	-0.1068
Melezitose	0.0732	**0.7631***	0.3526*	0.3986*	-0.2672	-0.3398*	-0.3701*
Maltotriose	0.3143*	0.5241*	0.4508*	-0.0097	0.1861	0.1187	-0.1098
Panose	0.4230*	0.5854*	0.5603*	0.4283*	-0.4791*	-0.5021*	-0.6707*
Isomatotriose	0.0995	0.2193	0.1634	0.3973*	-0.4168*	-0.4398*	-0.4529*
Maltotetraose	0.0290	0.1280	0.0723	0.0976	0.2005	0.0918	-0.0092
Total sugars	-0.0978	-0.2466	-0.1725	-0.5795*	**0.8669***	**0.8206***	0.6265*
Fructose+glucose	-0.4224*	-0.4608*	-0.5117*	-0.6118*	**0.8151***	**0.7990***	0.6098*
Total disaccharides		0.3708*	**0.9334***	0.2456	-0.1334	-0.1895	-0.0292
Total oligosaccharides	0.3708*		0.6793*	0.2635	-0.1539	-0.2132	-0.3952*
Total DS+OS	**0.9334***	0.6793*		0.2958*	-0.1649	-0.2321	-0.1757
F/G	0.2456	0.2635	0.2958*		**-0.7543***	**-0.8837***	-0.5670*
G/W	-0.1334	-0.1539	-0.1649	**-0.7543***		**0.9704***	0.5282*
(G-W)/F	-0.1895	-0.2132	-0.2321	**-0.8837***	**0.9704***		0.5813*
M/I	-0.0292	-0.3952*	-0.1757	-0.5670*	0.5282*	0.5813*	

**** Correlation is significant at the p<0.05; In bold type correlations with r>0.7***

TABLE 3

Literature data of bioactive compounds and other antioxidant-related parameters

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CHESTNUT	L*	39.40±3.10	-	-	-	*Castanea sativa*	González-Miret *et al.*, 2005	Spain	2002-2003	4
		48.30	-	-	-	*Castanea sativa*	Kropf *et al.*, 2010	Slovenia	2004-2006	37
		46.18±3.94	-	-	-	*Castanea sativa*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	29
		38.46±6.51	-	-	-	*Castanea sativa*	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	11
		63.00±4.80	-	-	-	*Castanea sativa*	Tuberoso *et al.*, 2014	Italy, Croatia, France	2009-2011	14
		36.83±0.01	-	-	-	*Castanea sativa*	Anjos *et al.*, 2015	Portugal and Spain	-	1
		47.59±1.52	-	-	-	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	a*	23.76±1.63	-	-	-	*Castanea sativa*	González-Miret *et al.*, 2005	Spain	2002-2003	4
		7.80	-	-	-	*Castanea sativa*	Kropf *et al.*, 2010	Slovenia	2004-2006	37
		9.15±2.62	-	-	-	*Castanea sativa*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	29
		21.05±5.85	-	-	-	*Castanea sativa*	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	11
		18.10±4.30	-	-	-	*Castanea sativa*	Tuberoso *et al.*, 2014	Italy, Croatia, France	2009-2011	14
		5.17±0.01	-	-	-	*Castanea sativa*	Anjos *et al.*, 2015	Portugal and Spain	-	1
		42.56±5.88	-	-	-	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	b*	20.38±3.64	-	-	-	*Castanea sativa*	González-Miret *et al.*, 2005	Spain	2002-2003	4
		40.83	-	-	-	*Castanea sativa*	Kropf *et al.*, 2010	Slovenia	2004-2006	37
		38.91±5.71	-	-	-	*Castanea sativa*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	29
		59.52±5.79	-	-	-	*Castanea sativa*	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	11
		82.70±8.80	-	-	-	*Castanea sativa*	Tuberoso *et al.*, 2014	Italy, Croatia, France	2009-2011	14
		0.23±0.01	-	-	-	*Castanea sativa*	Anjos *et al.*, 2015	Portugal and Spain	-	1
		81.52±4.20	-	-	-	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	C*	31.37±3.26	-	-	-	*Castanea sativa*	González-Miret *et al.*, 2005	Spain	2002-2003	4
		63.37±6.13	-	-	-	*Castanea sativa*	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	11
		84.70±9.30	-	-	-	*Castanea sativa*	Tuberoso *et al.*, 2014	Italy, Croatia, France	2009-2011	14

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CHESTNUT	h*	40.40±4.16	-	-	-	*Castanea sativa*	González-Miret *et al.*, 2005	Spain	2002-2003	4
		71.21±4.81	-	-	-	*Castanea sativa*	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	11
		77.80±2.20	-	-	-	*Castanea sativa*	Tuberoso *et al.*, 2014	Italy, Croatia, France	2009-2011	14
	Chlorogenic acid	0.55±0.02	µg/g	-	-	*Castanea sativa*	Pichichero *et al.*, 2009	Italy	2007	3
		4.38±6.00	µg/g	-	-	*Castanea sativa*	Perna *et al.*, 2013	Italy	2009	18
		ND	µg/g	-	-	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
		ND	µg/g	-	-	*Castanea sativa*	Petretto *et al.*, 2015	Italy	-	4
	Caffeic acid	0.87±0.38	µg/g	-	-	*Castanea sativa*	Tomás-Barberán *et al.*, 2001	Europe		9
		0.11±0.00	µg/g	-	-	*Castanea sativa*	Pichichero *et al.*, 2009	Italy	2007	3
		9.97±9.22	µg/g	-	-	*Castanea sativa*	Perna *et al.*, 2013	Italy	2009	18
		4.83±3.25	µg/g	-	-	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	Rutin	3.49±3.97	µg/g	-	-	*Castanea sativa*	Perna *et al.*, 2013	Italy	2009	18
		ND	µg/g	-	-	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
		ND	µg/g	-	-	*Castanea sativa*	Petretto *et al.*, 2015	Italy	-	4
	Coumaric acid	0.30±0.31	µg/g	-	-	*Castanea sativa*	Tomás-Barberán *et al.*, 2001	Europe		9
		2.03	µg/g	-	-	*Castanea sativa*	Dimitrova *et al.*, 2007	Europe	-	
		0.22±0.01	µg/g	-	-	*Castanea sativa*	Pichichero *et al.*, 2009	Italy	2007	3
		5.00±4.65	µg/g	-	-	*Castanea sativa*	Perna *et al.*, 2013	Italy	2009	18
		5.52±3.26	µg/g	-	-	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	Ferulic acid	16.66±33.71	µg/g	-	-	*Castanea sativa*	Perna *et al.*, 2013	Italy	2009	18
		1.64±0.70	µg/g	-	-	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
		ND	µg/g	-	-	*Castanea sativa*	Petretto *et al.*, 2015	Italy	-	4
	Luteolin	1.30±0.50	µg/g	-	-	*Castanea sativa*	Petretto *et al.*, 2015	Italy	-	4
	Quercetin	0.05±0.00	µg/g	-	-	*Castanea sativa*	Pichichero *et al.*, 2009	Italy	2007	3
		0.15±0.13	µg/g	-	-	*Castanea sativa*	Perna *et al.*, 2013	Italy	2009	18
		1.11±0.06	µg/g	-	-	*Castanea sativa*	Campillo *et al.*, 2015	Spain	-	1
		3.50±0.07	µg/g	-	-	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CHESTNUT	Quercetin	ND	µg/g	-	-	*Castanea sativa*	Petretto *et al.*, 2015	Italy	-	4
	Naringenin	0.01±0.00	µg/g	-	-	*Castanea sativa*	Campillo *et al.*, 2015	Spain	-	1
	Kaempferol	0.10±0.00	µg/g	-	-	*Castanea sativa*	Pichichero *et al.*, 2009	Italy	2007	3
		0.46±0.02	µg/g	-	-	*Castanea sativa*	Campillo *et al.*, 2015	Spain	-	1
		ND	µg/g	-	-	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
		ND	µg/g	-	-	*Castanea sativa*	Petretto *et al.*, 2015	Italy	-	4
	Chrysin	0.30±0.15	µg/g	-	-	*Castanea sativa*	Tomás-Barberán *et al.*, 2001	Europe		9
		0.08±0.00	µg/g	-	-	*Castanea sativa*	Pichichero *et al.*, 2009	Italy	2007	3
		0.28±0.03	µg/g	-	-	*Castanea sativa*	Campillo *et al.*, 2015	Spain	-	1
	Pinocembrin	1.12±1.00	µg/g	-	-	*Castanea sativa*	Tomás-Barberán *et al.*, 2001	Europe		9
		1.30±0.70	µg/g	-	-	*Castanea sativa*	Petretto *et al.*, 2015	Italy	-	4
	Galangin	0.15±0.00	µg/g	-	-	*Castanea sativa*	Pichichero *et al.*, 2009	Italy	2007	3
		5.80±1.10	µg/g	-	-	*Castanea sativa*	Petretto *et al.*, 2015	Italy	-	4
	PA_{HPLC}	62.32	µg/g	-	-	*Castanea sativa*	Perna *et al.*, 2013	Italy	2009	18
	TFC_{HPLC}	84.69	µg/g	-	-	*Castanea sativa*	Perna *et al.*, 2013	Italy	2009	18
	TPC_{HPLC}	3.10±0.15	µg/g	-	-	*Castanea sativa*	Pichichero *et al.*, 2009	Italy	2007	3
	TPC	21.12±0.55	mg gallic/ 100 g	Honey	-	*Castanea sativa*	Beretta *et al.*, 2005	Different geographical origins	2003	1
		19.99±3.41	mg gallic/ 100 g	Honey	-	*Castanea sativa*	Bertoncelj *et al.*, 2007	Slovenia	2004	10
		239.00	mg catechin/ 100 g	Honey	-	*Castanea sativa*	Küçük *et al.*, 2007	Turkey	2003	1
		19.46±1.17	mg gallic/ 100 g	Honey	-	*Castanea sativa*	Pichichero *et al.*, 2009	Italy	2007	3
		82.49±4.05	mg gallic/ 100 g	Honey	-	*Castanea sativa*	Giorgi *et al.*, 2011	Italy	2006	1
		60.50±13.21	mg gallic/ 100 g	Honey	-	Chestnut	Kumazawa *et al.*, 2012	Japan	-	3
		14.67±4.64	mg gallic/ 100 g	Honey	-	*Castanea sativa*	Perna *et al.*, 2012	Italy	2009	16
		131.80±29.00	mg gallic/ 100 g	Honey	-	*Castanea sativa*	Escuredo *et al.*, 2013	Northwest of Spain	-	34

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CHESTNUT	TPC	131.30±28.70	mg gallic/ 100 g	Honey	-	*Castanea sativa*	Escuredo *et al.*, 2013	Northwest of Spain	-	35
		113.69±42.99	mg gallic/ 100 g	Honey	-	*Castanea sativa*	León-Ruiz *et al.*, 2013	Castilla la Mancha (Spain)	-	5
		14.26±4.14	mg gallic/ 100 g	Honey	-	*Castanea sativa*	Perna *et al.*, 2013	Italy	2009	18
		98.26±17.77	mg gallic/ 100 g	Honey	-	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
		11.00±0.60	mg gallic/ 100 g	Honey	-	*Castanea sativa*	Petretto *et al.*, 2015	Italy	-	4
	TFC	14.36±0.86	mg quercetin/ 100 g	Honey	$NaNO_2$, 510 nm	*Castanea sativa*	Pichichero *et al.*, 2009	Italy	2007	3
		15.10±16.43	mg quercetin/ 100 g	Honey	$AlCl_3$, 420 nm	Chestnut	Kumazawa *et al.*, 2012	Japan	-	3
		14.05±8.03	mg quercetin/ 100 g	Honey	Dowd	*Castanea sativa*	Perna *et al.*, 2012	Italy	2009	16
		7.60±2.50	mg quercetin/ 100 g	Honey	Dowd $AlCl_3$, 425 nm, No colour correction	*Castanea sativa*	Escuredo *et al.*, 2013	Northwest of Spain	-	34
		7.70±2.50	mg quercetin/ 100 g	Honey	Dowd $AlCl_3$, 425 nm, No colour correction	*Castanea sativa*	Escuredo *et al.*, 2013	Northwest of Spain	-	35
		12.52±6.33	mg quercetin/ 100 g	Honey	Dowd	*Castanea sativa*	Perna *et al.*, 2013	Italy	2009	18
		8.10±2.56	mg quercetin/ 100 g	Extract	HCl, 415 nm	*Castanea sativa*	Can *et al.*, 2015	Turkey	2011-2012	7
	TEAC	60.42±2.12	% I	Honey	10 min, 734 nm	*Castanea sativa*	Perna *et al.*, 2012	Italy	2009	16
		90.81±51.05	µmol gallic/ 100 g	Honey	6 min, 734 nm	*Castanea sativa*	León-Ruiz *et al.*, 2013	Castilla la Mancha (Spain)	-	5
CLOVER	L*	41.21±0.28	-	-	-	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2006	1
		27.11±0.34	-	-	-	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2007	1
		70.35±3.82	-	-	-	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	a*	0.61±0.06	-	-	-	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2006	1
		0.13±0.03	-	-	-	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2007	1
		20.14 ± 1.55	-	-	-	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	b*	8.01±0.48	-	-	-	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2006	1
		0.22±0.20	-	-	-	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2007	1
		5.89±4.80	-	-	-	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CLOVER	Chlorogenic acid	ND	µg/g	-	-	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Caffeic acid	1.51±3.90	µg/g	-	-	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Rutin	ND	µg/g	-	-	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Coumaric acid	4.89±0.30	µg/g	-	-	*Melilotus* sp.	Gheldof *et al.*, 2002	Iowa, USA	2000	1
		ND	µg/g	-	-	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Ferulic acid	ND	µg/g	-	-	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Quercetin	1.57±0.15	µg/g	-	-	*Melilotus* sp.	Gheldof *et al.*, 2002	Iowa, USA	2000	1
		ND	µg/g	-	-	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Kaempferol	3.92±0.77	µg/g	-	-	*Melilotus* sp.	Gheldof *et al.*, 2002	Iowa, USA	2000	1
		ND	µg/g	-	-	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	Chrysin	2.05±0.14	µg/g	-	-	*Melilotus* sp.	Gheldof *et al.*, 2002	Iowa, USA	2000	1
	Pinocembrin	7.04±0.61	µg/g	-	-	*Melilotus* sp.	Gheldof *et al.*, 2002	Iowa, USA	2000	1
	Galangin	1.64±0.33	µg/g	-	-	*Melilotus* sp.	Gheldof *et al.*, 2002	Iowa, USA	2000	1
	TPC	12.80±11.00	mg gallic/ 100 g	Honey	-	*Melilotus* sp.	Gheldof and Engeseth, 2002	Iowa, USA	2000	1
		13.00±1.08	mg gallic/ 100 g	Honey	-	*Melilotus* sp.	Gheldof *et al.*, 2002	Iowa, USA	2000	1
		6.77±1.38	mg gallic/ 100 g	Honey	-	Clover	Wang *et al.*, 2004	Iowa, USA		1
		6.71±0.56	mg gallic/ 100 g	Honey	-	*Trifolium incarnatum*	Beretta *et al.*, 2005	Different geographical origins	2003	1
		70.00	mg gallic/ 100 g	Honey	-	*Medicago sativa*	Chang *et al.*, 2011	China	2009	1
		36.40±3.10	mg gallic/ 100 g	Honey	-	Clover	Kumazawa *et al.*, 2012	Japan	-	1
		49.00	mg gallic/ 100 g	Honey	-	*Medicago sativa*	Alqarni *et al.*, 2014	Saudi Arabia	2010	1
		46.00	mg gallic/ 100 g	Honey	-	*Trifolium alexandrinum*	Alqarni *et al.*, 2014	Saudi Arabia	2010	1
		82.73±28.04	mg gallic/ 100 g	Honey	-	*Trifolium* sp.	Ciappini and Stoppani, 2014	Argentina	2006-2010	53
		179.56±15.68	mg gallic/ 100 g	Honey	-	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2006	1
		183.17±21.94	mg gallic/ 100 g	Honey	-	*Trifolium* sp.	Özcan and Ölmez, 2014	Turkey	2007	1

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
CLOVER	TFC	3.33±0.60	mg gallic/ 100 g	Honey	-	*Medicago sativa*	Shafiee *et al.*, 2014	Iran		27
		661.00±0.02	mg gallic/ 100 g	Honey	-	*Medicago sativa*	Tofighi *et al.*, 2014	Irán	2012	1
		25.53±5.90	mg gallic/ 100 g	Extract	-	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
		1.85	mg rutin/100 g	Honey	$NaNO_3$	*Medicago sativa*	Chang *et al.*, 2011	China	2009	1
		18.10±0.00	mg quercetin/ 100 g	Honey	$AlCl_3$, 420 nm	Clover	Kumazawa *et al.*, 2012	Japan	-	1
		3.29±1.10	mg quercetin/ 100 g	Honey	$AlCl_3$, 425 nm	*Trifolium* sp.	Ciappini and Stoppani, 2014	Argentina	2006-2010	53
		0.65±0.42	mg quercetin/ 100 g	Extract	HCl, 415 nm	*Trifolium* sp.	Can *et al.*, 2015	Turkey	2011-2012	3
	TEAC	14.50±0.30	µg Trolox/ 100 g	Honey	6 min, 734 nm	*Medicago sativa*	Chang *et al.*, 2011	China	2009	1
		aprox. 55.00-125.00	mg Trolox/ 100 g	Honey	24 h, 734 nm	*Trifolium* sp.	Ciappini and Stoppani, 2014	Argentina	2006-2010	53
HEATHER	L*	1.80±0.90	-	-	-	*Erica*ceae (mainly *Erica* sp.)	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		25.46±3.45	-	-	-	Heather	Popek, 2002	Poland	-	8
		9.70±1.80	-	-	-	*E. arborea, E. australis*	Terrab *et al.*, 2003b	Morocco	-	3
		38.55±7.68	-	-	-	*Erica* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	5
		67.30±4.80	-	-	-	*Calluna vulgaris*	Kuś *et al.*, 2014	Poland	2009-2010	3
		66.10±3.80	-	-	-	*Erica* spp.	Tuberoso *et al.*, 2014	Italy, Spain	2010-2012	11
		28.40±6.68	-	-	-	*Erica* sp.	Anjos *et al.*, 2015	Portugal and Spain	-	7
		46.05±7.63	-	-	-	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	a*	1.06±0.48	-	-	-	Heather	Popek, 2002	Poland	-	8
		1.18±0.26	-	-	-	*E. arborea, E. australis*	Terrab *et al.*, 2003b	Morocco	-	3
		17.60±11.75	-	-	-	*Erica* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	5
		10.50±3.50	-	-	-	*Calluna vulgaris*	Kuś *et al.*, 2014	Poland	2009-2010	3
		12.00±2.80	-	-	-	*Erica* spp.	Tuberoso *et al.*, 2014	Italy, Spain	2010-2012	11
		3.73±1.98	-	-	-	*Erica* sp.	Anjos *et al.*, 2015	Portugal and Spain	-	7

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	a*	26.56±1.16	-	-	-	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	b*	6.57±1.39	-	-	-	Heather	Popek, 2002	Poland	-	8
		-3.20±0.55	-	-	-	*E. arborea, E. australis*	Terrab *et al.*, 2003b	Morocco	-	3
		16.22±12.11	-	-	-	*Erica* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	5
		68.00±2.30	-	-	-	*Calluna vulgaris*	Kuś *et al.*, 2014	Poland	2009-2010	3
		68.60±3.10	-	-	-	*Erica* spp.	Tuberoso *et al.*, 2014	Italy, Spain	2010-2012	11
		2.10±3.05	-	-	-	*Erica* sp.	Anjos *et al.*, 2015	Portugal and Spain	-	7
		68.00±2.12	-	-	-	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	C*	3.40±0.42	-	-	-	*E. arborea, E. australis*	Terrab *et al.*, 2003b	Morocco	-	3
		23.98±16.79	-	-	-	*Erica* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	5
		69.70±3.50	-	-	-	*Erica* spp.	Tuberoso *et al.*, 2014	Italy, Spain	2010-2012	11
	h*	-70.00±7.23	-	-	-	*E. arborea, E. australis*	Terrab *et al.*, 2003b	Morocco	-	3
		41.20±4.57	-	-	-	*Erica* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	5
		80.10±2.00	-	-	-	*Erica* spp.	Tuberoso *et al.*, 2014	Italy, Spain	2010-2012	11
	Chlorogenic acid	4.60±3.20	µg/g	-	-	*Erica* sp.	Andrade *et al.*, 1997b	Portugal	-	20
		0.28±0.17	µg/g	-	-	Heather	Sergiel *et al.*, 2014	Poland	-	2
		ND	µg/g	-	-	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Caffeic acid	0.50±0.50	µg/g	-	-	*Erica* sp.	Andrade *et al.*, 1997b	Portugal	-	20
		1.40±0.03	µg/g	-	-	Heather	Michalkiewicz *et al.*, 2008	Poland	-	3
		1.08±0.72	µg/g	-	-	*Calluna vulgaris*	Jasicka-Misiak, 2012	Poland	2009	15
		1.22±0.08	µg/g	-	-	Heather	Sergiel *et al.*, 2014	Poland	-	2
		0.76±1.50	µg/g	-	-	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Rutin	1.60±0.03	µg/g	-	-	Heather	Michalkiewicz *et al.*, 2008	Poland	-	3
		ND	µg/g	-	-	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	Ellagic acid	2.91±1.75	µg/g	-	-	*Erica* sp.	Ferreres *et al.*, 1996	Portugal	-	20
		2.90±1.80	µg/g	-	-	*Erica* sp.	Andrade *et al.*, 1997b	Portugal	-	20
		7.16±2.31	µg/g	-	-	*Erica* sp.	Tomás-Barberán *et al.*, 2001	Europe	-	10
		20.06±6.26	µg/g	-	-	*Calluna vulgaris*	Jasicka-Misiak, 2012	Poland	2009	15
	Coumaric acid	2.98±1.60	µg/g	-	-	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Coumaric acid	14.90±5.20	µg/g	-	-	*Erica* sp.	Andrade *et al.*, 1997b	Portugal	-	20
		10.50±7.72	µg/g	-	-	*Calluna vulgaris*	Jasicka-Misiak, 2012	Poland	2009	15
		7.73±1.06	µg/g	-	-	Heather	Sergiel *et al.*, 2014	Poland	-	2
	Ferulic acid	3.81±1.26	µg/g	-	-	*Calluna vulgaris*	Jasicka-Misiak, 2012	Poland	2009	15
		3.91±0.42	µg/g	-	-	Heather	Sergiel *et al.*, 2014	Poland	-	2
		ND	µg/g	-	-	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Luteolin	aprox. 0.08±0.11	µg/g	-	-	Heather	Sergiel *et al.*, 2014	Poland	-	2
	Quercetin	0.17±0.16	µg/g	-	-	*Erica* sp.	Ferreres *et al.*, 1996	Portugal	-	20
		0.40±0.01	µg/g	-	-	Heather	Michalkiewicz *et al.*, 2008	Poland	-	3
		0.84±2.12	µg/g	-	-	*Calluna vulgaris*	Jasicka-Misiak, 2012	Poland	2009	15
		aprox. 1.92±2.70	µg/g	-	-	Heather	Sergiel *et al.*, 2014	Poland	-	2
		21.05±0.51	µg/g	-	-	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Naringenin	ND	µg/g	-	-	Heather	Campillo *et al.*, 2015	Spain	-	1
	Kaempferol	0.13±0.94	µg/g	-	-	*Erica* sp.	Ferreres *et al.*, 1996	Portugal	-	20
		0.30±0.02	µg/g	-	-	Heather	Michalkiewicz *et al.*, 2008	Poland	-	3
		0.69±0.23	µg/g	-	-	*Calluna vulgaris*	Jasicka-Misiak, 2012	Poland	2009	15
		<0.05	µg/g	-	-	Heather	Sergiel *et al.*, 2014	Poland	-	2
		0.14±0.01	µg/g	-	-	Heather	Campillo *et al.*, 2015	Spain	-	1

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	Kaempferol	ND	µg/g	-	-	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	Chrysin	2.05±1.12	µg/g	-	-	*Erica* sp.	Tomás-Barberán *et al.*, 2001	Europe	-	10
		0.33±0.33	µg/g	-	-	*Calluna vulgaris*	Jasicka-Misiak, 2012	Poland	2009	15
		0.62±0.03	µg/g	-	-	Heather	Campillo *et al.*, 2015	Spain	-	1
	Pinocembrin	5.68±3.29	µg/g	-	-	*Erica* sp.	Tomás-Barberán *et al.*, 2001	Europe	-	10
	Galangin	0.10±0.06	µg/g	-	-	*Calluna vulgaris*	Jasicka-Misiak, 2012	Poland	2009	15
	TPC	154.44±40.89	mg gallic/ 100 g	Honey	-	*Calluna vulgaris*	Wilczyńska, 2010	Poland	2009	3
		68.49±2.83	mg gallic/ 100 g	Honey	-	*Erica* sp.	Estevinho *et al.*, 2012	Portugal	-	75
		72.78±0.02	mg gallic/ 100 g	Honey	-	*Erica australis*	Ferreira *et al.*, 2009	Portugal	2007	1
		20.42±0.06	mg gallic/ 100 g	Extract	-	*Erica australis*	Ferreira *et al.*, 2009	Portugal	2007	1
		657.00±20.00	mg gallic/ 100 g	Honey	-	*Erica arborea*	Ouchemoukh *et al.*, 2010	Algeria	2002	1
		70.12±4.48	mg gallic/ 100 g	Honey	-	*Calluna vulgaris*	Jasicka-Misiak, 2012	Poland	2009	15
		117.59±2.21	mg gallic/ 100 g	Honey	-	*Calluna vulgaris*	Aazza *et al.*, 2013	Portugal	2011	1
		181.00±62.60	mg gallic/ 100 g	Honey	-	*Erica* sp.	Escuredo *et al.*, 2013	Northwest of Spain	-	10
		178.90±59.80	mg gallic/ 100 g	Honey	-	*Erica* sp.	Escuredo *et al.*, 2013	Northwest of Spain	2008-2010	11
		54.22±0.43	mg gallic/ 100 g	Honey	-	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30
		51.70±1.80	mg gallic/ 100 g	Honey	-	Heather	Wieczorek *et al.*, 2014	Poland	2011-2012	1
		155.00±75.00	mg gallic/ 100 g	Honey	-	*Calluna vulgaris*	Wilczyńska, 2014	Poland	2009-2010	3
		105.45±26.08	mg gallic/ 100 g	Extract	-	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	TFC	58.74±0.05	mg catechin/ 100 g	Honey	$NaNO_2$, 510 nm	*Erica australis*	Ferreira *et al.*, 2009	Portugal	2007	1
		51.81±6.16	mg catechin/ 100 g	Honey	$NaNO_3$, 510 nm	*Erica* sp.	Estevinho *et al.*, 2012	Portugal	-	75
		21.16±0.80	mg galangin/ 100 g	Honey	$AlCl_3$, 420 nm	*Calluna vulgaris*	Aazza *et al.*, 2013	Portugal	2011	1

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HEATHER	TFC	6.00±1.40	mg quercetin/ 100 g	Honey	Dowd $AlCl_3$, 425 nm, No colour correction	*Erica* sp.	Escuredo *et al.*, 2013	Northwest of Spain	-	10
		6.50±2.00	mg quercetin/ 100 g	Honey	Dowd $AlCl_3$, 425 nm, No colour correction	*Erica* sp.	Escuredo *et al.*, 2013	Northwest of Spain	2008-2010	11
		59.11±0.32	mg quercetin/ 100 g	Honey	NaNO2, 510 nm	*Calluna vulgaris*	Moise *et al.*, 2013	Romania	-	30
		0.34±0.13	mg quercetin/ 100 g	Honey	Dowd $AlCl_3$, 415 nm, Colour correction	Heather	Wieczorek *et al.*, 2014	Poland	2011-2012	1
		5.84±1.80	mg quercetin/ 100 g	Extract	HCl, 415 nm	*Calluna vulgaris*	Can *et al.*, 2015	Turkey	2011-2012	6
	TEAC	25.45 ±5.34	%I	Honey	15 min, 734 nm	*Calluna vulgaris*	Wilczyńska, 2010	Poland	2009	3
		0.86 ± 0.03	IC50	Honey	Unknown	*Calluna vulgaris*	Aazza *et al.*, 2013	Portugal	2011	1
		1.42±0.08	µmol trolox/ 100 g	Honey	15 min, 734 nm	*Calluna vulgaris*	Wilczyńska, 2014	Poland	2009-2010	3
HONEYDEW	L*	9.10±1.30	-	-	-	*Quercus* sp. (forest honey)	Mateo and Boch-Reig, 1998	Spain	1980-1987	16
		23.70±3.01	-	-	-	Honeydew	Popek, 2002	Poland	-	10
		21.95±8.04	-	-	-	*Quercus* sp. and *Cedrus atlantica*	Terrab *et al.*, 2003a	Northwest Morocco	-	3
		24.81±2.47	-	-	-	*Quercus* sp.	Iglesias *et al.*, 2004	Central Spain	2000-2001	19
		41.52±6.14	-	-	-	*Quercus* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	33
		41.53±4.61	-	-	-	*Abies alba*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	30
		57.85±6.25	-	-	-	*Quercus* sp.	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	5
		31.49±0.03	-	-	-	*Pinus* sp.	Inan *et al.*, 2012	Turkey	-	1
		4.46±1.18	-	-	-	Honeydew	Kasperová *et al.*, 2012	Slovakia	2006-2008	8
		24.80±1.10	-	-	-	*Pinus* sp., *Abies* sp.	Oroian, 2012	Romania	2011	3
		71.06±2.77	-	-	-	*Abies* sp.	Karabagias *et al.*, 2014a	Greece	2011	31
		69.49±2.45	-	-	-	*Pinus* sp.	Karabagias *et al.*, 2014a	Greece	2011	39
		47.10±9.60	-	-	-	Honeydew	Tuberoso *et al.*, 2014	Italy, Croatia, France, Germany	2009-2010	22

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HONEYDEW	L*	34.31±0.01	-	-	-	*Quercus rotundifolia*	Anjos *et al.*, 2015	Portugal and Spain	-	1
		42.85±1.26	-	-	-	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		54.38±4.39	-	-	-	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	a*	0.17±0.30	-	-	-	Honeydew	Popek, 2002	Poland	-	10
		12.56±6.02	-	-	-	*Quercus* sp. and *Cedrus atlantica*	Terrab *et al.*, 2003a	Northwest Morocco	-	3
		-0.82±1.18	-	-	-	*Quercus* sp.	Iglesias *et al.*, 2004	Central Spain	2000-2001	19
		21.15±5.96	-	-	-	*Quercus* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	33
		8.74±1.88	-	-	-	*Abies alba*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	30
		20.55±4.84	-	-	-	*Quercus* sp.	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	5
		4.33±0.03	-	-	-	*Pinus* sp.	Inan *et al.*, 2012	Turkey	-	1
		1.30±2.39	-	-	-	Honeydew	Kasperová *et al.*, 2012	Slovakia	2006-2008	8
		1.20±0.10	-	-	-	*Pinus* sp., *Abies* sp.	Oroian, 2012	Romania	2011	3
		-4.49±1.20	-	-	-	*Abies* sp.	Karabagias *et al.*, 2014a	Greece	2011	31
		-3.69±0.63	-	-	-	*Pinus* sp.	Karabagias *et al.*, 2014a	Greece	2011	39
		23.30±7.30	-	-	-	Honeydew	Tuberoso *et al.*, 2014	Italy, Croatia, France, Germany	2009-2010	22
		4.38±0.02	-	-	-	*Quercus rotundifolia*	Anjos *et al.*, 2015	Portugal and Spain	-	1
		34.59±4.50	-	-	-	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		26.80±2.63	-	-	-	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	b*	4.25±0.86	-	-	-	Honeydew	Popek, 2002	Poland	-	10
		8.65±9.96	-	-	-	*Quercus* sp. and *Cedrus atlantica*	Terrab *et al.*, 2003a	Northwest Morocco	-	3
		2.79±1.53	-	-	-	*Quercus* sp.	Iglesias *et al.*, 2004	Central Spain	2000-2001	19
		22.12±10.18	-	-	-	*Quercus* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	33
		32.14±6.61	-	-	-	*Abies alba*	Bertoncelj *et al.*, 2011	Slovenia	2008-2009	30
		75.23±2.46	-	-	-	*Quercus* sp.	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	5

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	b*	-4.15±0.05	-	-	-	*Pinus* sp.	Inan *et al.*, 2012	Turkey	-	1
		5.28±1.57	-	-	-	Honeydew	Kasperová *et al.*, 2012	Slovakia	2006-2008	8
		2.30±0.50	-	-	-	*Pinus* sp., *Abies* sp.	Oroian, 2012	Romania	2011	3
		21.97±7.77	-	-	-	*Abies* sp.	Karabagias *et al.*, 2014a	Greece	2011	31
		18.76±4.52	-	-	-	*Pinus* sp.	Karabagias *et al.*, 2014a	Greece	2011	39
		65.80±7.60	-	-	-	Honeydew	Tuberoso *et al.*, 2014	Italy, Croatia, France, Germany	2009-2010	22
		-3.40±0.01	-	-	-	*Quercus rotundifolia*	Anjos *et al.*, 2015	Portugal and Spain	-	1
		71.65±4.10	-	-	-	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		89.45±4.93	-	-	-	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	C*	30.87±11.07	-	-	-	*Quercus* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	33
		78.02±2.65	-	-	-	*Quercus* sp.	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	5
		70.20±6.90	-	-	-	Honeydew	Tuberoso *et al.*, 2014	Italy, Croatia, France, Germany	2009-2010	22
	h*	44.33±7.39	-	-	-	*Quercus* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	33
		74.79±3.55	-	-	-	*Quercus* sp.	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	5
		70.40±6.70	-	-	-	Honeydew	Tuberoso *et al.*, 2014	Italy, Croatia, France, Germany	2009-2010	22
	Chlorogenic acid	0.50±0.03	µg/g	-	-	Honeydew	Biesaga and Pyrzynska, 2009	Poland	-	3
		0.76±0.02	µg/g	-	-	Honeydew	Pichichero *et al.*, 2009	Italy	2007	3
		0.57±0.91	µg/g	-	-	Honeydew	Silici *et al.*, 2013	Turkey	-	10
		ND	µg/g	-	-	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		ND	µg/g	-	-	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Caffeic acid	0.70±0.05	µg/g	-	-	Honeydew	Biesaga and Pyrzynska, 2009	Poland	-	3
		0.19±0.01	µg/g	-	-	Honeydew	Pichichero *et al.*, 2009	Italy	2007	3
		0.10±0.07	µg/g	-	-	Honeydew	Silici *et al.*, 2013	Turkey	-	10
		2.68±0.91	µg/g	-	-	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		3.90±3.39	µg/g	-	-	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	Rutin	0.40 ± 0.02	µg/g	-	-	Honeydew	Biesaga and Pyrzynska, 2009	Poland	-	3
		5.39 ± 1.24	µg/g	-	-	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		11.64 ± 6.92	µg/g	-	-	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Coumaric acid	2.70±0.09	µg/g	-	-	Honeydew	Biesaga and Pyrzynska, 2009	Poland	-	3
		0.25±0.01	µg/g	-	-	Honeydew	Pichichero *et al.*, 2009	Italy	2007	3
		2.08±9.30	µg/g	-	-	Honeydew	Silici *et al.*, 2013	Turkey	-	10
		15.95±2.22	µg/g	-	-	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		4.41±3.46	µg/g	-	-	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Ferulic acid	0.40±0.02	µg/g	-	-	Honeydew	Biesaga and Pyrzynska, 2009	Poland	-	3
		17.38±0.85	µg/g	-	-	Honeydew	Biesaga and Pyrzynska, 2009	Poland	-	3
		0.19±0.05	µg/g	-	-	Honeydew	Silici *et al.*, 2013	Turkey	-	10
		4.19±3.00	µg/g	-	-	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		2.14±1.30	µg/g	-	-	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Quercetin	0.10±0.01	µg/g	-	-	Honeydew	Biesaga and Pyrzynska, 2009	Poland	-	3
		0.09±0.00	µg/g	-	-	Honeydew	Pichichero *et al.*, 2009	Italy	2007	3
		1.07±1.70	µg/g	-	-	*Abies* sp.	Karabagias *et al.*, 2014b	Greece	2011	31
		0.23±0.53	µg/g	-	-	*Pinus* sp.	Karabagias *et al.*, 2014b	Greece	2011	39
		ND	µg/g	-	-	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		11.77±2.32	µg/g	-	-	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Kaempferol	0.08±0.00	µg/g	-	-	Honeydew	Pichichero *et al.*, 2009	Italy	2007	3
		2.56±2.69	µg/g	-	-	*Abies* sp.	Karabagias *et al.*, 2014b	Greece	2011	31
		0.39±1.36	µg/g	-	-	*Pinus* sp.	Karabagias *et al.*, 2014b	Greece	2011	39
		ND	µg/g	-	-	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		7.50±0.11	µg/g	-	-	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
	Chrysin	0.23±0.02	µg/g	-	-	Honeydew	Pichichero *et al.*, 2009	Italy	2007	3
		0.41±1.05	µg/g	-	-	*Abies* sp.	Karabagias *et al.*, 2014b	Greece	2011	31

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	Chrysin	0.14±0.34	µg/g	-	-	*Pinus* sp.	Karabagias *et al.*, 2014b	Greece	2011	39
	Galangin	0.32±0.02	µg/g	-	-	Honeydew	Pichichero *et al.*, 2009	Italy	2007	3
	TPC_{HPLC}	3.28±0.19	µg/g	-	-	Honeydew	Pichichero *et al.*, 2009	Italy	2007	3
	TPC	25.56±0.75	mg gallic/ 100 g	Honey	-	Honeydew	Beretta *et al.*, 2005	Different geographical origins	2003	1
		113.90±1.20	mg gallic/ 100 g	Honey	-	Honeydew	Meda *et al.*, 2005	Burkina Faso	2002-2003	2
		24.14±3.95	mg gallic/ 100 g	Honey	-	*Abies alba*	Bertoncelj *et al.*, 2007	Slovenia	2004	10
		21.75±2.06	mg gallic/ 100 g	Honey	-	*Picea* sp.	Bertoncelj *et al.*, 2007	Slovenia	2004	10
		23.39±2.17	mg gallic/ 100 g	Honey	-	Forest	Bertoncelj *et al.*, 2007	Slovenia	2004	10
		1304.00±5.00	mg gallic/ 100 g	Honey	-	Honeydew	Ouchemoukh *et al.*, 2007	Algeria	2002	1
		103.00±13.00	mg gallic/ 100 g	Honey	-	*Quercus* sp.	Pérez *et al.*, 2007	Madrid, Spain	2002	5
		113.00	mg gallic/ 100 g	Honey	-	*Quercus* sp.	Vela *et al.*, 2007	Spain	-	17
		101.00±32.00	mg gallic/ 100 g	Honey	-	*Quercus* sp.	González-Lorente *et al.*, 2008	Spain	-	8
		23.00-125.00	mg gallic/ 100 g	Honey	-	Honeydew	Mărghitaş *et al.*, 2009	Romania	2005-2006	7
		27.60±1.38	mg gallic/ 100 g	Honey	-	Honeydew	Pichichero *et al.*, 2009	Italy	2007	3
		21.52±1.72	mg gallic/ 100 g	Honey	-	Honeydew	Lachman *et al.*, 2010	Czech Republic	2006	6
		11.15±2.57	mg gallic/ 100 g	Extract	-	Forest	Lachman *et al.*, 2010	Czech Republic	2006	8
		12.61±2.56	mg gallic/ 100 g	Extract	-	Honeydew	Lachman *et al.*, 2010	Czech Republic	2006	6
		15.56±0.20	mg gallic/ 100 g	Honey	-	*Pinus* sp.	Özkök *et al.*, 2010	Turkey	2004-2006	50
		65.46±5.68	mg gallic/ 100 g	Honey	-	Honeydew	Wilczyńska, 2010	Poland	2009	4
		6.08±0.14	mg gallic/ 100 g	Extract	-	Honeydew	Socha *et al.*, 2011	Poland	2007	1
		119.79±1.41	mg gallic/ 100 g	Honey	-	*Salix* sp.	Tuberoso *et al.*, 2011	Croatia	2009	2
		23.75±7.41	mg gallic/ 100 g	Honey	-	Forest	Cimpoiu *et al.*, 2013	Romania	-	4

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	TPC	140.60±43.10	mg gallic/ 100 g	Honey	-	*Quercus* sp.	Escuredo *et al.*, 2013	Northwest of Spain	-	13
		508.55±28.49	mg gallic/ 100 g	Honey	-	*Pinus Pinus*a	Özcan and Ölmez, 2014	Turkey	2006	1
		446.65±39.35	mg gallic/ 100 g	Honey	-	*Cedrus* sp.	Özcan and Ölmez, 2014	Turkey	2006	1
		442.94±36.52	mg gallic/ 100 g	Honey	-	*Pinus Pinus*a	Özcan and Ölmez, 2014	Turkey	2007	1
		685.42±31.79	mg gallic/ 100 g	Honey	-	*Cedrus* sp.	Özcan and Ölmez, 2014	Turkey	2007	1
		66.25±10.54	mg gallic/ 100 g	Honey	-	Honeydew	Wieczorek *et al.*, 2014	Poland	2011-2012	2
		87.40±21.40	mg gallic/ 100 g	Honey	-	Honeydew	Wilczyńska, 2014	Poland	2009-2010	9
		120.04±18.56	mg gallic/ 100 g	Extract	-	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		61.42±5.59	mg gallic/ 100 g	Extract	-	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
		132.30±30.00	mg gallic/ 100 g	Honey	-	*Quercus* sp.	Rodríguez-Flores *et al.*, 2015	Northwest of Spain	2009-2011	32
	TFC	2.74±1.25	mg quercetin/ 100 g	Honey	Dowd $AlCl_3$, 415 nm, Colour correction	Honeydew	Meda *et al.*, 2005	Burkina Faso	2002-2003	2
		5.46-28.25	mg quercetin/ 100 g	Honey	$NaNO_2$, 510 nm	Honeydew	Mărghitaş *et al.*, 2009	Romania	2005-2006	7
		16.43±0.82	mg quercetin/ 100 g	Honey	$NaNO_2$, 510 nm	Honeydew	Pichichero *et al.*, 2009	Italy	2007	3
		0.65±0.12	mg quercetin/ 100 g	Extract	$NaNO_2$, 510 nm	Forest	Lachman *et al.*, 2010	Czech Republic	2006	8
		0.83±0.24	mg quercetin/ 100 g	Extract	$NaNO_2$, 395 nm	Honeydew	Lachman *et al.*, 2010	Czech Republic	2006	6
		2.28±2.45	mg quercetin/ 100 g	Honey	$AlCl_3$, 415 nm	*Pinus* sp.	Özkök *et al.*, 2010	Turkey	2004-2006	50
		9.60±2.70	mg quercetin/ 100 g	Honey	Dowd $AlCl_3$, 425 nm	*Quercus* sp	Escuredo *et al.*, 2013	Northwest of Spain	-	13
		1.67±0.73	mg quercetin/ 100 g	Honey	Dowd $AlCl_3$, 415 nm, Colour correction	Honeydew	Wieczorek *et al.*, 2014	Poland	2011-2012	2
		3.10±0.56	mg quercetin/ 100 g	Extract	HCl, 415 nm	*Quercus robur*	Can *et al.*, 2015	Turkey	2011-2012	3
		1.58±1.30	mg quercetin/ 100 g	Extract	HCl, 415 nm	*Pinus brutia*	Can *et al.*, 2015	Turkey	2011-2012	4
		9.50± 1.70	mg quercetin/ 100 g	Honey	Dowd $AlCl_3$, 425 nm	*Quercus* sp	Rodríguez-Flores *et al.*, 2015	Northwest of Spain	2009-2011	32

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
HONEYDEW	TEAC	19.05±6.65	%I	Honey	15 min, 734 nm	Honeydew	Wilczyńska, 2010	Poland	2009	4
		982.93±32.18	mg ascorbic acid/kg	Honey	20 min, 734 nm	Honeydew	Lachman *et al.*, 2010	Czech Republic	2006	6
		29.27±0.39	%I	Extract	15 min, 734 nm	Honeydew	Socha *et al.*, 2011	Poland	2007	1
		8.64±3.52	µmol Trolox/g	Honey	15 min, 734 nm	Forest	Cimpoiu *et al.*, 2013	Romania		4
		5.82±0.04	µmol Trolox/g	Honey	FIA	Forest	Gorjanovic *et al.*, 2013	Serbia	2009	1
		5.06±0.02	µmol Trolox/g	Honey	FIA	*Pinus* sp.	Gorjanovic *et al.*, 2013	Serbia	2009	1
		1.29±0.12	µmol Trolox/100 g	Honey	15 min, 734 nm	Honeydew	Wilczyńska, 2014	Poland	2009-2010	9
LAVENDER	L*	42.70±3.20	-	-	-	*Lavandula latifolia*	Mateo and Boch-Reig, 1998	Spain	1980-1987	12
		67.20±5.47	-	-	-	*Lavandula* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	4
		78.13±4.68	-	-	-	Lavender	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	9
		80.93±4.99	-	-	-	*Lavandula latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		77.39±8.49	-	-	-	Lavandin	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		30.28±4.95	-	-	-	*Lavandula* sp.	Anjos *et al.*, 2015	Portugal and Spain	-	
		62.55±2.73	-	-	-	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	a*	20.97±4.40	-	-	-	*Lavandula* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	4
		4.54±3.15	-	-	-	Lavender	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	9
		2.08±3.37	-	-	-	*Lavandula latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		4.59±14.95	-	-	-	Lavandin	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		0.74±1.08	-	-	-	*Lavandula* sp.	Anjos *et al.*, 2015	Portugal and Spain	-	
		15.20±0.25	-	-	-	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	b*	59.48±5.79	-	-	-	*Lavandula* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	4
		60.39±13.48	-	-	-	Lavender	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	9

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
LAVENDER	b*	52.75±14.30	-	-	-	*Lavandula latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		54.33±14.95	-	-	-	Lavandin	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		8.61±4.89	-	-	-	*Lavandula* sp.	Anjos *et al.*, 2015	Portugal and Spain	-	
		10.08±2.78	-	-	-	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	C*	63.25±4.69	-	-	-	*Lavandula* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	4
		60.56±13.59	-	-	-	Lavender	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	9
		52.86±14.40	-	-	-	*Lavandula latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		54.58±15.09	-	-	-	Lavandin	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
	h*	70.41±5.06	-	-	-	*Lavandula* sp.	González-Miret *et al.*, 2005	Spain	2002-2003	4
		85.81±2.97	-	-	-	Lavender	León-Ruiz *et al.*, 2011	Castilla la Mancha (Spain)	-	9
		88.45±3.29	-	-	-	*Lavandula latifolia*	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
		85.70±3.16	-	-	-	Lavandin	Castro-Vázquez *et al.*, 2014	La Alcarria (Spain)	-	10
	Chlorogenic acid	5.00±0.60	µg/g	-	-	*Lavandula stoechas*	Andrade *et al.*, 1997a	Portugal	-	20
		ND	µg/g	-	-	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
		1.00±1.00	µg/g	-	-	*Lavandula stoechas*	Petretto *et al.*, 2015	Italy	-	4
	Caffeic acid	0.60±0.10	µg/g	-	-	*Lavandula stoechas*	Andrade *et al.*, 1997a	Portugal	-	20
		0.80±0.31	µg/g	-	-	*Lavandula* sp.	Tomás-Barberán *et al.*, 2001	Europe	-	2
		3.88±2.73	µg/g	-	-	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
LAVENDER	Rutin	1.19±1.34	µg/g	-	-	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
		3.70±1.90	µg/g	-	-	*Lavandula stoechas*	Petretto *et al.*, 2015	Italy	-	4
	Coumaric acid	0.40±0.20	µg/g	-	-	*Lavandula stoechas*	Andrade *et al.*, 1997a	Portugal	-	20
		0.78±0.53	µg/g	-	-	*Lavandula* sp.	Tomás-Barberán *et al.*, 2001	Europe	-	2
		1.01	µg/g	-	-	*Lavandula* sp.	Dimitrova *et al.*, 2007	Europe	-	2
		2.56±1.11	µg/g	-	-	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	Ferulic acid	1.90±0.80	µg/g	-	-	*Lavandula stoechas*	Andrade *et al.*, 1997a	Portugal	-	20
		ND	µg/g	-	-	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
		ND	µg/g	-	-	*Lavandula stoechas*	Petretto *et al.*, 2015	Italy	-	4
	Luteolin	0.30	µg/g	-	-	*Lavandula* sp.	Truchado *et al.*, 2009	Italy	-	1
		2.60±1.10	µg/g	-	-	*Lavandula stoechas*	Petretto *et al.*, 2015	Italy	-	4
	Quercetin	0.67±0.42	µg/g	-	-	*Lavandula* sp.	Tomás-Barberán *et al.*, 2001	Europe	-	2
		ND	µg/g	-	-	*Lavandula* sp.	Campillo *et al.*, 2015	Spain	-	1
		ND	µg/g	-	-	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
		3.50±1.70	µg/g	-	-	*Lavandula stoechas*	Petretto *et al.*, 2015	Italy	-	4
	Naringenin	ND	µg/g	-	-	*Lavandula* sp.	Campillo *et al.*, 2015	Spain	-	1
	Kaempferol	0.26	µg/g	-	-	*Lavandula* sp.	Truchado *et al.*, 2009	Italy	-	1
		ND	µg/g	-	-	*Lavandula* sp.	Campillo *et al.*, 2015	Spain	-	1

HONEY	PARAMETER	DATA	UNITS	HONEY/ EXTRACT	METHOD	FLORAL ORIGIN	AUTHOR	GEOGRAPHICAL ORIGIN	YEAR	SAMPLES
LAVENDER	Kaempferol	ND	µg/g	-	-	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
		ND	µg/g	-	-	*Lavandula stoechas*	Petretto *et al.*, 2015	Italy	-	4
	Chrysin	1.50±1.69	µg/g	-	-	*Lavandula* sp.	Tomás-Barberán *et al.*, 2001	Europe	-	2
		1.26	µg/g	-	-	*Lavandula* sp.	Truchado *et al.*, 2009	Italy	-	1
		0.21±0.02	µg/g	-	-	*Lavandula* sp.	Campillo *et al.*, 2015	Spain	-	1
	Pinocembrin	2.21	µg/g	-	-	*Lavandula* sp.	Truchado *et al.*, 2009	Italy	-	1
		16.00±2.00	µg/g	-	-	*Lavandula stoechas*	Petretto *et al.*, 2015	Italy	-	4
	Galangin	0.70	µg/g	-	-	*Lavandula* sp.	Truchado *et al.*, 2009	Italy	-	1
		3.50±0.10	µg/g	-	-	*Lavandula stoechas*	Petretto *et al.*, 2015	Italy	-	4
	TPC	20.57±2.62	mg gallic/ 100 g	Honey	-	*Lavandula* sp.	Gomes *et al.*, 2011	Portugal	2009	73
		32.99±1.61	mg gallic/ 100 g	Honey	-	*Lavandula stoechas*	Aazza *et al.*, 2013	Portugal	2011	2
		52.92±3.86	mg gallic/ 100 g	Honey	-	*Lavandula latifolia*	León-Ruiz *et al.*, 2013	Castilla la Mancha (Spain)	-	5
		53.39±23.34	mg gallic/ 100 g	Extract	-	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
		7.30±1.60	mg gallic/ 100 g	Extract	-	*Lavandula stoechas*	Petretto *et al.*, 2015	Italy	-	4
	TFC	12.47±1.57	mg catechin/ 100 g	Honey	$NaNO_2$, 510 nm	*Lavandula* sp.	Gomes *et al.*, 2011	Portugal	2009	73
		3.12±0.04	mg galangin/ 100 g	Honey	$AlCl_3$, 425 nm	*Lavandula stoechas*	Aazza *et al.*, 2013	Portugal	2011	2
		2.20±1.54	mg quercetin/ 100 g	Extract	HCl, 415 nm	*Lavandula stoechas*	Can *et al.*, 2015	Turkey	2011-2012	5
	TEAC	4.01±0.04	IC50	Honey	Unknown	*Lavandula stoechas*	Aazza *et al.*, 2013	Portugal	2011	2
		79.86±13.87	µmol gallic/ 100 g	Honey	6 min, 734 nm	*Lavandula latifolia*	León-Ruiz *et al.*, 2013	Castilla la Mancha (Spain)	-	5

REFERENCES

AAZZA, S; LYOUSSI, B; ANTUNES, D; MIGUEL, M G (2013) Physicochemical characterization and antioxidant activity of commercial Portuguese honeys. *Journal of Food Science* 78(8): C1159-C1165. http://dx.doi.org/10.1111/1750-3841.12201

ALQARNI, A S; OWAYSS, A A; MAHMOUD, A A; HANNAN, M A (2014) Mineral content and physical properties of local and imported honeys in Saudi Arabia. *Journal of Saudi Chemical Society* 18(5): 618-625. http://dx.doi.org/10.1016/j.jscs.2012.11.009

ANDRADE, P; FERRERES, F; AMARAL, M T (1997a) Analysis of honey phenolic acids by HPLC, its application to honey botanical characterization. *Journal of Liquid Chromatography & Related Technologies* 20(14): 2281-2288. http://dx.doi.org/10.1080/10826079708006563http://dx.doi.org/

ANDRADE, P; FERRERES, F; GIL, M I; TOMÁS-BARBERÁN, F A (1997b) Determination of phenolic compounds in honeys with different floral origin by capillary zone electrophoresis. *Food Chemistry* 60(1): 79-84. http://dx.doi.org/10.1016/S0308-8146(96)00313-5

ANJOS, O; CAMPOS, M G; RUIZ, P C; ANTUNES, P (2015) Application of FTIR-ATR spectroscopy to the quantification of sugar in honey. *Food Chemistry* 169: 218-223. http://dx.doi.org/10.1016/j.foodchem.2014.07.138

BERETTA, G; GRANATA, P; FERRERO, M; ORIOLI, M; FACINO, R M (2005) Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytica Chimica Acta* 533(2): 185-191. http://dx.doi.org/ 10.1016/j.aca.2004.11.010

BERTONCELJ, J; DOBERŠEK, U; JAMNIK, M; GOLOB, T (2007) Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chemistry* 105(2): 822-828. http://dx.doi.org/10.1016/j.foodchem.2007.01.060

BERTONCELJ, J; POLAK, T; KROPF, U; KOROŠEC, M; GOLOB, T (2011) LC-DAD-ESI/MS analysis of flavonoids and abscisic acid with chemometric approach for the classification of Slovenian honey. *Food Chemistry* 127(1): 296-302. http://dx.doi.org/10.1016/j.foodchem.2011.01.003

BIESAGA, M; PYRZYNSKA, K (2009) Liquid chromatography/tandem mass spectrometry studies of the phenolic compounds in honey. *Journal of Chromatography A* 1216(38): 6620-6626. http://dx.doi.org/10.1016/j.chroma.2009.07.066

CAMPILLO, N; VIÑAS, P; FÉREZ-MELGAREJO, G; HERNÁNDEZ-CÓRDOBA, M (2015) Dispersive liquid–liquid microextraction for the determination of flavonoid aglycone compounds in honey using liquid chromatography with diode array detection and time-of-flight mass spectrometry. *Talanta* 131: 185-191. http://dx.doi.org/10.1016/j.talanta.2014.07.083

CAN, Z; YILDIZ, O; SAHIN, H; AKYUZ-TURUMTAY, E; SILICI, S; KOLAYLI, S (2015) An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles. *Food Chemistry* 180: 133-141. http://dx.doi.org/10.1016/j.foodchem.2015.02.024

CASTRO-VÁZQUEZ, L; LEÓN-RUIZ, V; ALAÑÓN, M E; PÉREZ-COELLO, M S; GONZÁLEZ-PORTO, A V (2014) Floral origin markers for authenticating Lavandin honey (*Lavandula angustifolia x latifolia*). Discrimination from Lavender honey (*Lavandula latifolia*). *Food Control* 37: 362-370. http://dx.doi.org/10.1016/j.foodcont.2013.09.003

CHANG, X; WANG, J; YANG, S; CHEN, S; SONG, Y (2011) Antioxidative, antibrowning and antibacterial activities of sixteen floral honeys. *Food & Function* 2(9): 541. http://dx.doi.org/10.1039/c1fo10072f

CIAPPINI, M C; STOPPANI, F (2014) Determination of antioxidant capacity, flavonoids, and total phenolic content in eucalyptus and clover honeys. *Journal of Apicultural Science* 58(1): 103-111.

CIMPOIU, C; HOSU, A; MICLAUS, V; PUSCAS, A (2013) Determination of the floral origin of some Romanian honeys on the basis of physical and biochemical properties. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 100: 149-154. http://dx.doi.org/10.1016/j.saa.2012.04.008

DIMITROVA, B; GEVRENOVA, R; ANKLAM, E (2007) Analysis of phenolic acids in honeys of different floral origin by solid-pase extraction and high-performance liquid chromatography. *Phytochemical Analysis* 18(1): 24-32. http://dx.doi.org/10.1002/pca.948

ESCUREDO, O; MÍGUEZ, M; FERNÁNDEZ-GONZÁLEZ, M; CARMEN SEIJO, M (2013) Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. *Food Chemistry* 138(2-3): 851-856. http://dx.doi.org/10.1016/j.foodchem.2012.11.015

ESTEVINHO, L M; FEÁS, X; SEIJAS, J A; VÁZQUEZ-TATO, M P (2008) Organic honey from *Trás-Os-Montes* region (Portugal): Chemical, palynological, microbiological and bioactive compounds characterization. *Food and Chemical Toxicology* 50(2): 258-264. http://dx.doi.org/10.1016/j.fct.2011.10.034

FERREIRA, I C F R; AIRES, E; BARREIRA, J C M; ESTEVINHO, L M (2009) Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry* 114(4): 1438-1443. http://dx.doi.org/10.1016/j.foodchem.2008.11.028

FERRERES, F; ANDRADE, P; GIL, M I; TOMÁS-BARBERÁN, F A (1996) Floral nectar phenolics as biochemical markers for the botanical origin of heather honey. *Zeitschrift für Lebensmittel-Untersuchung und Forshung* 202(1): 40-44. http://dx.doi.org/10.1007/BF01229682

GHELDOF, N; ENGESETH, N J (2002) Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *Journal of Agricultural and Food Chemistry* 50(10): 3050-3055. http://dx.doi.org/10.1021/jf0114637

GHELDOF, N; WANG, X H; ENGESETH, N J (2002) Identification and quantification of antioxidant components of honeys from various floral sources. *Journal of Agricultural and Food Chemistry* 50(21): 5870-5877. http://dx.doi.org/10.1021/jf0256135

GIORGI, A; MADEO, M; BAUMGARTNER, J; LOZZIA, G C (2011) The relationships between phenolic content, pollen diversity, physicochemical information and radical scavenging activity in honey. *Molecules* 16: 336-347. http://dx.doi.org/10.3390/molecules16010336

GOMES, T; FEÁS, X; IGLESIAS, A; ESTEVINHO, L M (2011) Study of organic honey from the Northeast of Portugal. *Molecules* 16(12): 5374-5386. http://dx.doi.org/10.3390/molecules16075374

GONZÁLEZ-MIRET, M L; TERRAB, A; HERNANZ, D; FERNÁNDEZ-RECAMALES, M A.; HEREDIA, F J (2005) Multivariate correlation between colour and mineral composition of honeys and by their botanical origin. *Journal of Agricultural and Food Chemistry* 53: 2574-2580. http://dx.doi.org/10.1021/jf048207p

GONZÁLEZ-LORENTE, M; DE LORENZO-CARRETERO, C; PÉREZ-MARTÍN, R A (2008) Sensory attributes and antioxidant capacity of spanish honeys. *Journal of Sensory Studies* 23(3): 293-302. http://dx.doi.org/10.1111/j.1745-459X.2008.00156.x

GORJANOVIĆ, S Ž; ÁLVAREZ-SUÁREZ, J M; NOVAKOVIĆ, M M; PASTOR, F T; PEZO, L; BATTINO, M; SUŽNJEVIĆ, D Ž (2013) Comparative analysis of antioxidant activity of honey of different floral sources using recently developed polarographic and various spectrophotometric assays. *Journal of Food Composition and Analysis* 30(1): 13-18. http://dx.doi.org/10.1016/j.jfca.2012.12.004

IGLESIAS, M T; DE LORENZO, C; POLO, M D; MARTIN-ALVEREZ, P J; PUEYO, E (2004) Usefulness of amino acid composition to discriminate between honeydew and floral honeys. Application to honeys from a small geographic area. *Journal of Agricultural and Food Chemistry* 52(1): 84-89. http://dx.doi.org/10.1021/jf030454q

İNAN, Ö; ÖZCAN, M M; ARSLAN, D; ÜNVER, A (2012) Some physico-chemical and sensory properties of heat treated commercial pine and blossom honey. *Journal of Apicultural Research* 51(4): 347-352. http://dx.doi.org/10.3896/IBRA.1.51.4.09

JASICKA-MISIAK, I; POLIWODA, A; DEREŃ, M; KAFARSKI, P (2012) Phenolic compounds and abscisic acid as potential markers for the floral origin of two Polish unifloral honeys. *Food Chemistry* 131(4): 1149-1156. http://dx.doi.org/10.1016/j.foodchem.2011.09.083

KARABAGIAS, I K; BADEKA, A; KONTAKOS, S; KARABOURNIOTI, S; KONTOMINAS, M G (2014a) Characterisation and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical parameters and chemometrics. *Food Chemistry* 146: 548-557. http://dx.doi.org/10.1016/j.foodchem.2013.09.105

KARABAGIAS, I K; VAVOURA, M V; NIKOLAOU, C; BADEKA, A V; KONTAKOS, S; KONTOMINAS, M G (2014b) Floral authentication of Greek unifloral honeys based on the combination of phenolic compounds, physicochemical parameters and chemometrics. *Food Research International* 62: 753-760. http://dx.doi.org/10.1016/j.foodres.2014.04.015

KASPEROVÁ, J; NAGY, J; POPELKA, P; DICAKOVÁ, Z; NAGYOVÁ, A; MAL'A, P (2012) Physico-chemical indicators and identification of selected Slovak honeys based on colour measurement. Acta Veterinaria Brno 81: 57-61. http://dx.doi.org/10.2754/avb201281010057

KROPF, U; KOROŠEC, M; BERTONCELJ, J; OGRINC, N; NEČEMER, M; KUMP, P; GOLOB, T (2010) Determination of the geographical origin of Slovenian black locust, lime and chestnut honey. *Food Chemistry* 121(3): 839-846. http://dx.doi.org/10.1016/j.foodchem.2009.12.094

KÜÇÜK, M; KOLAYLI, S; KARAOĞLU, Ş; ULUSOY, E; BALTACI, C; CANDAN, F (2007) Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chemistry* 100(2): 526-534. http://dx.doi.org/10.1016/j.foodchem.2005.10.010

KUMAZAWA, S; OKUYAMA, Y; MURASE, M; AHN, M-R; NAKAMURA, J; TATEFUJI, T (2012) Antioxidant activity in honeys of various floral origins: Isolation and identification of antioxidants in peppermint honey. *Food Science and Technology Research* 18(5): 679-685. http://dx.doi.org/10.3136/fstr.18.679

KUŚ, P M; CONGIU, F; TEPER, D; SROKA, Z; JERKOVIC, I; TUBEROSO, C I G (2014) Antioxidant activity, color characteristics, total phenol content and general HPLC fingerprints of six polish unifloral honey types. *LWT - Food Science and Technology* 55(1): 124-130. http://dx.doi.org/10.1016/j.lwt.2013.09.016

LACHMAN, J; ORSÁK, M; HEJTMÁNKOVÁ, A; KOVÁŘOVÁ, E (2010) Evaluation of antioxidant activity and total phenolics of selected Czech honeys. *LWT-Food Science and Technology* 43(1): 52-58. http://dx.doi.org/10.1016/j.lwt.2009.06.008

LEÓN-RUIZ, V; VERA, S; GONZÁLEZ-PORTO, A V; SAN ANDRÉS, M P (2011) Vitamin C and sugar levels as simple markers for discriminating Spanish honey sources. *Journal of Food Science* 76(3): C356-C361. http://dx.doi.org/10.1111/j.1750-3841.2011.02041.x

LEÓN-RUIZ, V; GONZÁLEZ-PORTO, A V.; AL-HABSI, N; VERA, S; SAN ANDRÉS, M P; JAUREGI, P (2013) Antioxidant, antibacterial and ACE-inhibitory activity of four monofloral honeys in relation to their chemical composition. *Food & Function* 4(11): 1617-1624. http://dx.doi.org/10.1039/c3fo60221d

MĂRGHITAŞ, L A; DEZMIREAN, D; BOBIS, O; LASLO, L; BOGDANOV, S (2009) Physico-chemical and bioactive properties of different floral origin honeys from Romania. *Food Chemistry* 112(4): 863-867. http://dx.doi.org/10.1016/j.foodchem.2008.06.055

MATEO, R; BOSCH-REIG, F (1998) Classification of Spanish unifloral honeys by discriminant analysis of electrical conductivity, colour, water content, sugars, and pH. *Journal of Agricultural and Food Chemistry* 46(2): 393-400. http://dx.doi.org/10.1021/jf970574w

MICHALKIEWICZ, A; BIESAGA, M; PYRZYNSKA, K (2008) Solid-phase extraction procedure for determination of phenolic acids and some flavonols in honey. *Journal of Chromatography A* 1187(1-2): 18-24. http://dx.doi.org/10.1016/j.chroma.2008.02.001

MOISE, A; MĂRGHITAŞ, L A; DEZMIREAN, D; BOBIS, O (2013) Nutraceutical properties of Romanian heather honey. *Nutrition & Food Science* 43(3): 218-227. http://dx.doi.org/10.1108/00346651311327864

OROIAN, M (2012) Physicochemical and rheological properties of Romanian honeys. *Food Biophysics* 7(4): 296-307. http://dx.doi.org/10.1007/s11483-012-9268-x

OUCHEMOUKH, S; SCHWEITZER, P; LOUAILECHE, H (2007) Physicochemical characteristics and pollen spectrum of some Algerian honeys. *Food Control* 18(1): 52-58. http://dx.doi.org/10.1016/j.foodcont.2005.08.007

OUCHEMOUKH, S; SCHWEITZER, P; BACHIR BEY, M; DJOUDAD-KADJI, H; LOUAILECHE, H (2010) HPLC sugar profiles of Algerian honeys. *Food Chemistry* 121(2): 561-568. http://dx.doi.org/10.1016/j.foodchem.2009.12.047

ÖZCAN, M M; ÖLMEZ, Ç (2014) Some qualitative properties of different monofloral honeys. *Food Chemistry* 163: 212-218. http://dx.doi.org/10.1016/j.foodchem.2014.04.072

PÉREZ, R A; IGLESIAS, M T; PUEYO, E; GONZALEZ, M; DE LORENZO, C (2007) Amino acid composition and antioxidant capacity of Spanish honeys. *Journal of Agricultural and Food Chemistry* 55(2): 360-365. http://dx.doi.org/10.1021/jf062055b

PERNA, A; INTAGLIETTA, I; SIMONETTI, A; GAMBACORTA, E (2013) A comparative study on phenolic profile, vitamin C content and antioxidant activity of Italian honeys of different botanical origin. *International Journal of Food Science & Technology* 48(9): 1899-1908. http://dx.doi.org/10.1111/ijfs.12169

PETRETTO, G L; COSSU, M; ALAMANNI, M C (2015) Phenolic content, antioxidant and physico-chemical properties of Sardinian monofloral honeys. *International Journal of Food Science & Technology* 50(2): 482-491. http://dx.doi.org/10.1111/ijfs.12652

PICHICHERO, E; CANUTI, L; CANINI, A (2009) Characterisation of the phenolic and flavonoid fractions and antioxidant power of Italian honeys of different botanical origin. *Journal of the Science of Food and Agriculture* 89(4): 609-616. http://dx.doi.org/10.1002/jsfa.3484

POPEK, S (2002) A procedure to identify a honey type. *Food Chemistry* 79(3): 401-406. http://dx.doi.org/10.1016/S0308-8146(02)00391-6

RODRÍGUEZ-FLORES, M S; ESCUREDO, O; SEIJO, M C (2015) Assessment of physicochemical and antioxidant characteristics of Quercus pyrenaica honeydew honeys. *Food Chemistry* 166: 101-106. http://dx.doi.org/10.1016/j.foodchem.2014.06.005

SERGIEL, I; POHL, P; BIESAGA, M (2014) Characterization of honeys according to their content of phenolic compounds using high performance liquid chromatography/tandem mass spectrometry. *Food Chemistry* 145: 404-408. http://dx.doi.org/10.1016/j.foodchem.2013.08.068

SHAFIEE, S; MINAEI, S; MOGHADDAM-CHARKARI, N; BARZEGAR, M (2014) Honey characterization using computer vision system and artificial neural networks. *Food Chemistry* 159: 143-150. http://dx.doi.org/10.1016/j.foodchem.2014.02.136

SILICI, S; SARIOGLU, K; KARAMAN, K (2013) Determination of polyphenols of some Turkish honeydew and nectar honeys using HPLC-DAD. *Journal of Liquid Chromatography & Related Technologies* 36(July 2014): 2330-2341. http://dx.doi.org/10.1080/10826076.2012.720332

SOCHA, R; JUSZCZAK, L; PIETRZYK, S; GAŁKOWSKA, D; FORTUNA, T; WITCZAK, T (2011) Phenolic profile and antioxidant properties of Polish honeys. *International Journal of Food Science & Technology* 46(3): 528-534. http://dx.doi.org/10.1111/j.1365-2621.2010.02517.x

TERRAB, A; DÍEZ, M J; HEREDIA, F J (2003a) Palynological, physico-chemical and colour characterization of Moroccan honeys: III. Other unifloral honey types. *International Journal of Food Science and Technology* 38: 395-402.

TERRAB, A; GONZÁLEZ, A G; DÍEZ, M J; HEREDIA, F J (2003b) Characterisation of Moroccan unifloral honeys using multivariate analysis. *European Food Research and Technology* 218(1): 88-95. http://dx.doi.org/10.1007/s00217-003-0797-x

TOFIGHI, Z; ES-HAGHI, A; ASL, M M; TAJIC, A R; NAVAI, M S; TAVAKOLI, S; HADJIAKHOONDI, A; YASSA, N (2014) Investigation of chemical keys for relationship between plants and their unifloral honeys by hydrodistillation and SPME and biological activities of honeys. *European Food Research Technology* 238(4): 665-673.

TOMÁS-BARBERÁN, F A.; MARTOS, I; FERRERES, F; RADOVIC, B S; ANKLAM, E (2001) HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *Journal of the Science of Food and Agriculture* 81(5): 485-496. http://dx.doi.org/10.1002/jsfa.836

TRUCHADO, L; FERRERES, F; TOMÁS-BARBERÁN, F A (2009a) Liquid chromatography-tandem mass spectrometry reveals the widespread occurrence of flavonoid glycosides in honey, and their potential as floral origin markers. *Journal of Chromatography A* 1216(43): 7241-7248. http://dx.doi.org/10.1016/j.chroma.2009.07.057

TUBEROSO, C I G; JERKOVIĆ, I; BIFULCO, E; MARIJANOVIĆ, Z (2011) Biodiversity of *Salix* spp. honeydew and nectar honeys determined by RP-HPLC and evaluation of their antioxidant capacity. *Chemistry & Biodiversity* 8(5): 872-879. http://dx.doi.org/10.1002/cbdv.201000359

TUBEROSO, C I G; JERKOVIĆ, I; SARAIS, G; CONGIU, F; MARIJANOVIĆ, Z; KÚS, P M (2014) Color evaluation of seventeen European unifloral honey types by means of spectrophotometrically determined CIE L* C^*_{ab} h°_{ab} chromaticity coordinates. *Food chemistry* 145: 284-291. http://dx.doi.org/10.1016/j.foodchem.2013.08.032

VELA, L; DE LORENZO, C; PÉREZ, R A (2007) Antioxidant capacity of Spanish honeys and its correlation with polyphenol content and other physicochemical properties. *Journal of the Science of Food and Agriculture* 87(6): 1069-1075. http://dx.doi.org/10.1002/jsfa.2813

WANG, X H; GHELDOF, N; ENGESETH, N J (2004) Effect of processing and storage on antioxidant capacity of honey. *Journal of Food Science* 69(2): C96-C101. http://dx.doi.org/10.1111/j.1365-2621.2004.tb15509.x

WIECZOREK, J; PIETRZAK, M; POMIANOWSKI, J; WIECZOREK, Z (2014) Honey as a source of bioactive compounds. *Polish Journal of Natural Sciences* 29(3): 275-285.

WILCZYŃSKA, A (2010) Phenolic content and antioxidant activity of different types of Polish honey-A short report. *Polish Journal of Food and Nutrition Sciences* 60 (4): 309-313.

WILCZYŃSKA, A (2014) Effect of filtration on colour, antioxidant activity and total phenolics of honey. *LWT - Food Science and Technology* 57(2): 767-774. http://dx.doi.org/10.1016/j.lwt.2014.01.034

TABLE 4

Pearson's correlation matrix of bioactive compounds and other antioxidant-related parameters

	L*	a*	b*	C*	h*	Chlorogenic acid	Caffeic acid	Rutin	Ellagic acid
L*		**-0.8467***	-0.0399	-0.2031	**0.9541***	0.6233*	0.0566	-0.159	-0.5289*
a*	**-0.8467***		0.5239*	0.6610*	**-0.9517***	-0.5522*	0.0037	0.2227	0.3363*
b*	-0.0399	0.5239*		**0.9849***	-0.2616	-0.0173	0.1371	0.1994	-0.2153
C*	-0.2031	0.6610*	**0.9849***		-0.4180*	-0.1173	0.1196	0.2208	-0.1292
h*	**0.9541***	**-0.9517***	-0.2616	-0.4180*		0.6143*	0.0355	-0.1954	-0.4809*
Chlorogenic acid	0.6233*	-0.5522*	-0.0173	-0.1173	0.6143*		0.1169	0.0144	-0.4548*
Caffeic acid	0.0566	0.0037	0.1371	0.1196	0.0355	0.1169		0.1547	-0.1006
Rutin	-0.159	0.2227	0.1994	0.2208	-0.1954	0.0144	0.1547		-0.3267*
Ellagic acid	-0.5289*	0.3363*	-0.2153	-0.1292	-0.4809*	-0.4548*	-0.1006	-0.3267*	
Coumaric acid	-0.2832*	0.1359	-0.1546	-0.1079	-0.2166	-0.0968	0.1613	0.4645*	-0.0253
Sinapic acid	-0.0099	0.0956	0.1783	0.1786	-0.0541	0.0495	-0.1942	0.0434	0.0182
Ferulic acid	-0.003	0.0568	0.1795	0.1672	-0.0194	-0.0627	0.6891*	0.2210	-0.0305
Luteolin	-0.0941	0.1449	0.0695	0.0931	-0.128	-0.0016	-0.064	0.4173*	-0.3794*
Quercetin	0.6143*	-0.5640*	-0.0851	-0.1835	0.5988*	**0.7281***	0.1676	-0.2202	-0.2965*
Naringenin	-0.137	0.1618	0.1264	0.1456	-0.1445	0.0701	-0.0815	0.2771*	-0.0228
Kaempferol	0.2097	-0.1832	0.0031	-0.0373	0.2074	0.1120	0.1035	-0.1457	-0.0897
Chrysin	0.1385	0.0788	0.2314	0.2276	0.0290	0.2034	0.3560*	-0.1114	-0.0253
Pinocembrin	0.2258	-0.0128	0.1976	0.1781	0.1184	0.2753*	0.2274	-0.232	0.0046
Galangin	-0.3307*	0.3149*	0.0984	0.1498	-0.3220*	-0.2981*	0.3348*	0.3840*	-0.0296
PA_{HPLC}	-0.5191*	0.2947*	-0.251	-0.169	-0.4521*	-0.3928*	0.0951	-0.1688	**0.9190***
TFC_{HPLC}	0.2122	-0.0127	0.2230	0.2003	0.1167	0.3268*	0.3554*	0.0409	-0.156
TPC_{HPLC}	-0.4681*	0.2915*	-0.1978	-0.1213	-0.4240*	-0.3147*	0.1795	-0.1589	**0.8811***
TPCh	**-0.7527***	**0.7586***	0.1689	0.2954*	**-0.7973***	-0.5778*	-0.1293	-0.1416	0.6093*
TPCe	**-0.7673***	**0.7181***	0.0999	0.2276	**-0.7840***	-0.4657*	0.0849	0.2596	0.4000*
TFCe	-0.0233	0.2722*	0.4196*	0.4226*	-0.177	0.0491	0.4022*	0.1309	-0.0365
TEACh	**-0.8323***	0.6101*	-0.174	-0.0381	**-0.7685***	-0.5200*	-0.0889	-0.0347	**0.7087***
TEACe	**-0.7922***	0.5931*	-0.173	-0.0395	**-0.7439***	-0.4345*	-0.0057	0.2395	0.5278*

**** Correlation is significant at the p<0.05; In bold type correlations with r>0.7***

	Coumaric acid	Sinapic acid	Ferulic acid	Luteolin	Quercetin	Naringenin	Kaempferol	Chrysin	Pinocembrin
L*	-0.2832*	-0.0099	-0.003	-0.0941	0.6143*	-0.137	0.2097	0.1385	0.2258
a*	0.1359	0.0956	0.0568	0.1449	-0.5640*	0.1618	-0.1832	0.0788	-0.0128
b*	-0.1546	0.1783	0.1795	0.0695	-0.0851	0.1264	0.0031	0.2314	0.1976
C*	-0.1079	0.1786	0.1672	0.0931	-0.1835	0.1456	-0.0373	0.2276	0.1781
h*	-0.2166	-0.0541	-0.0194	-0.128	0.5988*	-0.1445	0.2074	0.0290	0.1184
Chlorogenic acid	-0.0968	0.0495	-0.0627	-0.0016	**0.7281***	0.0701	0.1120	0.2034	0.2753*
Caffeic acid	0.1613	-0.1942	0.6891*	-0.064	0.1676	-0.0815	0.1035	0.3560*	0.2274
Rutin	0.4645*	0.0434	0.2210	0.4173*	-0.2202	0.2771*	-0.1457	-0.1114	-0.232
Ellagic acid	-0.0253	0.0182	-0.0305	-0.3794*	-0.2965*	-0.0228	-0.0897	-0.0253	0.0046
Coumaric acid		-0.2238	0.3645*	0.2837*	-0.1063	-0.0203	-0.1672	-0.0065	-0.1349
Sinapic acid	-0.2238		-0.1066	0.0949	-0.0914	0.1671	0.0238	-0.14	-0.1381
Ferulic acid	0.3645*	-0.1066		-0.1728	-0.0727	-0.1064	-0.0373	0.0267	-0.114
Luteolin	0.2837*	0.0949	-0.1728		-0.1782	0.0665	-0.0386	-0.1268	-0.1695
Quercetin	-0.1063	-0.0914	-0.0727	-0.1782		-0.0757	0.3633*	0.3701*	0.4813*
Naringenin	-0.0203	0.1671	-0.1064	0.0665	-0.0757		-0.1624	0.1419	0.0137
Kaempferol	-0.1672	0.0238	-0.0373	-0.0386	0.3633*	-0.1624		0.1583	0.1651
Chrysin	-0.0065	-0.14	0.0267	-0.1268	0.3701*	0.1419	0.1583		**0.9141***
Pinocembrin	-0.1349	-0.1381	-0.114	-0.1695	0.4813*	0.0137	0.1651	**0.9141***	
Galangin	0.5637*	-0.1229	0.4382*	0.2900*	-0.2426	0.0956	0.0140	0.1322	-0.1621
PA_{HPLC}	0.2174	-0.0129	0.1866	-0.3974*	-0.2381	-0.0804	-0.0948	-0.0144	-0.0133
TFC_{HPLC}	0.0611	-0.1281	0.0206	0.0018	0.5420*	0.2563	0.3824*	**0.9123***	**0.8420***
TPC_{HPLC}	0.2317	-0.0434	0.1914	-0.3966*	-0.109	-0.0193	-0.0037	0.2026	0.1870
TPCh	-0.0032	0.0297	-0.1512	-0.0112	-0.4551*	0.0938	-0.2113	0.1670	0.1909
TPCe	0.1241	0.0408	-0.0148	0.3019*	-0.5599*	0.2040	-0.2329	-0.0682	-0.1306
TFCe	0.0422	0.2074	0.2129	0.1596	0.2203	0.1450	0.3007*	0.5596*	0.4475*
TEACh	0.1377	-0.0064	-0.0403	-0.0565	-0.4968*	0.0942	-0.2735*	-0.1664	-0.2017
TEACe	0.2430	-0.0531	-0.0554	0.2188	-0.5291*	0.1621	-0.3275*	-0.2054	-0.2494

**** Correlation is significant at the p<0.05; In bold type correlations with r>0.7***

	Galangin	PA_{HPLC}	TFC_{HPLC}	TPC_{HPLC}	TPCh	TPCe	TFCe	TEACh	TEACe
L*	-0.3307*	-0.5191*	0.2122	-0.4681*	**-0.7527***	**-0.7673***	-0.0233	**-0.8323***	**-0.7922***
a*	0.3149*	0.2947*	-0.0127	0.2915*	**0.7586***	**0.7181***	0.2722*	0.6101*	0.5931*
b*	0.0984	-0.251	0.2230	-0.1978	0.1689	0.0999	0.4196*	-0.174	-0.173
C*	0.1498	-0.169	0.2003	-0.1213	0.2954*	0.2276	0.4226*	-0.0381	-0.0395
h*	-0.3220*	-0.4521*	0.1167	-0.4240*	**-0.7973***	**-0.7840***	-0.177	**-0.7685***	**-0.7439***
Chlorogenic acid	-0.2981*	-0.3928*	0.3268*	-0.3147*	-0.5778*	-0.4657*	0.0491	-0.5200*	-0.4345*
Caffeic acid	0.3348*	0.0951	0.3554*	0.1795	-0.1293	0.0849	0.4022*	-0.0889	-0.0057
Rutin	0.3840*	-0.1688	0.0409	-0.1589	-0.1416	0.2596	0.1309	-0.0347	0.2395
Ellagic acid	-0.0296	**0.9190***	-0.156	**0.8811***	0.6093*	0.4000*	-0.0365	**0.7087***	0.5278*
Coumaric acid	0.5637*	0.2174	0.0611	0.2317	-0.0032	0.1241	0.0422	0.1377	0.2430
Sinapic acid	-0.1229	-0.0129	-0.1281	-0.0434	0.0297	0.0408	0.2074	-0.0064	-0.0531
Ferulic acid	0.4382*	0.1866	0.0206	0.1914	-0.1512	-0.0148	0.2129	-0.0403	-0.0554
Luteolin	0.2900*	-0.3974*	0.0018	-0.3966*	-0.0112	0.3019*	0.1596	-0.0565	0.2188
Quercetin	-0.2426	-0.2381	0.5420*	-0.109	-0.4551*	-0.5599*	0.2203	-0.4968*	-0.5291*
Naringenin	0.0956	-0.0804	0.2563	-0.0193	0.0938	0.2040	0.1450	0.0942	0.1621
Kaempferol	0.0140	-0.0948	0.3824*	-0.0037	-0.2113	-0.2329	0.3007	-0.2735	-0.3275
Chrysin	0.1322	-0.0144	**0.9123***	0.2026	0.1670	-0.0682	0.5596*	-0.1664	-0.2054
Pinocembrin	-0.1621	-0.0133	**0.8420***	0.1870	0.1909	-0.1306	0.4475*	-0.2017	-0.2494
Galangin		0.1232	0.2125	0.1736	0.0740	0.2604	0.3270*	0.2530	0.2554
PA_{HPLC}	0.1232		-0.1155	**0.9717***	0.5322*	0.3334*	0.0481	0.7044*	0.5231*
TFC_{HPLC}	0.2125	-0.1155		0.1225	0.0149	-0.1142	0.6256*	-0.2541	-0.25
TPC_{HPLC}	0.1736	**0.9717***	0.1225		0.5353*	0.3060*	0.1969	0.6434*	0.4632*
TPCh	0.0740	0.5322*	0.0149	0.5353*		0.6270*	0.1972	**0.7574***	0.6215*
TPCe	0.2604	0.3334*	-0.1142	0.3060*	0.6270*		0.1600	**0.7168***	**0.8507***
TFCe	0.3270*	0.0481	0.6256*	0.1969	0.1972	0.1600		-0.0022	-0.0041
TEACh	0.2530	**0.7044***	-0.2541	0.6434*	**0.7574***	**0.7168***	-0.0022		**0.8652***
TEACe	0.2554	0.5231*	-0.25	0.4632*	0.6215*	**0.8507***	-0.0041	**0.8652***	

**** Correlation is significant at the p<0.05; In bold type correlations with r>0.7***

ANNEX 2

SCIENTIFIC ARTICLES DERIVED FROM THE PHD THESIS

SCIENTIFIC ARTICLES DERIVED FROM THE PHD THESIS

Sancho, M T; Pascual-Maté, A; Rodríguez-Morales, E G; Osés, S M; Escriche, I; Periche, A; Fernández-Muiño, M A (2016) **Critical assessment of antioxidant-related parameters of honey.** *International Journal of Food Science and Technology* 51(1): 30-36 (Annex 2, Article 1). http://dx.doi.org/10.1111/ijfs.12988

Osés, S M; Pascual-Maté, A; De la Fuente, D; De Pablo, A; Fernández-Muiño, M A; Sancho, M T. **Comparison of methods to determine antibacterial activity of honeys against *Staphylococcus aureus*.** Submitted to *Wageningen Journal of Life Sciences* (Chapter 7).

Osés, S M; Pascual-Maté, A; Fernández-Muiño, M A; López-Díaz, T M; Sancho, M T (2015) **Bioactive properties of honey with propolis.** *Food Chemistry* 196: 1215-1223 (Annex 2, Article 2). http://dx.doi.org/10.1016/j.foodchem.2015.10.050

ARTICLE 1

Critical assessment of antioxidant-related parameters of honey

Sancho, M T; Pascual-Maté, A; Rodríguez-Morales, E G; Osés, S M; Escriche, I; Periche, A; Fernández-Muiño, M A (2016) *International Journal of Food Science and Technology* 51(1): 30-36. http://dx.doi.org/10.1111/ijfs.12988

International Journal of Food Science +Technology

Institute of Food Science +Technology ifst

Original article

Critical assessment of antioxidant-related parameters of honey

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Summary In this study, several antioxidant-related parameters were researched on 56 Spanish honeys, setting up and optimising some assays. Melissopalynology and colour (L^*, a^*, b^*) were determined. Solid-phase extraction (SPE) was used to obtain honeys' phenolic extracts. Total phenolics, total flavonoids and trolox equivalent antioxidant capacity (TEAC) were determined in both honeys and extracts. It was verified that total flavonoids determination in neutral media must be carried out on extracts instead of on honeys, because of sugars' interferences; likewise, extracts' colours must be corrected in this assay. The endpoint for honeys' trolox equivalent antioxidant capacity (TEAC) was researched. Significant linear relationships were found between TEAC values of honeys and honeys' phenolic extracts, as well as between the results of TEAC measured at different times. Therefore, it would be possible to reliably calculate TEAC at 60 min (endpoint), measuring the absorbance at 6 min, thus saving analysis time and reducing costs.

Keywords Antioxidant activity, colour, flavonoids, honey, phenols.

Introduction

Honey has a wide range of phenolic compounds, and therefore, it has been reported to possess an antioxidant ability, which greatly depends on its composition that is, in turn, conditioned by the botanical source of this foodstuff. Studies about the antioxidant potential of various unifloral, multifloral and honeydew honeys are interesting to later check whether some honeys have actually antioxidant effects when they are ingredients of other food products, and/or within the body after consumption (*in vivo* assays). The latter research is of particular interest because the European Food Safety Authority denied the health claims with regard to antioxidant-related properties of honey because this food '…has not been sufficiently characterized in relation to the claimed effects' (European Food Safety Authority, 2010, 2011). Flavonoids and other phenolics are the main compounds responsible for honey antioxidant activity (Malenica-Staver *et al.*, 2014). Honey flavonoids, as a whole, are usually determined by aluminium chloride chelation methods that must be carried out after sugars' removal, because these substances hamper proper chelation (Denni & Mammen, 2012). However, in most published papers, authors determine total flavonoids in neutral media directly on honeys, and sometimes, with no sample's colour correction. Trolox equivalent antioxidant capacity (TEAC) is a simple and widely used procedure to determine antioxidant activity of foods. Nevertheless, before using it to measure the antioxidant capacity of a particular food, the endpoint of the assay should be previously researched (van den Berg *et al.*, 1999; Prior *et al.*, 2005).

The aims of this work were as follows: first, to study antioxidant-related features of honeys from different botanical origins analysing such parameters as colour (L^*, a^*, b^*), total phenolics, total flavonoids and TEAC. Second, to go in depth in the method for honeys' total flavonoids analysis carried out in neutral media. Finally, to research the endpoint for honey's TEAC determination, in order to set up a reliable honey's antioxidant activity analysis by TEAC method.

Materials and methods

Samples

This work was carried out on fifty-six representative artisanal and unpasteurised Spanish honeys, whose botanical origins had been determined by melissopalynology (von der Ohe *et al.*, 2004), with the result of

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twenty-one multifloral, sixteen honeydew, ten heather (*Erica* sp. and *Calluna vulgaris*), five lavender (*Lavandula* sp.), three clover (Leguminosae Type *Trifolium* sp.) and one sainfoin (Leguminosae Type *Onobrychis* sp.) honeys. Sampling was carried out within the region of Castile-Leon, covering an area larger than 94 200 km^2. Samples were stored at 4 °C until analysis in dark conditions.

Apparatus

Colour parameters were determined with a Hunter Lab colorimeter (ColorFlex EZ System®, Reston, VA, USA). Total phenolics, total flavonoids and TEAC assays were carried out by visible spectrophotometry with a 400Bio UV–visible spectrophotometer (Varian®, Mulgrave, Vic., Australia).

Procedures

Phenolic extracts were obtained by solid-phase extraction (SPE). Ten grams of honey was mixed with 15 mL of acidified water and loaded onto Strata-X SPE cartridge (Phenomenex®, Torrance, CA, USA) previously conditioned with methanol and water. Sugars and other polar honey's constituents were completely removed with acidified and ultrapure water. After vacuum drying, phenolic fractions were eluted from the cartridge with 3 mL 2:1 (v/v) methanol: acetonitrile (Bertoncelj *et al*., 2011).

Colour parameters L^* (lightness), a^* and b^* (chromaticity coordinates) were determined using illuminant D65 and 10° observer. Specimens were illuminated at 45° (Commission Internationale de L'eclairage, 2004).

Total phenolics (mg gallic acid/100 g) were determined by Folin–Ciocalteu method (Meda *et al*., 2005). 0.5 mL of a filtered honey solution (100 mg mL^{-1}) or 0.5 mL of 1:25 diluted extract was mixed with 2.5 mL of 0.2 N Folin–Ciocalteu reagent. After 5 min, 2 mL of saturated sodium carbonate solution was added, and the mixtures were kept in the dark for 120 min. Then, the absorbance was read at 760 nm. Gallic acid was used to adjust the standard curve.

Total flavonoids (mg quercetin/100 g) were determined in the extracts by the Dowd aluminium chloride colorimetric assay in neutral media (Dowd, 1959; Meda *et al*., 2005; Isla *et al*., 2011) and adapted for the analysed samples. One millilitre of a diluted honey extract (300 μL extract diluted to 5 mL with methanol) was mixed with the same volume of 2% aluminium trichloride in methanol. After 10 min, absorbance (A_1) was read at 415 nm against a blank constituted by 1 mL of 2% $AlCl_3$ in methanol and 1 mL of methanol instead of the diluted honey extract. Colour of extracts was corrected by determining the absorbance (A_2) of a solution containing 1 mL of a diluted honey extract mixed with the same volume of methanol against a blank of methanol. A_2 was substracted from A_1 before calculating. Quercetin was used to adjust the standard curve that was read against a blank of methanol. The same procedure was also applied to honey solutions (0.01 mg mL^{-1}).

TEAC antioxidant activity (μmol trolox equivalent per g) was determined by measuring the scavenging ability of antioxidants to the radical $ABTS^{\bullet+}$ (Re *et al*., 1999). TEAC was analysed in both honeys and extracts, measuring the absorbance at 734 nm after 6, 30 and 60 min. The radical cation $ABTS^{\bullet+}$ was produced by the reaction of 7 mM ABTS stock solution with 2.45 mM potassium persulfate in the dark for 16 h. Then, the $ABTS^{\bullet+}$ solution was diluted to obtain an absorbance between 0.70 and 0.80 at 734 nm. For honey solutions (100 mg mL^{-1}), 10 μL of each honey solution was mixed with 990 μL of the diluted $ABTS^{\bullet+}$ solution. For honey extracts, first, 300 μL extract was diluted to 5 mL with methanol, and finally, 10 μL of each diluted extract was mixed with 990 μL of the diluted $ABTS^{\bullet+}$ solution. Blank was distilled water for honeys and methanol for honey extracts. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used to adjust the standard curve.

Statistical determinations were carried out with Statgraphics Centurion XVI (2010).

All analytical procedures were carried out in triplicate.

Results

The results of colour (L^*, a^*, b^*), total phenolics, total flavonoids and TEAC antioxidant activity (measuring the absorbance at different times) are summarised in Table 1. Total flavonoids results are those corresponding to honey extracts, after verifying the unfeasibility of the method when diluted honeys were used instead of their extracts, because for more than 95% honeys, the absorbance of the colour correction (1 mL of honey solution plus 1 mL of methanol) was higher than the absorbance of the sample (1 mL of honey solution plus 1 mL of 2% $AlCl_3$ in methanol).

In respect of unifloral honeys, lavender samples showed the highest values for lightness (ranging from 43.16 to 71.01), and heather honeys the lowest ones (ranging from 29.8 to 57.36). Heather samples showed intense reddish tonalities (ranging from 7.19 to 15.64), whereas a^* values widely ranged in the other honeys. b^* values were in all cases positive, meaning that all samples were in the yellow area. Heather honeys showed the highest values of total phenolics (ranging from 112.32 to 183.35 mg gallic acid per 100 g), extracts' total phenolics (ranging from 15.62 to

Table 1 Mean, median, standard deviation, minimum and maximum values of L^*, a^*, b^*, total phenolics of honeys and extracts, total flavonoids of extracts and TEAC antioxidant activity of honeys and extracts

	Colour			Honeys' total phenolics (mg gallic acid per 100 g)	Extracts' total phenolics (mg gallic acid per 100 g)	Extracts' total flavonoids (mg quercetin per 100 g)	Honeys' TEAC (μmol trolox per g)			Extracts' TEAC (μmol trolox per g)		
	L^*	a^*	b^*				6 min	30 min	60 min	6 min	30 min	60 min
Mean	48.24	8.94	33.82	119.24	26.29	3.44	4.35	5.90	6.92	1.92	2.34	2.58
Median	45.45	9.55	33.71	130.05	27.51	3.41	4.55	6.23	7.52	1.99	2.45	2.68
Standard deviation	10.78	3.59	3.54	39.42	8.95	1.15	1.88	2.35	2.57	0.68	0.79	0.85
Minimum	26.07	0.17	23.80	29.10	7.98	0.93	0.97	1.39	1.64	0.49	0.62	0.76
Maximum	71.01	15.64	43.46	183.35	60.30	6.98	7.46	9.49	10.65	3.16	3.85	4.43

60.30 mg gallic acid per 100 g), extracts' total flavonoids (ranging from 3.41 to 6.98 mg quercetin per 100 g) and TEAC (ranging from 5.0 to 10.0 μmol trolox equivalent per gram, reading the absorbance at 60 min), whereas lavender samples showed the lowest values for total phenolics (ranging from 51.52 to 101.48 mg gallic acid per 100 g), extracts' total phenolics (ranging from 8.41 to 27.34 mg gallic acid per 100 g), extracts' total flavonoids (ranging from 1.10 to 4.49 mg quercetin per 100 g) and TEAC (ranging from 1.83 to 6.37 μmol trolox equivalent per gram, reading the absorbance at 60 min).

Results of most parameters fulfilled all assumptions necessary for one-way ANOVA test (90% confidence level). For a^* and b^* colour results, the nonparametric Kruskal–Wallis test was applied (90% confidence level). L^* values grouped heather and honeydew honeys. a^*, b^* and total flavonoids grouped multifloral, honeydew, clover and lavender honeys. Total phenolics and TEAC grouped, on the one hand, sainfoin and lavender samples, and on the other hand, multifloral, honeydew, clover and heather samples.

Values of L^* were significantly correlated with those of honeys' total phenolics ($r = -0.7925$), extracts' total phenolics ($r = -0.7859$), honeys' TEAC ($r = -0.8882$) and extracts' TEAC ($r = -0.8389$); values of honeys' total phenolics were significantly correlated with those of extracts' total phenolics ($r = 0.6849$), honeys' TEAC ($r = 0.8373$) and extracts' TEAC ($r = 0.7029$); values of extracts' total phenolics were significantly correlated with those of honeys' TEAC ($r = 0.7684$) and extracts' TEAC ($r = 0.8647$). Similar correlations among colour features, phenolics and antioxidant activities were described in the literature for both honeys from different botanical and geographical origins (Perna *et al.*, 2012; Kamboj *et al.*, 2013; Gambacorta *et al.*, 2014; Petretto *et al.*, 2015) and honey-based dairy products (Bansal *et al.*, 2014).

TEAC antioxidant activity (μmol trolox equivalent per gram) of both honeys and extracts progressively increased with time up to 60 min. Significant linear relationships (90% confidence level) were found among all TEAC results (Table 2). Linear relationships were obtained between TEAC of honeys and TEAC of honeys' phenolic extracts, as well as between TEAC of honeys and extracts measuring absorbance at different times (Fig. 1).

With the results of honeys' TEAC at 6 min and the equations of Table 2, TEAC values at 60 min were calculated. Then, actual and calculated values of TEAC at 60 min were compared with *t*-test and one-way ANOVA (90% confidence level). Both procedures showed that there were no differences between actual TEAC values (μmol trolox equivalent per gram) of both honeys (Table 3) and extracts, measuring the absorbance at 60 min, and calculated TEAC values (μmol trolox equivalent per gram) at 60 min, by measuring absorbance at 6 min.

Discussion

The variability of colour among the samples was mainly related to honeys' botanical origin. According to the literature, the mean value for L^* (<50) obtained in this study is typical of dark honeys, such as heather (González-Miret *et al.*, 2005) and honeydew (González-Miret *et al.*, 2005; Tuberoso *et al.*, 2014). These results are in concordance with the fact that the majority of the analysed samples (55%) were heather unifloral, honeydew honeys or honeys rich in heather and honeydew. a^* and b^* data were particularly high in heather samples and honeys rich in heather, so that in addition to other parameters, a^* and b^* values could help characterise heather honeys.

As expected, honeys' total phenolics were higher than extracts' total phenolics because the method of analysis (Meda *et al.*, 2005) actually determines total reducing capacity and apart from phenolic compounds, honeys possess different reducing substances such as ascorbic acid, and reducing sugars, among others (Ferreira *et al.*, 2009). The relationships found between values of total phenolics and those of L^* and

Table 2 Linear relationships among TEAC results of honeys and honeys' phenolic extracts

Relationships	Correlation coefficient (*r*)
TEAC-Extracts = 0.59 + 0.30 × TEAC-Honeys	0.9043
TEAC-Extracts-60 = 0.22 + 1.23 × TEAC-Extracts-6	0.9879
TEAC-Extracts-60 = 0.08 + 1.07 × TEAC-Extracts-30	0.9978
TEAC-Extracts-30 = 0.12 + 1.16 × TEAC-Extracts-6	0.9937
TEAC-Honeys-60 = 1.00 + 1.35 × TEAC-Honeys-6	0.9818
TEAC-Honeys-60 = 0.50 + 1.09 × TEAC-Honeys-30	0.9959
TEAC-Honeys-30 = 0.49 + 1.24 × TEAC-Honeys-6	0.9945
TEAC-Honeys & Extracts-60 = −0.03 + 1.52 × TEAC-Honeys & Extracts-6	0.9817
TEAC-Honeys & Extracts-60 = 0.50 + 1.09 × TEAC-Honeys & Extracts-30	0.9959
TEAC-Honeys & Extracts-30 = 0.49 + 1.24 × TEAC-Honeys & Extracts-6	0.9945

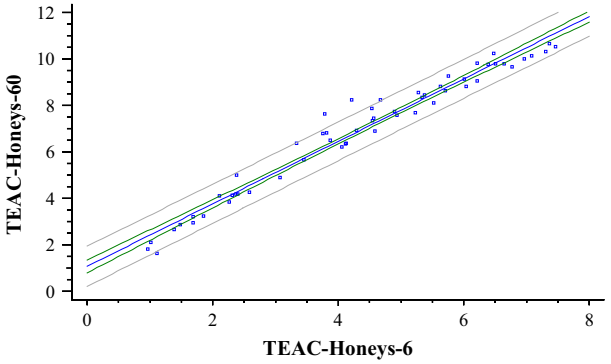

Figure 1 Relationships between TEAC values (μmol trolox equivalent per g) of honeys at 6 and 60 min. 'TEAC-Honeys-60' is TEAC antioxidant activity measuring the absorbance at 60 min; 'TEAC-Honeys-6' is TEAC antioxidant activity measuring the absorbance at 6 min). Equation: TEAC-Honeys-60 = 1.00 + 1.35 × TEAC-Honeys-6. Correlation coefficient = 0.9818.

TEAC antioxidant activity agreed with literature (Wilczynska, 2014).

The moderately significant relationship found between total phenolics of honeys and extracts is interesting, because it shows that the proportion of total reducing substances in honeys and their corresponding extracts appears to be somehow constant, even for very different honey samples from various botanical origins.

With regard to flavonoids' contents, the values of this work were in general lower than those described for different honeys by other authors. After a thorough literature revision, it must be explained that in most published papers, the spectrophotometric assay based on aluminium complex formation conducted in neutral media was carried out directly on a honey solution with no sugars' removal and, in some cases, with no sample's colour correction. The reason is likely due to the fact that those papers followed previous references that were in turn, based on procedures (Arvouet-Grand *et al.*, 1994; Popova *et al.*, 2005) published for propolis extracts in which there were no sugars' interferences. Thus, if the assay is carried out on a honey solution, the results depend on the specific flavonoid

Table 3 Summary of the results of *t*-test (90% confidence level) and variance check of one-way ANOVA (90% confidence level) applied to the results of honeys' TEAC actual values (μmol trolox equivalent per gram) measuring the absorbance at 60 min (TEAC-Honeys-60), and the results of honeys' TEAC calculated values at 60 min (μmol trolox equivalent per gram), measuring the absorbance at 6 min (TEAC-Honeys-6). t = 0.113464. P-value = 0.90987

	Actual TEAC-Honeys-60	Calculated TEAC-Honeys-60
Sample size	56	56
Average	6.91875	6.86411
Standard deviation	2.56686	2.52967
Coefficient of variation	37.10%	36.85%
Minimum	1.64	2.31
Maximum	10.65	11.06
Range	9.01	8.75
Stnd. Skewness	−1.44897	−0.543526
Stnd. Kurtosis	−1.36357	−1.59705
Variance	6.58875	6.39924
Degrees of freedom	55	55

Variance check	Test	*P*-Value
Levene's	0.010297	0.91936

Comparison	Sigma1	Sigma2	*F*-Ratio	*P*-Value
ActualTEAC-Honeys-60/Calculated TEAC-Honeys-60	2.56686	2.52967	1.02961	0.9142

composition of the sample because, on the one hand, flavonoids do not react uniformly and, on the other hand, glycosylation prevents chelation of Al(III) with some flavonoids, but not with all flavonoids (Pekal & Pyrzynska, 2014).

In addition, in the literature, there is no agreement about the blank when total flavonoids are analysed directly on a honey solution in neutral media. As it has been commented above, the assays of all manuscripts were based on others, being the common principle for all of them the initial spectrophotometric Dowd's procedure with some modifications. The original method (Dowd, 1959) used an aluminium chloride reagent blank. Nevertheless, for honey solutions, in some articles, the blank employed was methanol, thereby neither the reagents nor the samples colour were corrected, so that the final flavonoids' values could be overestimated. Despite the fact that a blank of reagents (as in Dowd, 1959) is usual in spectrophotometric measurements, very few authors employed such blank for the analysis of honeys' flavonoids in neutral media, and their manuscripts cited Isla *et al.* (2011) as a reference, which was in turn based on Popova *et al.* (2005) procedure that had been set up and applied to 6 poplar Turkish propolis, in which the colour of the extracts could have not interfered. Values of honey solutions' total flavonoids of those papers (based on the Popova *et al.*, 2005 manuscript) might also be overestimated because matrix interferences were not substracted. When a honey solution is used, a sample's colour correction is compulsory, because the results are based on the absorbance measurement at 415 nm (or 425 nm), and at this (these) wavelength(s), there is a colour interference of the honey itself, which is particularly important for dark samples. In most published papers about total flavonoids analysis on honey solutions, authors claimed that they followed Meda *et al.* (2005) procedure, based in turn on Arvouet-Grand *et al.* (1994) assay for propolis extracts, which used as blank a solution of the sample and the solvent, thus only correcting the colour of the samples, but not the interference in the absorbance recording due to the aluminium chloride. Therefore, data of honey solutions' total flavonoids of those manuscripts based on Arvouet-Grand *et al.*, 1994 paper could be overestimated, as well.

Both for honeys' extracts and for honeys' solutions, we followed the procedure described in this manuscript, in which a blank of reagents was used, and then absorbance of the colour of the samples was substracted, in a similar way to that described in the official method of AOAC (2005) for the analysis of proline in honey.

We verified that, when using honey solutions (instead of honey extracts), for the vast majority of samples, the absorbance values at 415 nm (and also at 425 nm) were considerably higher for colour correction than for the sample with flavonoid–aluminium complex, showing the unreliability of the procedure if sugars and other interferences were not removed. Therefore, spectrophotometric analysis of honeys' flavonoids in neutral media must be always carried out after getting rid of sugars; otherwise, the results could be specious.

Our TEAC results were similar to the values described in the literature for Brazilian honeys (Sant'Ana *et al.*, 2012) and slightly lower than the antioxidant activities described for South African samples (Serem & Bester, 2012). Our TEAC data were also similar to those described in the literature for other honeys from different botanical and geographical origins that were analysed by another method combined to a flow injection analysis (Álvarez-Suárez *et al.*, 2010a,b; Gorjanovic *et al.*, 2013).

In respect of TEAC antioxidant activity, in the literature, no agreement was found regarding the proper endpoint for the absorbance measurement. Some researchers determined the absorbance at 1, 4, 6 and 10 min (Baltrusaityté *et al.*, 2007; Escriche *et al.*, 2014), whereas other scientists considered the endpoint at 1 minute (Tuberoso *et al.*, 2013), at 6 min (Vit *et al.*, 2009; Sant'Ana *et al.*, 2012), at 7 min (Habib *et al.*, 2014), at 10 min (da Silva *et al.*, 2013), at 15 min (Socha *et al.*, 2009; Kowalski, 2013; Wilczynska, 2014), at 20 min (Lachman *et al.*, 2010) and at 30 min (Serem & Bester, 2012). Our work shows that absorbance values at different times change proportionally for both honeys from different botanical origins and their corresponding extracts, so that it would be possible to calculate the TEAC antioxidant activity at 60 min, measuring the absorbance at 6 min, thus saving analysis time and reducing costs. However, it would be necessary to study if similar relationships occur in other honeys from different origins and harvested in different years, in order to propose an appropriate analytical procedure for the determination of honey's TEAC antioxidant activity.

Conclusion

This research has shown that honeys from different botanical origins share common antioxidant-related features, being the most important of which total phenolics and TEAC. Total flavonoids analysis with aluminium trichloride in neutral media must be carried out on honey extracts, because honey sugars interfere; furthermore, in this determination, extracts' colours must be corrected. In respect of TEAC assay, if similar equations are obtained for honeys from different botanical and geographical origins, it would be possible to save analysis time and money, calculating the

values corresponding to the endpoint from the data of absorbance at 6 min.

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References

Álvarez-Suárez, J.M., González-Paramás, A.M., Santos-Buelga, C. & Battino, M. (2010a). Antioxidant characterization of native monofloral Cuban honeys. *Journal of Agricultural and Food Chemistry*, **8**, 9817–9824.

Álvarez-Suárez, J.M., Tulipani, S., Díaz, D. *et al*. (2010b). Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. *Food and Chemical Toxicology*, **48**, 2490–2499.

AOAC (2005). Proline in honey (method 979.20). In: *Official Methods of Analysis of AOAC International* (edited by W. Horwitz). Pp. 25–37. Gaithersburg, MD, USA: AOAC

Arvouet-Grand, A., Vennat, B., Pourrat, A. & Legret, P. (1994). Standardisation d'un extrait de propolis et identification des principaux constituants. *Journal de pharmacie de Belgique*, **49**, 462–468.

Baltrusaityté, V., Venskutonis, P.R. & Čeksteryté, V. (2007). Radical scavenging activity of different floral origin honey and beebread phenolic extracts. *Food Chemistry*, **101**, 502–514.

Bansal, V., Sharma, H.K. & Nanda, V. (2014). Optimisation of spray drying process parameters for low-fat honey-based milk powder with antioxidant activity. *International Journal of Food Science and Technology*, **49**, 1196–1202.

van den Berg, R., Haenen, G.R.M.M., van den Berg, H. & Bast, A. (1999). Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chemistry*, **66**, 511–517.

Bertoncelj, J., Polak, T., Kropf, U., Korosec, M. & Golob, T. (2011). LC-DAD-ESI/MS analysis of flavonoids and abscisic acid with chemometric approach for the classification of Slovenian honey. *Food Chemistry*, **127**, 296–302.

Commission Internationale de L'eclairage-CIE. (2004). *Technical report*. 3rd Edition. CIE 15:2004.

Denni, M. & Mammen, D. (2012). A critical evaluation on the reliability of two aluminum chloride chelation methods for quantification of flavonoids. *Food Chemistry*, **135**, 1365–1368.

Dowd, L.E. (1959). Spectrophotometric determination of quercetin. *Analytical Chemistry*, **31**, 1184–1187.

Escriche, I., Kadar, M., Juan-Borras, M. & Domenech, E. (2014). Suitability of antioxidant capacity, flavonoids and phenolic acids for floral authentication of honey. Impact of industrial thermal treatment. *Food Chemistry*, **42**, 135–143.

European Food Safety Authority. (2010). Scientific opinion on the substantiation of health claims related to honey. *EFSA Journal*, **8**, 1484.

European Food Safety Authority. (2011). Scientific opinion on the substantiation of health claims related to honey. *EFSA Journal*, **9**, 2243.

Ferreira, I.C.F.R., Aires, E., Barreira, J.C.M. & Estevinho, L.M. (2009). Antioxidant activity of Portuguese honey samples: different contributions of the entire honey and phenolic extract. *Food Chemistry*, **114**, 1438–1443.

Gambacorta, E., Simonetti, A., Garrisi, N., Intaglietta, I. & Perna, A. (2014). Antioxidant properties and phenolic content of sulla (*Hedysarum* spp.) honeys from Southern Italy. *International Journal of Food Science and Technology*, **49**, 2260–2268.

González-Miret, M.L., Terrab, A., Hernanz, D., Fernández-Recamales, M.A. & Heredia, F.J. (2005). Multivariate correlation between color and mineral composition of honeys and their botanical origin. *Journal of Agricultural and Food Chemistry*, **53**, 2574–2580.

Gorjanovic, S.Ž., Álvarez-Suárez, J.M., Novakovic, M.M. *et al*. (2013). Comparative analysis of antioxidant activity of honey of different floral sources using recently developed polarographic and various spectrophotometric assays. *Journal of Food Composition and Analysis*, **30**, 13–18.

Habib, H.M., Al Meqbali, F.T., Kamal, H., Souka, U.D. & Ibrahim, W.H. (2014). Bioactive components, antioxidant and DNA damage inhibitory activities of honeys from arid regions. *Food Chemistry*, **153**, 28–34.

Isla, M.I., Craig, A., Ordoñez, R. *et al*. (2011). Physico chemical and bioactive properties of honeys from Northwestern Argentina. *LWT-Food Science and Technology*, **44**, 1922–1930.

Kamboj, R., Bera, M.B. & Nanda, V. (2013). Evaluation of physicochemical properties, trace metal content and antioxidant activity of Indian honeys. *International Journal of Food Science and Technology*, **48**, 578–587.

Kowalski, S. (2013). Changes of antioxidant activity and formation of 5-hydroxymethylfurfural in honey during thermal and microwave processing. *Food Chemistry*, **141**, 1378–1382.

Lachman, J., Orsák, M., Hejtmánková, A. & Kovárová, E. (2010). Evaluation of antioxidant activity and total phenolics of selected Czech honeys. *LWT-Food Science and Technology*, **43**, 52–58.

Malenica-Staver, M., Ratkaj, I., Broznic, D. *et al*. (2014). Bioactivity of *Satureja Montana* L. honey extracts and their profile screening. *RSC Advances*, **4**, 47329–47340.

Meda, A., Lamien, C.E., Romito, M., Millogo, J. & Nacoulma, O.G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry*, **91**, 571–577.

von der Ohe, W., Persano-Oddo, L., Piana, M.L., Morlot, M. & Martin, P. (2004). Harmonized methods of melissopalynology. *Apidologie*, **35**, S18–S25.

Pekal, A. & Pyrzynska, K. (2014). Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Analytical Methods*, **7**, 1776–1782.

Perna, A., Simonetti, A., Intaglietta, I., Sofo, A. & Gambacorta, E. (2012). Metal content of southern Italy honey of different botanical origins and its correlation with polyphenol content and antioxidant activity. *International Journal of Food Science and Technology*, **47**, 1909–1917.

Petretto, G.L., Cossu, M. & Alamanni, M.C. (2015). Phenolic content, antioxidant and physico-chemical properties of Sardinian monofloral honeys. *International Journal of Food Science and Technology*, **50**, 482–491.

Popova, M., Silici, S., Kaftanoglu, O. & Bankova, V. (2005). Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. *Phytomedicine*, **12**, 221–228.

Prior, R.L., Wu, X. & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, **53**, 4290–4302.

Re, R., Pellegrini, N., Proteggente, A., Pannal, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, **26**, 1231–1237.

Sant'Ana, L.D., Sousa, J.P.L.M., Salgueiro, F.B., Lorenzon, M.C.A. & Castro, R.N. (2012). Characterization of monofloral honeys with multivariate analysis of their chemical profile and antioxidant activity. *Journal of Food Science*, **77**, C135–C140.

Serem, J.C. & Bester, M.J. (2012). Physicochemical properties, antioxidant activity and cellular protective effects of honeys from southern Africa. *Food Chemistry*, **133**, 1544–1550.

da Silva, I.A.A., da Silva, T.M.S., Camara, C.A. *et al*. (2013). Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas, Northern Brazil. *Food Chemistry*, **141**, 3552–3558.

Socha, R., Juszczak, L., Pietrzyk, S. & Fortuna, T. (2009). Antioxidant activity and phenolic composition of herb honeys. *Food Chemistry*, **113**, 568–574.

Statgraphics centurion XVI. (2010). *User Manual*. Warrenton, VA: StatPoint Technologies, Inc.

Tuberoso, C.I.G., Boban, M., Bifulco, E., Budimir, D. & Pirisi, F.M. (2013). Antioxidant capacity and vasodilatory properties of Mediterranean food: the case of Cannonau wine, myrtle berries liqueur and strawberry-tree honey. *Food Chemistry*, **140**, 686–691.

Tuberoso, C.I.G., Jerković, I., Sarais, G., Congiu, F., Marijanović, Z. & Kuś, P.M. (2014). Color evaluation of seventeen European unifloral honey types by means of spectrophotometrically determined CIE L*, C^*_{ab}, h°_{ab} chromaticity coordinates. *Food Chemistry*, **145**, 284–291.

Vit, P., Rodríguez-Malaver, A., Roubik, D.W. *et al*. (2009). Expanded parameters to assess the quality of honey from Venezuelan *Apis mellifera*. *Journal of ApiProduct and ApiMedical Science*, **1**, 72–81.

Wilczynska, A. (2014). Effect of filtration on colour, antioxidant activity and total phenolics of honey. *LWT-Food Science and Technology*, **57**, 767–774.

ARTICLE 2

Bioactive properties of honey with propolis

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Bioactive properties of honey with propolis

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ABSTRACT

Nowadays, propolis is used as an innovative preservative and as a bioactive food supplement. Due to its bitter and astringent flavour, propolis is hardly accepted by consumers. The aim of this study was to obtain a likeable food product made with honey and propolis, whose antimicrobial, antioxidant and anti-inflammatory properties were enhanced in comparison with those of the base honeys used. 0.1%, 0.3% and 0.5% soft propolis extracts were added to honeys and the products that most appealed to the users were subjected to further research. Total phenolics, flavonoids, ABTS free radical and hydroxyl radicals scavenging and anti-inflammatory activities increased in all mixtures. Antimicrobial activity of the combined products showed synergic effects, resulting in higher results than those of the base honeys and propolis extracts. Therefore, honeys enriched with small amounts of propolis extracts are promising functional foods.

1. Introduction

Honey is the natural sweet food produced by *Apis mellifera* from nectar and honeydew. Apart from sugars, honey contains small amounts of interesting compounds, such as phenolics, that provide potential beneficial properties (Isla et al., 2011; White, 1975). Propolis is a resinous substance that bees collect from bud and exudates of plants and transform with enzymes, being used to seal beehives' holes. Propolis is mainly constituted of resin, wax and essential oils (Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2008), acting as an effective antiseptic agent in the hive. The composition of honey and propolis varies depending on factors such as the botanical origin, climate, and environmental conditions (Isla et al., 2011; Viuda-Martos et al., 2008).

Since ancient times, honey and propolis have somehow been considered as therapeutic agents, due to several reported functional actions (Gómez-Caravaca, Gómez-Romero, Arráez-Román, Segura-Carretero, & Fernández-Gutierrez, 2006). Honey have shown antibacterial, antifungal and antiviral action due to pH, sugars, hydrogen peroxide and the presence of some phytochemicals, mainly phenolic compounds (Isla et al., 2011). Propolis contain higher amounts of phenolic compounds than honeys, having shown considerably higher antimicrobial and antioxidant activities (Banskota, Tezuka, & Kadota, 2001; Meda, Lamien, Romito, Millogo, & Nacoulma, 2005; Socha, Galkowska, Bugaj, & Juszczak, 2015).

Propolis is currently used as an ingredient of candies, biopharmaceuticals and as a constituent of cosmetics, gaining popularity as a natural preservative and a source of bioactive compounds for foods and drinks, in which it helps improve shelf-life and consumers' health (Duman & Özpolat, 2015; Gutiérrez-Cortés & Suarez Mahecha, 2014; Spinelli, Conte, Lecce, Incoronato, & del Nobile, in press). However, the high concentration of propolis' phenolic compounds was reported as primarily responsible for the bitterness and astringency (Naczk & Shahidi, 2004), providing with strong and unpleasant flavours that alter the sensory characteristics of other foods combined with them (Banskota et al., 2001). Therefore, the consumers' acceptability of foods in which propolis is an ingredient is determined by the propolis concentration, which should be carefully researched in order not to negatively modify the sensory characteristics of those foods.

Recently there has been a growing interest for new food products with functional properties. With regard to honeys, there are already on the markets products labelled as to contain 3% propolis. The commercial success of them strongly depends on the degree of liking and quality expectations. However, there is little scientific data about the possible advantages of adding propolis to foodstuffs. Juszczak, Galkowska, Ostrowska, and Socha (in press) showed an increase in total phenolics and antioxidant activity of a multifloral honey after propolis addition. The aim of this study was to make likeable products of honey and propolis and to research such functional components and properties as total phenols, total flavonoids, antimicrobial, antioxidant and anti-inflammatory activities in the base honeys, base propolis extracts,

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as well as in the honey and propolis mixtures that appealed to the consumers, in order to assess if those blends have functional advantages in respect of the initial commodities.

2. Materials and methods

2.1. Standards, reagents and apparatus

Hydrochloric acid, Folin–Ciocalteu reagent, quercetin, 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), hydrogen peroxide, thiobarbituric acid, hyaluronic acid sodium salt from *Streptococcus equi* (HA), bovine serum albumin (BSA), hyaluronidase from bovine testes type IV-S (HAase), N-acetyl-D-glucosamine (NAG) and glycerol (Sigma–Aldrich, Steinheim, Germany). Food grade ethanol, methanol, sodium carbonate, sodium chloride, sodium hydroxide, potassium persulfate, Fe $(NH_4)_2(SO_4)_2$, EDTA, acetic acid, formic acid and p-dimethylaminebenzaldehyde (VWR-Prolabo, Fontenay-sous-Bois, France). Gallic acid, $NaNO_2$ and catechin (Panreac, Barcelona, Spain). $AlCl_3$ (Fluka analytical, Buch SG, Switzerland). Na_2HPO_4, and NaH_2PO_4 (Scharlau, Barcelona, Spain). Potassium tetraborate, sodium benzoate and uric acid (Alfa Aesar, Karlsruhe, Germany). Nutrient broth No. 2 (NB), MEB (Malt Extract broth), Agar bacteriological No. 1 and RINGER (OXOID, Hampshire, England). MRS "DeMan, Rogosa and Sharpe" (MERCK, Darmstadt, Germany). Water was deionized using a Milli-Q water purification system (Millipore, Bedford, USA).

Absorbance was measured with a Varian Cary Bio 400 spectrophotometer.

2.2. Honey and propolis samples

Samples were four honeys and four propolis harvested in Spain in 2014. Honeys' melissopalynology (Von der Ohe, Peresano-Oddo, Piana, Morlot, & Martin, 2004) showed that samples H1 and H2 were heather (*Erica* sp. and *Calluna vulgaris* (L.) Hull) honeys; sample H3 was a chestnut (*Castanea sativa* Miller) honey with honeydew elements; and sample H4 was a multifloral honey. High quality propolis (A–D) were collected using a plastic propolis trap placed on top of the beehive, that beekeepers withdrew, freezed and shook.

All samples were stored in the dark at 4 °C (honey), and at −20 °C (propolis) until use.

2.3. Soft propolis extracts

Propolis samples were grounded in a marble mortar at −30 °C. Twenty grams of pulverized propolis were extracted with 75 ml of 90% food grade ethanol during 48 h at room temperature and in the absence of light, using a magnetic stirring system. Subsequently, the extracts were filtered through a funnel using Whatman filter paper No. 40. After filtration, each ethanol extract was transferred to a round bottom flask and subjected to a rotary evaporation to dryness at 25 °C. Eventually, in order to complete the evaporation of ethanol, it was left in a vacuum oven for 24 h at 30 °C. The soft propolis extract (SPrEx) was frozen until further use.

2.4. Preparation of honey with propolis

Each SPrEx was added to each honey at 0.1%, 0.3% and 0.5%. 100 g of each mixture were prepared by weighting the corresponding amounts of propolis and honey and mixing them by mechanical stirring until complete homogenization. All mixtures were stored at 4 °C until their use.

2.5. Sensory analysis

First, a panel of 5 trained assessors tested mixtures of honey and SPrEx with concentrations of SPrEx between 0.1% and 3%, choosing the concentrations of 0.1%, 0.3% and 0.5% SPrEx as suitable to be mixed with honey. All products with more than 0.5% SPrEx were rejected due to their unpleasant organoleptic characteristics, agreeing with a preliminary research done the previous year with one honey and two propolis. Second, the panel chose the two most likeably propolis concentrations for each honey. Finally, an acceptance test was carried out by sixty-five untrained adult panellists, who assessed each honey sample with the two different SPrEx concentrations that had been chosen among 0.1%, 0.3% and 0.5%, using a 9-point hedonic scale, where 9 was like a lot and 1 was dislike intensely (ISO 4121: 2003). The assessed samples were 1H, 1HA (0.3% and 0.5%), 1HB (0.1% and 0.3%), 1HC (0.1% and 0.3%), 1HD (0.1% and 0.3%), 2H, 2HA (0.3% and 0.5%), 2HB (0.1% and 0.3%), 2HC (0.1% and 0.3%), 2HD (0.1% and 0.3%), 3H, 3HA (0.1% and 0.3%), 3HB (0.3% and 0.5%), 3HC (0.1% and 0.3%), 3HD (0.3% and 0.5%) and 4H, 4HA (0.1% and 0.3%), 4HB (0.3% and 0.5%), 4HC (0.1% and 0.3%), 4HD (0.3% and 0.5%). The samples were prepared 1 h before the test run, and the analysis was performed following the conditions established by ISO 6658:2008. The panellists were students and staff of the University, who received instructions about the research and the products. Each sample was coded with three random digits and honeys without SPrEx were also included, evaluating 9 samples in each session. Unsalted crackers and water at room temperature were provided to clean the palate between samples. The assessors chose for each honey the most likeable product of honey with propolis (H-SPrEx).

2.6. Phenolics extraction

Phenolic compounds were extracted following the method described by Escriche, Kadar, Juan-Borrás, and Domenech (2014). For honeys and H-SPrEx, 25 g of sample was dissolved in 250 ml of distilled water, while in the case of propolis, 0.2 g of SPrEx was dissolved in 100 ml of 90% ethanol (v/v) and diluted 1/20 with distilled water. Subsequently, the pH of the solutions was adjusted to pH 2.0 with 2 M HCl. Solutions were slowly filtered through a column with Amberlite XAD-2 resin (Supelco, Bellefonte, PA, USA), preconditioned with methanol and distilled water. The column was washed with 250 ml of acidified water (pH 2 with 2 M HCl) and rinsed with 300 ml of neutral distilled water to remove all sugars and other polar compounds of honey and propolis. The phenol compounds were eluted with 250 ml of methanol.

2.7. Total phenolics content

Total phenolics were determined in raw samples and methanolic extracts by the Folin–Ciocalteu assay (Meda et al., 2005). SPrEx (0.2000 g) were dissolved to 100 ml with ethanol 90% (v/v), 1 ml of this solution was diluted to 50 ml with distilled water. Each honey and H-SPrEx (5.0000 g), was diluted to 50 ml with distilled water. All samples were filtered through Whatman No. 1 paper. 0.5 ml of each sample (raw and methanolic extracts), was mixed with 2.5 ml of 0.2 N Folin–Ciocalteu reagent. After 5 min, 2 ml of 75 g/l sodium carbonate was added and the tubes were incubated 2 h in dark at room temperature. Then, absorbance was read at 760 nm against

a blank of distilled water. The standard for the calibration curve was gallic acid (5–260 μg/ml), expressing the results as mg gallic acid (GA)/100 g sample.

2.8. Total flavonoids content

The total flavonoids content was determined according to the two most applied spectrophotometric methods based on the formation of aluminium–flavonoid complexes (Meda et al., 2005; Pękal & Pyrzynska, 2014). Total flavonoids content was determined in methanolic extracts without sugars' interferences.

Procedure 1: 1 ml of methanolic extract was mixed with 0.3 ml of $NaNO_2$ (5% w/v). After 5 min, 0.5 ml of $AlCl_3$ (2% w/v, in methanol) was added. 6 min later, the mixtures were neutralized with 0.5 ml of 1 M NaOH solution. After 10 min at room temperature, absorbance was read at 510 nm against a blank of methanol. The standard for the calibration curve was catechin (2.55–200 μg/ml), expressing the results as mg catechin (C)/100 g sample.

Procedure 2: 1 ml of methanolic extract was mixed with 1 ml of $AlCl_3$ (2% w/v, in methanol). After 10 min at room temperature, absorbance was read at 415 nm against a blank sample consisting of a 1 ml methanolic extract with 1 ml methanol without $AlCl_3$. The standard for the calibration curve was quercetin (2.5–200 μg/ml), expressing the results as mg quercetin (Q)/100 g sample.

2.9. Antioxidant activity

2.9.1. ABTS scavenging activity test (TEAC assay)

Antioxidant activity of honeys, SPrEx and H-SPrEx was evaluated by TEAC assay, using ABTS as the source of free radicals (Miguel, Doughmi, Aazza, Antunes, & Lyoussi, 2014). Honeys and H-SPrEx were prepared at 100 g/l with distilled water, while SPrEx samples were prepared at 0.1%, 0.3% and 0.5% (w/v) with 90% ethanol. 1490 μl of $ABTS^{\cdot+}$ was mixed with 10 μl of sample, standard or blank. Trolox was used as standard for the calibration curve (0.625–5 mM). The percentage inhibition after 6 min was calculated, expressing the results as μmol Trolox (T)/g sample (for honeys and H-SPrEx), and as μmol Trolox (T)/100 ml of SPrEx (for propolis extracts).

2.9.2. Radical-scavenging effect on hydroxyl radicals (AOA assay)

Hydroxyl radicals scavenging ability of honeys, SPrEx and H-SPrEx was assayed following the procedure of Koracevic, Koracevic, Djordjevic, Andrejevic, and Cosic (2001). Honeys and H-SPrEx were diluted in distilled water 1/25 (w/v), while SPrEx were diluted in ethanol 90% at 0.1%, 0.3% and 0.5% (w/v). Samples (10 μl) were mixed with 490 μl of sodium phosphate buffer (0.1 M, pH 7.4), 500 μl of sodium benzoate (0.01 M), 200 μl of $FeSO_4$–EDTA (2 mM) and 200 μl of hydrogen peroxide (0.01 M). After 1 h incubation at 37 °C, the reaction was stopped adding 1 ml of acetic acid (20%) and 1 ml of thiobarbituric acid (0.8% w/v) in NaOH (50 mM). The solution was boiled throughout 10 min and then cooled in ice. The absorbance was measured at 532 nm against distilled water. Each sample (A_1) had its own control (A_0), in which acetic acid (20%) was added before Fe–EDTA and H_2O_2. For each series of analysis a negative control (K_1 and K_0) was prepared, containing the same reagents as *A*, except the sample, which had been replaced with phosphate buffer. 1 mM uric acid in NaOH (5 mM) (U_1 and U_0) was used as standard for calibration. Antioxidant activity was calculated for honeys and H-SPrEx as mmol uric acid/100 g = $2.5 \times (C_U) \times (K - A)/(K - U)$, and for SoPrEx as mmol uric acid/100 ml = $0.1 \times (C_U) \times (K - A)/(K - U)$, where C_U is the concentration of uric acid (1 mM), *K* is the absorbance of control ($K_1 - K_0$), *A* is the absorbance of sample ($A_1 - A_0$) and *U* is the absorbance of uric acid solution ($U_1 - U_0$).

2.10. Antimicrobial activity

2.10.1. Culture media and microbial strains

Six bacterial and six fungi species were used for the experiment: *Escherichia coli* (CECT 434), *Lactobacillus plantarum* (CECT 220), *Staphylococcus aureus* TA1, *S. aureus* NB1, *Pseudomonas aeruginosa* M1 and *Listeria innocua* TA1 (bacterial collection of the Department of Biotechnology and Food Science, at Burgos University, isolated from foods), *Aspergillus flavus* (CECT 2687), *Penicillium nordicum* (CECT 20766), *Penicillium expansum* MP75, *Penicillium commune* M35 (fungi collection of the Department of Food Hygiene and Food Technology, at León University, isolated from foods and identified by T.M. López-Díaz et al.), *Fusarium* sp. NB1 and *Aspergillus niger* NB1 (fungi collection of the Department of Biotechnology and Food Science, at Burgos University, isolated from bee pollen). Stock cultures were maintained on Nutrient broth No. 2 (NB) for bacterium, MRS (DeMan, Rogosa and Sharpe) for *Lb. plantarum* and MEB (Malt Extract broth) for fungi with glycerol (20%) at −20 °C.

Bacterial inoculum was prepared by growing cells in NB or MRS for 24 h at 37 °C. Cell suspensions were diluted in sterile RINGER to provide initial cell counts of about 8 log cfu/ml. While fungi was prepared from sporulating 7-day-old cultures grown on MEA (Malt Extract Agar) at 25 °C. Colonies were covered with 5 ml sterile Tween 80 at 0.05% (v/v) and surface scraped with a sterile loop. The mixtures of conidia were counted at microscope in a Neubauer chamber (Brand, Wertheim, Germany). Dilutions with sterile Tween 80 at 0.05% were used to adjust the suspension to 5 log conidia/ml.

2.10.2. Agar well diffusion

Agar plates (NA, MRS, MEA) were inoculated with bacterium suspensions (overnight cultures grown at 37 °C on nutrient broth or MRS and further diluted in RINGER to obtain a final concentration of 8 log cfu/ml), or fungi suspensions (at 5 log conidia/ml) over the entire surface of the plate. Two hours later, sterile discs (6.0 mm diameter, Oxoid) impregnated in each sample (honey, SPrEx at 0.1%, 0.3% and 0.5% and H-SprEx) were placed on the surface of the agar using a sterile tweezer. Plates were incubated at 37 °C for 24 h for bacterium and at 25 °C for 5 days for fungi. Ethanol was also tested because propolis was diluted in it. Inhibition zones were measured using a Vernier calliper. The diameter of zones, including the diameter of the discs, was recorded.

2.11. Anti-inflammatory activity

Anti-inflammatory activity of honeys, SPrEx and H-SPrEx was assessed by hyaluronidase inhibition assay (Ferreres et al., 2012), slightly modified, based in the mechanism of the Morgan–Elson reaction. Dilutions of samples at 75% and 50% (w/v, in distilled water) were prepared for the assay. A stock solution of 5 mg/ml HA sodium salt from *Streptococcus equi* was prepared in water and stored at 4 °C. HA stock solution (70 μl) and 100 μl of buffer (0.2 M sodium formate, 0.1 M NaCl and 0.2 mg/ml BSA, pH adjusted to 3.68 with formic acid) were added to 200 μl milliQ water and 50 μl sample. The mixture was heated at 37 °C for 10 min before starting the reaction by addition of 50 μl of hyaluronidase from bovine testes type IV-S (600 U/ml) prepared in 0.9% NaCl. The mixture was incubated 1 h at 37 °C in a water bath. The enzymatic reaction was stopped by adding 100 μl of 0.8 M potassium tetraborate and then, incubated 3 min in water-bath at ebullition. The test tubes were cooled at room temperature and 750 μl of DMAB (2 g of DMAB dissolved in a mixture of 2.5 ml of 10 N HCl and 17.5 ml of glacial acetic acid, further diluted 1:2 with glacial acetic acid immediately before use), was added. The tubes were incubated 20 min at 37 °C and the colour of the

resulting product was read at 586 nm against a blank sample (where enzyme and samples were substituted by buffer). Enzyme activity was defined as 1 unit (U) of hyaluronidase that catalyzes the liberation of 1 μmol N-acetyl-D-glucosamine (NAG) per min under specified conditions. N-acetyl-D-glucosamine standard solutions (in the range between 0 and 2 μmol per test), were used as standard for calibration curves. With the NAG formed in each enzymatic reaction and using the linear regression equation, the percentage of enzyme inhibition was calculated as % Inhibition = $(A - B/A) \times 100$, where A was μmol of NAG in the positive control (where μl of sample was substituted by buffer) and B was μmol of NAG of each sample reaction.

2.12. Statistical analysis

Each assay was carried out in triplicate. All results were evaluated by multiple range tests. Sensory analysis was assessed by Turkey's tests ($p < 0.05$), and the other analyses by LSD test ($p < 0.05$). Principal Component Analysis (PCA) and Pearson correlations were applied to the results. Statistical software Stagraphics Ceturion XVI was used.

3. Results and discussion

3.1. Sensory analysis

All averages obtained after the sensory analysis for all honeys, alone or with different concentrations of SPrEx were higher to 4.5, meaning that all tested samples had been accepted by the panel. The samples that appealed most to the consumers were the honeys alone, and the honeys with 0.1% SPrEx. Significant differences ($p < 0.05$) were observed among samples. As we were interested in making an edible product with honey and the highest amount of SPrEx as possible, we chose for further analyses the following mixtures that the consumers had liked the most: H1 + 0.5% propolis A (H1A), H1 + 0.3% propolis B (H1B), H1 + 0.1% propolis C (H1C) and H1 + 0.1% propolis D (H1D), H2 + 0.5% propolis A (H2A), H2 + 0.3% propolis B (H2B), H2 + 0.1% propolis C (H2C) and H2 + 0.1% propolis D (H2D), H3 + 0.1% propolis A (H3A), H3 + 0.5% propolis B (H3B), H3 + 0.3% propolis C (H3C) and H3 + 0.3% propolis D (H3D), H4 + 0.1% propolis A (H4A), H4 + 0.5% propolis B (H4B), H4 + 0.3% propolis C (H4C) and H4 + 0.3% propolis D (H4D).

Several studies showed that concentrations of propolis below 0.5% added to different foods (fish, sausages) were acceptable for the consumers (Duman & Özpolat, 2015; Gutiérrez-Cortés & Suarez Mahecha, 2014). However, Spinelli et al. (in press) increased the concentration of propolis up to 5% in fresh fish burgers, adding new ingredients such as potato flakes and extra virgin olive oil to give a final product with acceptable sensory characteristics.

3.2. Total phenolics contents

Total phenolics of propolis ranged from 21,188 to 22,762 mg GA/100 g. Values of methanolic propolis extracts ranged from 20,552 to 21,814 mg GA/100 g. No significant differences were found between total phenolics of propolis and their methanolic extracts, as well as among the propolis samples. The values obtained in this study were higher than those obtained by Socha et al. (2015) in Polish propolis (15,005–19,714 mg GA/100 g), although they were similar to the ranges obtained by other researchers (Kalogeropoulos, Konteles, Troullidou, Mourtzinos, & Karathanos, 2009; Moreira, Dias, Pereira, & Estevinho, 2008) in Greek and Portuguese propolis.

Fig. 1A shows total phenolics of honeys, H-SPrEx, and their corresponding methanolic extracts. Agreeing with literature, total phenolics of entire samples were higher than those of methanolic extracts (Ferreira, Aires, Barreira, & Estevinho, 2009). Significant differences were obtained between total phenolics of entire samples and their methanolic extracts. Higher values were obtained for samples H1 and H3 (99.73 and 103.48 mg GA/100 g) than for samples H2 and H4 (79.67 and 79.05 mg GA/100 g, respectively). Our values were similar than those obtained by other researchers for different entire honeys. Thus, Can et al. (2015) found that the total phenolic content was 105.46 mg GA/100 g and 98.26 mg GA/100 g in heather and chestnut honeys, respectively, while Meda et al. (2005) obtained total phenolic contents in multifloral honeys between 32.59 and 93.66 mg GA/100 g. However, other authors described lower total phenolic values. Beretta, Granata, Ferrero, Orioli, and Facino (2005) found that total phenolics average was 17.04 mg GA/100 g in multifloral honeys, and 21.12 mg GA/100 g in chesnut honeys. Kuś et al. (2014) reported that phenolics average for heather honeys was 30.62 mg GA/100 g, result that is similar to our data for total phenolics in methanolic extracts; nevertheless, those authors did not carry out any extraction.

Fig. 1A shows that, because of propolis addition, H-SPrEx had higher amounts of total phenolics than the base honey samples. These results agree with those obtained by Juszczak et al. (in press) who also reported higher total phenolics' content in honey with propolis in comparison with honey, though their values were lower than our data.

3.3. Total flavonoids' contents of methanolic extracts

By procedure 1, propolis total flavonoids ranged from 10,406 mg C/100 g to 13,796 mg C/100 g. By procedure 2, total flavonoids ranged from 7965 mg Q/100 g to 9686 mg Q/100 g. According to Pękal and Pyrzynska (2014), procedure 1 in alkaline medium allowed the altogether quantification of rutin, luteolin and catechins, whereas procedure 2 conducted in neutral media allowed the altogether quantification of such flavonols as quercetin, morin, kaempferol and rutin as well as the flavone luteolin. Besides, these authors also concluded that procedure 1 were considerably less selective for the determination of total flavonoids in comparison with procedure 2, because other non-flavonoid compounds were quantified by the former procedure (i.e. cholorogenic acid). Our propolis samples showed higher levels of total flavonoids by procedure 1 (expressed as catechin equivalents), than total flavonoids by procedure 2 (expressed as quercetin equivalents). Our values agree with literature data. Ahn et al. (2007) reported total flavonoids' values ranging from 830 to 16,200 mg Q/100 g propolis and Socha et al. (2015) found that the total flavonoids content in propolis samples ranged from 3564 to 6204 mg Q/100 g.

Total flavonoids contents of methanolic extracts of honeys and H-SPrEx are shown in Fig. 1B. In general, the amount of flavonoids by procedure 1 was higher than the quantity of flavonoids by procedure 2. Chestnut (H3) honey showed the highest values of flavonoids by procedure 1 (3.70 mg C/100 g honey), and the multifloral (H4) sample the highest values of flavonoids by procedure 2 (2.39 mg Q/100 g honey). Our results for total flavonoids were lower than those obtained by other authors. Meda et al. (2005) reported that the total flavonoids content varied from 0.17 to 8.35 mg Q/100 g, while Isla et al. (2011) obtained higher flavonoid content (around 15 mg Q/100 g) in multifloral honeys. Also, Ferreira et al. (2009) obtained a mean of 58.7 mg C/100 g for heather honey, which is considerably higher that the results of our study (1.56 mg C/100 g in H1 and 3.01 mg C/100 g in H2). It is important to highlight the fact that most researchers did not extract flavonoids from honey, so that at the wavelength of

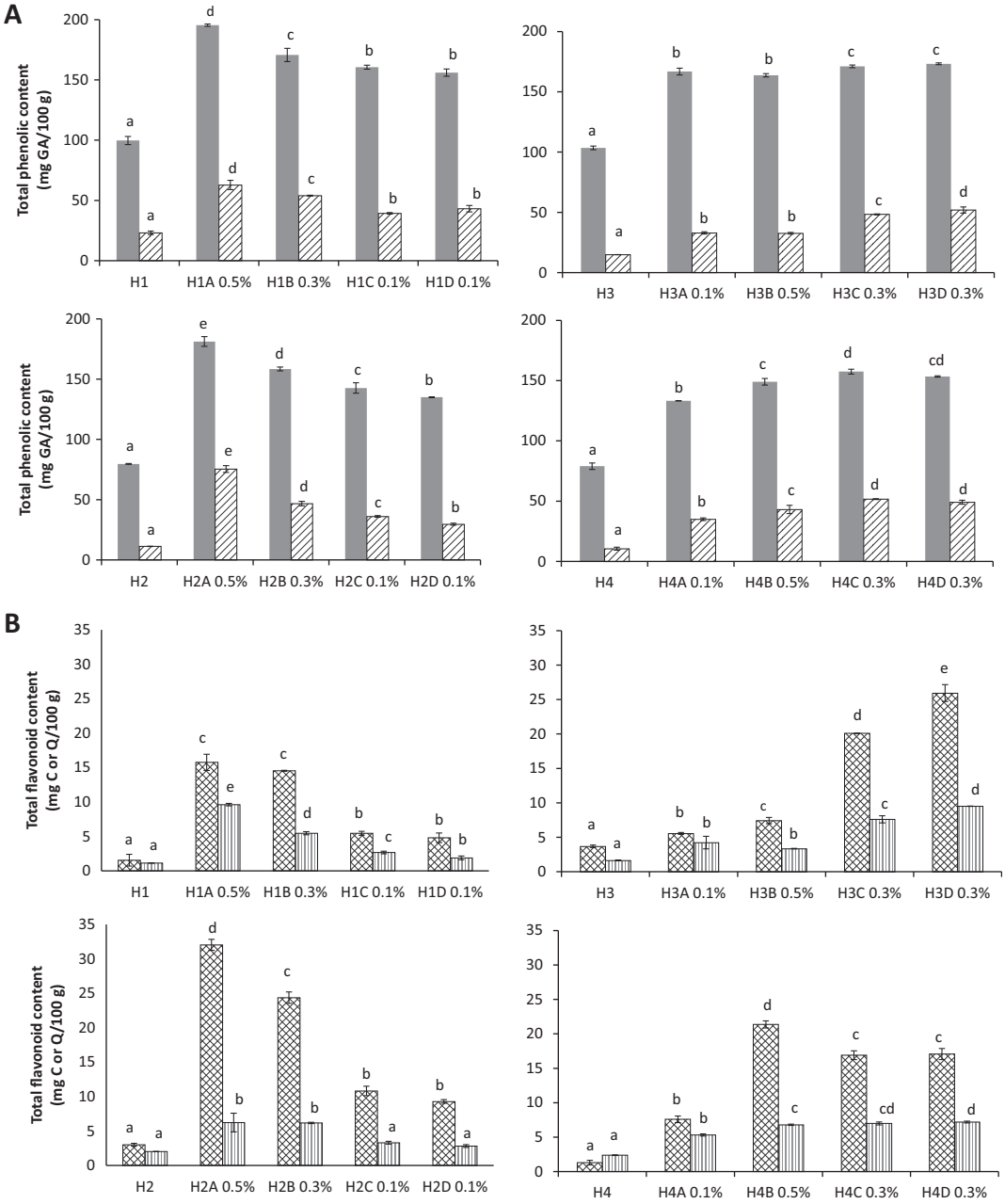

Fig. 1. Total phenolics (A) of original samples (■) and methanolic extract (▨) and total flavonoids of methanolic extract (B) by procedure 1 (▩) expressed as mg C/100 g and procedure 2 (|||) expressed as mg Q/100 g of honeys and H-"SPrEx". Different superscript letters (a–e) by each sample indicate significant differences ($p < 0.05$).

measurement, there are interferences of both honey colours and sugars. These interferences must be removed (Sancho et al., 2015).

Regarding methanolic extracts of H-SPrEx, values obtained by both procedures 1 and 2 were considerably higher than those of the base honeys. Similar results were obtained by Juszczak et al. (in press).

3.4. Antioxidant activity

3.4.1. ABTS scavenging activity test (TEAC assay)

The undiluted soft propolis extracts showed antioxidant activities ranging from 1184.66 to 1400.86 μmol Trolox/g, similar to those obtained by Serra-Bonvehí and LaCalle-Gutiérrez (2011) for Basque (North Spain) propolis. TEAC values of SPrEx diluted with ethanol ranged for 0.1% dilution between 131.64 and 156.85 μmol Trolox/100 ml; for 0.3% dilution from 355.40 to 420.26; and for 0.5% dilution from 466.12 to 470.94. As expected, antioxidant activity increased with the amount of added SPrEx not showing significant differences ($p > 0.05$) among them.

TEAC of honeys ranged between 62.50 and 825.34 μmol Trolox/100 g (Fig. 2A), values that were similar to those obtained by other researchers (Stanislava et al., 2013). When SPrEx was added at different concentrations, TEAC increased in all samples but in honey H1-SPrEx-D. These results are concordant with those of other study, in which propolis was added to fish burgers, thus increasing antioxidant activity in the original product (Spinelli et al., in press). Chestnut honey (H3) and the products made with it showed the highest TEAC results.

3.4.2. Radical-scavenging effect on hydroxyl radicals (AOA assay)

All soft propolis extracts showed a very similar radical-scavenging effect on hydroxyl radicals (0.12 mmol UA/100 ml),

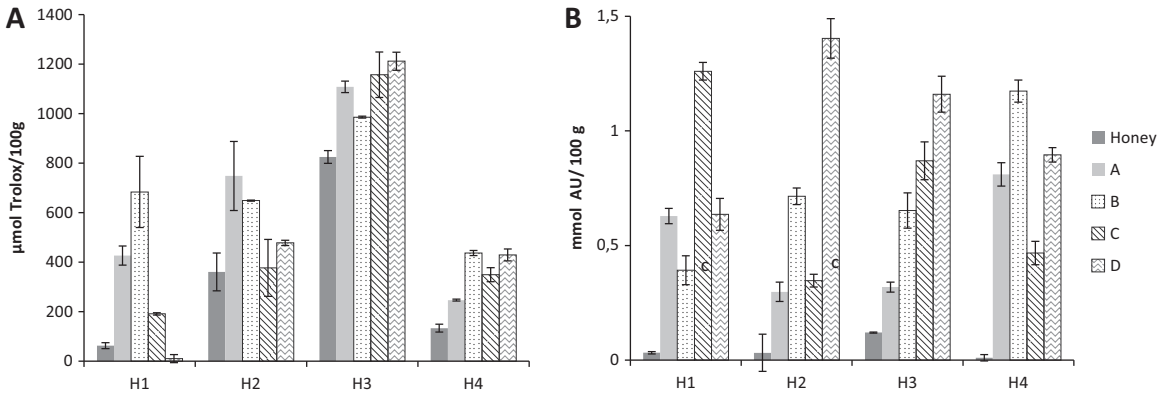

Fig. 2. Mean values ($n = 3$) of antioxidant activity by TEAC assay (A) expressed as µmol Trolox/100 g of honeys and H-"SPrEx", and radical-scavenging effect on hydroxyl radicals (AOA assay) (B), expressed as mmol UA/100 g of honey and H-"SPrEx". Error bars represent the standard deviation for each data point.

Table 1
Antimicrobial activity of different concentrations of propolis, honeys and H-"SPrEx" expressed as inhibition diameter (mm) including disc (6.0 mm) by agar well diffusion method against six fungi and six bacterium ($n = 3$).

	P. expansum	*P. nordicum*	*P. commune*	*A. flavus*	*A. niger*	*Fusarium* sp.	*St. aureus* TA1	*St. aureus* NB1	*Lb. plantarum*	*L. innocua*	*E. coli*	*Ps. aeruginosa*
$A_{0.5\%}$	$_{A}6.00^{a}$	$_{CD}10.35^{a}$	$_{CD}10.08^{a}$	$_{BC}8.42^{a}$	$_{AB}7.85^{a}$	$_{A}6.00^{a}$	$_{CD}10.30^{a}$	$_{F}15.80^{b}$	$_{A}6.25^{a}$	$_{DE}11.95^{b}$	$_{BC}8.50^{b}$	$_{BC}8.95^{b}$
$A_{0.3\%}$	$_{A}6.00^{a}$	$_{ABC}7.90^{a}$	$_{BC}9.75^{a}$	$_{ABC}8.75^{a}$	$_{AB}7.05^{a}$	$_{A}6.00^{a}$	$_{ABC}8.35^{a}$	$_{AB}7.05^{a}$	$_{C}10.65^{b}$	$_{ABC}8.65^{ab}$	$_{BC}9.50^{b}$	$_{A}6.00^{a}$
$A_{0.1\%}$	$_{A}6.00^{a}$	$_{BC}7.68^{a}$	$_{BC}7.63^{a}$	$_{C}8.07^{a}$	$_{AB}6.55^{a}$	$_{A}6.00^{a}$	$_{C}8.25^{a}$	$_{A}6.00^{a}$	$_{D}9.95^{b}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$
$B_{0.5\%}$	$_{AB}7.80^{a}$	$_{DE}11.23^{b}$	$_{ABCD}8.55^{a}$	$_{CDE}10.45^{a}$	$_{BCD}9.35^{b}$	$_{AB}6.65^{b}$	$_{ABCD}8.20^{ab}$	$_{A}6.40^{a}$	$_{CDE}9.90^{a}$	$_{BCDE}9.75^{a}$	$_{BCD}9.55^{b}$	$_{E}11.95^{b}$
$B_{0.3\%}$	$_{AB}6.90^{a}$	$_{E}10.30^{ab}$	$_{DE}9.72^{a}$	$_{CD}8.65^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{E}10.50^{b}$	$_{A}6.00^{a}$	$_{BC}7.80^{a}$	$_{CD}8.60^{a}$	$_{CDE}9.15^{b}$	$_{A}6.00^{a}$
$B_{0.1\%}$	$_{A}6.52^{a}$	$_{B}8.87^{a}$	$_{AB}7.83^{a}$	$_{AB}8.03^{a}$	$_{AB}6.95^{ab}$	$_{A}6.00^{a}$	$_{A}6.08^{a}$	$_{A}6.00^{a}$	$_{C}12.20^{a}$	$_{AB}7.65^{a}$	$_{A}6.50^{a}$	$_{AB}7.00^{a}$
$C_{0.5\%}$	$_{ABC}9.08^{b}$	$_{D}12.50^{a}$	$_{BCD}10.20^{a}$	$_{ABC}9.10^{a}$	$_{A}6.58^{a}$	$_{AB}8.20^{a}$	$_{AB}8.25^{b}$	$_{AB}7.90^{b}$	$_{D}12.60^{b}$	$_{CD}11.50^{a}$	$_{AB}8.35^{a}$	$_{BC}9.45^{a}$
$C_{0.3\%}$	$_{ABC}9.20^{b}$	$_{BCD}10.23^{a}$	$_{BCD}10.00^{a}$	$_{ABCD}9.52^{a}$	$_{A}6.00^{a}$	$_{CD}12.45^{b}$	$_{AB}8.30^{b}$	$_{A}6.00^{a}$	$_{D}13.15^{b}$	$_{BCD}10.80^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$
$C_{0.1\%}$	$_{AB}6.55^{a}$	$_{CD}9.48^{a}$	$_{ABCD}8.60^{a}$	$_{ABCD}7.93^{a}$	$_{ABC}7.63^{a}$	$_{ABC}7.73^{a}$	$_{A}6.00^{a}$	$_{AB}6.70^{ab}$	$_{ABC}7.65^{a}$	$_{D}10.40^{a}$	$_{A}6.00^{a}$	$_{BCD}9.00^{a}$
$D_{0.5\%}$	$_{A}8.60^{b}$	$_{D}14.10^{b}$	$_{A}9.75^{a}$	$_{ABC}10.83^{b}$	$_{AB}10.35^{b}$	$_{A}9.05^{b}$	$_{A}8.05^{a}$	$_{A}9.70^{a}$	$_{CD}13.35^{c}$	$_{BCD}12.90^{c}$	$_{A}8.60^{b}$	$_{A}9.30^{b}$
$D_{0.3\%}$	$_{AB}6.98^{a}$	$_{E}13.85^{b}$	$_{ABC}7.53^{a}$	$_{BCD}8.93^{ab}$	$_{D}10.70^{b}$	$_{ABC}7.83^{ab}$	$_{ABC}7.27^{a}$	$_{BCD}9.65^{a}$	$_{BCD}9.40^{b}$	$_{CD}9.75^{b}$	$_{A}6.00^{a}$	$_{A}6.10^{a}$
$D_{0.1\%}$	$_{A}6.00^{a}$	$_{C}10.83^{a}$	$_{C}9.77^{a}$	$_{B}7.35^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{AB}6.43^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{AB}6.40^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$
1H	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}7.05^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$
$1HA_{0.5\%}$	$_{BCDE}14.85^{c}$	$_{DE}16.13^{c}$	$_{BCD}11.85^{b}$	$_{A}6.78^{ab}$	$_{ABC}10.55^{b}$	$_{AB}10.00^{b}$	$_{CDE}15.20^{c}$	$_{E}17.40^{b}$	$_{BCD}13.50^{c}$	$_{BCD}11.75^{b}$	$_{BCD}13.40^{b}$	$_{AB}10.25^{bc}$
$1HB_{0.3\%}$	$_{BCDE}10.38^{b}$	$_{DE}12.30^{b}$	$_{EF}13.75^{b}$	$_{AB}7.28^{ab}$	$_{A}6.00^{a}$	$_{ABCD}9.35^{ab}$	$_{F}14.75^{c}$	$_{F}14.45^{b}$	$_{CDEF}11.70^{b}$	$_{EF}13.75^{b}$	$_{ABC}8.30^{a}$	$_{A}6.00^{a}$
$1HC_{0.1\%}$	$_{BC}8.95^{b}$	$_{CD}10.05^{b}$	$_{E}12.13^{b}$	$_{BC}8.55^{b}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{DE}10.95^{b}$	$_{F}14.60^{b}$	$_{DE}11.60^{b}$	$_{F}14.25^{b}$	$_{AB}7.75^{a}$	$_{DE}11.85^{c}$
$1HD_{0.1\%}$	$_{BC}8.88^{b}$	$_{DE}10.80^{b}$	$_{CD}10.28^{b}$	$_{B}7.58^{ab}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{FG}12.65^{bc}$	$_{G}13.50^{b}$	$_{DEF}11.60^{b}$	$_{EFG}12.00^{b}$	$_{DE}10.85^{ab}$	$_{B}8.65^{b}$
2H	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{B}8.70^{a}$	$_{C}12.45^{b}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$
$2HA_{0.5\%}$	$_{DE}11.65^{b}$	$_{ABCD}9.00^{ab}$	$_{CDE}11.03^{b}$	$_{A}6.43^{a}$	$_{AB}7.10^{a}$	$_{ABC}8.75^{b}$	$_{ABCD}9.00^{a}$	$_{BCD}9.35^{a}$	$_{E}13.20^{c}$	$_{E}13.6^{b}$	$_{ABCD}9.10^{b}$	$_{BCD}9.60^{b}$
$2HB_{0.3\%}$	$_{BC}11.00^{b}$	$_{AB}7.93^{ab}$	$_{BC}11.20^{b}$	$_{A}7.03^{ab}$	$_{AB}7.88^{a}$	$_{AB}8.00^{b}$	$_{ABC}10.05^{a}$	$_{ABC}10.10^{ab}$	$_{C}12.65^{c}$	$_{C}12.75^{b}$	$_{ABC}9.95^{b}$	$_{A}7.50^{ab}$
$2HC_{0.1\%}$	$_{ABC}8.25^{ab}$	$_{CD}9.63^{b}$	$_{CD}9.95^{b}$	$_{ABC}7.68^{b}$	$_{A}6.00^{a}$	$_{AB}6.73^{a}$	$_{BC}8.50^{a}$	$_{CD}9.70^{a}$	$_{DE}11.25^{bc}$	$_{E}12.85^{b}$	$_{BC}8.80^{ab}$	$_{CD}9.30^{b}$
$2HD_{0.1\%}$	$_{AB}8.03^{ab}$	$_{BC}9.38^{ab}$	$_{BC}10.38^{b}$	$_{A}6.33^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{BC}10.55^{a}$	$_{C}11.00^{ab}$	$_{BC}10.50^{b}$	$_{BC}10.40^{b}$	$_{BC}9.80^{b}$	$_{A}6.00^{a}$
3H	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.50^{a}$	$_{A}6.50^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$
$3HA_{0.1\%}$	$_{BC}9.80^{ab}$	$_{ABC}8.88^{b}$	$_{CD}11.98^{b}$	$_{AB}7.25^{a}$	$_{A}6.00^{a}$	$_{AB}8.00^{a}$	$_{DE}14.55^{b}$	$_{DE}15.20^{b}$	$_{A}6.00^{a}$	$_{E}17.00^{bc}$	$_{BC}9.85^{bc}$	$_{ABC}8.55^{a}$
$3HB_{0.5\%}$	$_{DE}13.93^{b}$	$_{BCD}10.48^{c}$	$_{E}15.08^{b}$	$_{BCD}10.88^{b}$	$_{BC}10.30^{b}$	$_{AB}9.05^{b}$	$_{E}15.25^{bc}$	$_{E}14.50^{b}$	$_{A}6.00^{a}$	$_{CDE}13.60^{b}$	$_{BCD}12.00^{c}$	$_{A}6.00^{a}$
$3HC_{0.3\%}$	$_{ABC}11.68^{ab}$	$_{AB}10.10^{c}$	$_{BCD}14.78^{b}$	$_{A}8.58^{ab}$	$_{A}8.88^{b}$	$_{A}6.00^{a}$	$_{CD}17.45^{c}$	$_{BCD}15.10^{b}$	$_{A}6.00^{a}$	$_{E}18.85^{c}$	$_{AB}11.3^{bc}$	$_{AB}9.55^{a}$
$3HD_{0.3\%}$	$_{CDE}13.05^{b}$	$_{BCD}10.27^{c}$	$_{DE}13.70^{b}$	$_{ABC}8.93^{ab}$	$_{ABC}9.45^{b}$	$_{ABC}9.33^{b}$	$_{AB}6.65^{a}$	$_{BCD}11.00^{ab}$	$_{A}6.00^{a}$	$_{E}16.90^{bc}$	$_{ABC}9.10^{b}$	$_{A}6.00^{a}$
4H	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{B}7.55^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{A}6.55^{a}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$
$4HA_{0.1\%}$	$_{C}10.80^{bc}$	$_{AB}8.20^{ab}$	$_{BC}9.68^{b}$	$_{BC}10.53^{bc}$	$_{A}6.00^{a}$	$_{A}6.00^{a}$	$_{BC}9.45^{a}$	$_{E}15.95^{d}$	$_{A}6.00^{a}$	$_{D}13.35^{b}$	$_{BC}9.95^{ab}$	$_{A}6.00^{a}$
$4HB_{0.5\%}$	$_{BC}9.50^{ab}$	$_{DEF}13.08^{c}$	$_{EF}15.00^{c}$	$_{BCD}11.95^{c}$	$_{AB}8.88^{b}$	$_{ABC}9.15^{ab}$	$_{BCD}10.35^{a}$	$_{CDE}12.40^{b}$	$_{BCD}11.20^{b}$	$_{F}15.85^{bc}$	$_{CDE}12.10^{b}$	$_{A}6.00^{a}$
$4HC_{0.3\%}$	$_{EF}13.55^{c}$	$_{DE}12.05^{c}$	$_{EF}13.30^{c}$	$_{BC}9.58^{b}$	$_{B}7.90^{b}$	$_{E}12.55^{b}$	$_{BC}9.75^{a}$	$_{FG}15.00^{cd}$	$_{E}12.35^{b}$	$_{G}16.10^{c}$	$_{CD}10.35^{ab}$	$_{A}6.00^{a}$
$4HD_{0.3\%}$	$_{DEF}12.55^{bc}$	$_{CDE}11.30^{bc}$	$_{F}14.90^{c}$	$_{CD}10.60^{bc}$	$_{AB}7.88^{b}$	$_{CD}10.48^{ab}$	$_{BC}9.30^{a}$	$_{DEF}13.00^{bc}$	$_{CDE}11.60^{b}$	$_{EF}13.25^{b}$	$_{CD}10.65^{b}$	$_{A}6.00^{a}$

Different superscript letters (a–c) in the same column for each microorganism indicate significant differences ($p < 0.05$). Different capital letters (A–G) in the same row for each sample indicate significant differences ($p < 0.05$) between microorganisms.

which was slightly higher than that of the uric acid standard 1 mM. Furthermore, there were no significant differences ($p > 0.05$) among different concentrations. Honey samples showed values lower than 0.15 mmol UA/100 g (Fig. 2B), which were similar to the results obtained by Pérez, Rodríguez-Malaver, and Vit (2006). When SoPrEx was added to honey, radical-scavenging effect on hydroxyl radicals significantly increased in all samples.

3.5. Antimicrobial activity

Table 1 shows the antimicrobial activity of different concentrations of SPrEx, honeys and H-SPrEx. Ethanol was used as control sample, not having shown inhibitory effects against fungi. However ethanol showed a small effect against bacteria, therefore the halo obtained by ethanol was subtracted to the halo obtained by

Table 2
Anti-inflammatory activity of propolis, honeys and H-"SPrEx" (diluted at 50% and 75%) expressed as % hyaluronidase inhibition.

		PA	PB	PC	PD
Propolis	0.1%	$_{AB}17.07^{a}$	$_{A}13.79^{a}$	$_{B}24.55^{ab}$	$_{AB}20.37^{a}$
	0.3%	$_{AB}20.20^{a}$	$_{C}42.49^{ab}$	$_{B}27.25^{b}$	$_{A}15.55^{a}$
	0.5%	$_{A}21.39^{a}$	$_{B}53.64^{b}$	$_{A}18.54^{a}$	$_{B}45.31^{b}$
		H1	H2	H3	H4
H and H-"SPrEx" 50%	Honey	$_{A}-11.26^{a}$	$_{C}7.37^{a}$	$_{C}9.64^{a}$	$_{B}-3.78^{a}$
	PA	$_{B}33.33^{c}$	$_{B}25.43^{b}$	$_{A}13.87^{ab}$	$_{B}29.07^{d}$
	PB	$_{A}7.63^{b}$	$_{B}28.87^{b}$	$_{B}26.95^{c}$	$_{A}14.16^{bc}$
	PC	$_{B}26.59^{c}$	$_{AB}20.18^{ab}$	$_{C}41.59^{d}$	$_{A}16.72^{c}$
	PD	$_{B}30.66^{c}$	$_{AB}19.68^{ab}$	$_{AB}17.77^{b}$	$_{A}7.86^{b}$
H and H-"SPrEx" 75%	Honey	$_{C}61.53^{a}$	$_{A}2.31^{b}$	$_{B}30.47^{a}$	$_{C}65.61^{a}$
	PA	$_{B}62.38^{a}$	$_{A}12.77^{c}$	$_{B}65.75^{b}$	$_{B}58.02^{a}$
	PB	$_{AB}56.34^{a}$	$_{A}40.36^{d}$	$_{B}68.66^{b}$	$_{B}65.22^{a}$
	PC	$_{AB}58.31^{a}$	$_{A}37.76^{d}$	$_{B}74.27^{b}$	$_{AB}53.54^{a}$
	PD	$_{C}69.84^{a}$	$_{A}-14.86^{a}$	$_{B}32.80^{a}$	$_{C}62.54^{a}$

Different superscript letters (a–d) in the same column indicate significant differences ($p < 0.05$). Different capital letters (A–C) in the same row for each sample indicate significant differences ($p < 0.05$). PA, PB, PC, PD: propolis samples; H1, H2, H3, H4: honeys samples; H: honey; H-"SPrEx": honey with soft propolis extract.

SPrEx. Regarding propolis samples, in general the three different concentrations (0.1%, 0.3% and 0.5%), showed similar antimicrobial activities, with no significant differences ($p > 0.05$) among halo diameters, although in some cases, SPrEx at 0.5% showed higher antimicrobial activities ($p < 0.05$), as expected. Only propolis A against *Lb. plantarum* showed higher antimicrobial activity at 0.1% than at 0.5%. In general, propolis C and D appeared to have higher activities. The microorganisms more sensitive to SPrEx were *P. nordicum* and *Lb. plantarum*, while the most resistant were *P. expansum* and *Ps. aeruginosa*. Other researchers obtained higher halos against bacteria, although they used higher propolis extract concentrations than those of this study. However, we obtained higher halos against *A. niger* if compared to the halos of other authors (Kujumgiev et al., 1999). Several researchers obtained higher antimicrobial activities of propolis against Gram-positive than against Gram-negative bacteria (Campos et al., 2014; Silici & Kutluca, 2005).

Focusing on honeys and H-SPrEx, no honeys' antimicrobial activity against most microorganisms was observed. There was only a weak activity against *St. aureus*. These results were quite different that those obtained in other studies, in which honeys had shown high antimicrobial activities (Fidaleo, Zuorro, & Lavecchia, 2011; Sherlock et al., 2010). The differences are likely due to the honeys' composition and botanical origins. When SPrEx were added to honey, both antifungal and antibacterial activity dramatically increased. Nevertheless, the products made with honeys H3 and H4 did not show antibacterial activities against *Lb. plantarum* and *Ps. aeruginosa*. Feás, Pacheco, Iglesias, and Estevinho (2014)

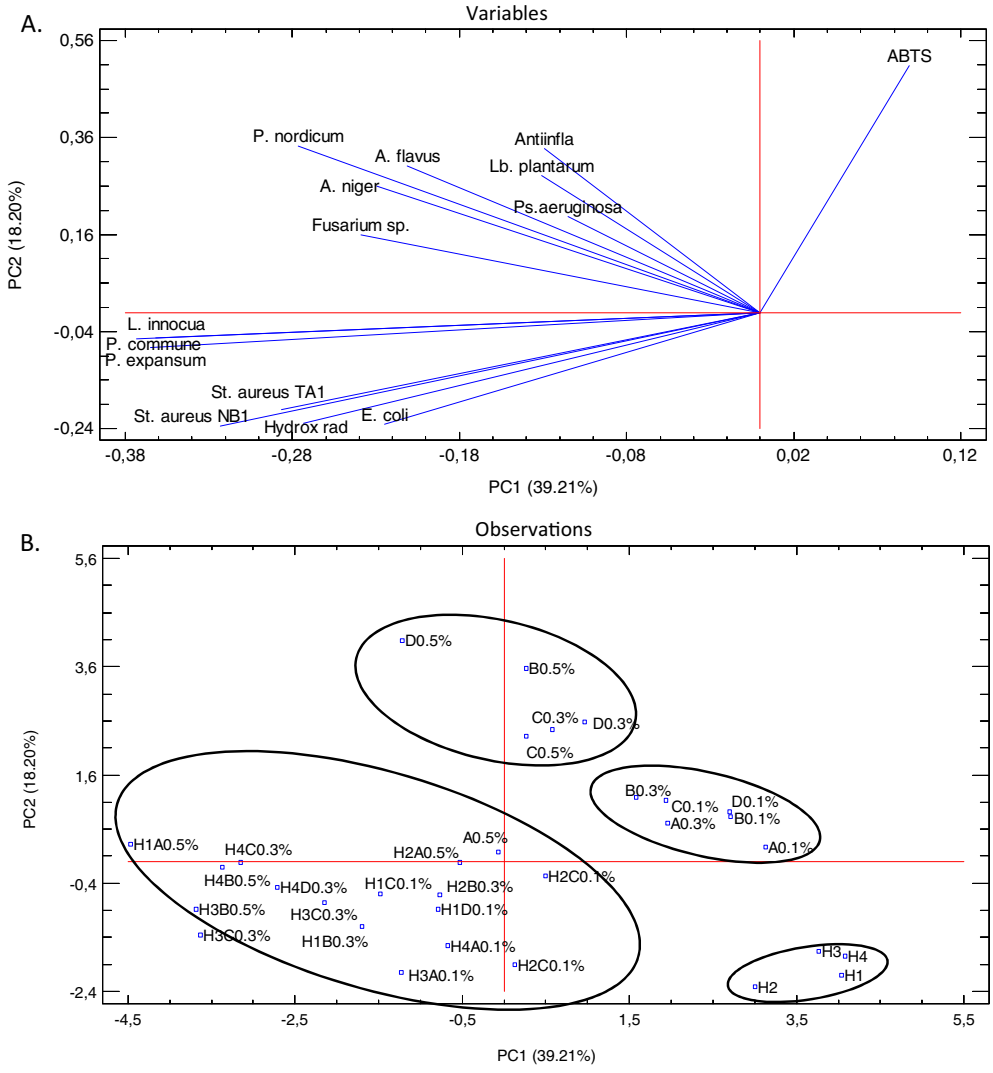

Fig. 3. PCA analysis of antimicrobial, antioxidant and anti-inflammatory properties of "SPrEx", at different concentrations (0.1%, 0.3% and 0.5%), honeys and H-"SPrEx".

obtained a high effectiveness in reducing the lettuce microbial load when propolis was added to that food. In our study, the most sensitive microorganisms were *St. aureus*, *L. innocua* and *P. commune*, while the most resistant were *Ps. aeruginosa*, *Fusarium* sp. and *A. niger*. Our results are in agreement with other studies, where *St. aureus*, *L. innocua* and *P. commune* showed to be more sensitive to honey than other microorganisms. In contrast, our honeys were more efficient against *E. coli* than other honeys, because the sensitivity against this bacterium was higher than the values found in the literature, in which gram negative bacterium are described as the most resistant to honey (Al-Waili, Al-Ghamdi, Ansari, Al-Attal, & Salom, 2012; Fidaleo et al., 2011; Sherlock et al., 2010). Voidarou et al. (2011) also obtained bigger inhibition halo for *E. coli* than for some strains of *St. aureus*.

Finally, a synergic effect was observed in H-SPrEx, in agreement with the results of Al-Waili et al. (2012), because higher halo inhibition zones were observed in all products than in honeys or SPrEx alone.

3.6. Anti-inflammatory activity

Table 2 shows the anti-inflammatory activity of the SPrEx (at 0.1%, 0.3% and 0.5%), honeys, and H-SPrEx, having been all samples diluted at 50% and 75% in distilled water and expressing the results as % hyaluronidase inhibition.

In general, anti-inflammatory activity of SPrEx increased as propolis concentration did, in agreement with the results of Silva, Rodrigues, Feás, and Estevinho (2012). In respect of honeys, samples at 50% showed null or very low activity, in contrast with the significant activities found in honeys at 75% (except for honey H2), agreeing with other studies (Hadagali & Chua, 2014) where anti-inflammatory activity of honeys was also described. In all samples diluted at 50%, independently of the SPrEx, additions of SPrEx to honeys led to an increase of anti-inflammatory activities. In samples diluted at 75%, such increases were observed only in samples H2 and H3, probably because honeys H1 and H4 had a relatively high anti-inflammatory activities and the activities of SPrEx were overlapped.

3.7. Principal components analysis and correlations

Principal Component Analysis (Fig. 3), divided the samples into 4 groups, honeys (with lower antioxidant, antimicrobial and anti-inflammatory activity), SPrEx at 0.1% and two SPrEx at 0.3% (with low antimicrobial and anti-inflammatory activity), SPrEx at 0.5% and two SPrEx at 0.3% (with higher total-antioxidant activity-ABTS, antimicrobial and anti-inflammatory activity), and H-SPrEx (with higher antimicrobial activity against *St. aureus*, *E. coli*, *L. innocua*, *P. commune* and *P. expansum* and higher hydroxyl radical scavenging activity). In H-SPrEx it was also observed that the activity increased in samples where SPrEx were present at 0.5% (samples placed leftmost). Therefore, honey samples were clearly separated from H-SPrEx.

The correlation matrix showed a significant correlation among the biochemical and antioxidant parameters (p-value <0.05). A strong correlation was found between total phenolics and total flavonoids determined by procedures 1 and 2 ($r = 0.727$ and $r = 0.824$) and among these three parameters and the antifungal and anti-inflammatory activities ($r > 0.554$ and $r > 0.396$), in agreement with the results of other studies (Isla et al., 2011; Miguel et al., 2014). It is important to highlight the negative correlation found between TEAC antioxidant activity and antimicrobial and hydroxyl radical scavenging activity (r between −0.298 and −0.467). It is likely due to the fact that in H-SPrEx, there was a synergic effect regarding antimicrobial activity, but not in respect of antioxidant activity. Furthermore, antioxidant activity was correlated with total flavonoids contents obtained by procedures 1 and 2 ($r = 0.428$ and $r = 0.446$; p-value <0.05) but not with total phenolics ($r = 0.251$; p-value >0.05), which is contradictory to other studies (Ferreira et al., 2009; Isla et al., 2011; Miguel et al., 2014). However, this research confirmed Meda et al. (2005) results, in which the radical scavenging activity could not be predicted by total phenolics, because antioxidant capacity is the result of the combined activity of a wide range of compounds.

4. Conclusion

Honey and propolis mixtures with concentrations of SPrEx higher than 0.5% are not sensory acceptable because of their unpleasant organoleptic characteristics.

The preference of consumers in respect of the propolis concentration added to honey (0.1–0.5%) was variable depending of the type of honey.

SPrEx added to honey, even at a concentration as low as 0.1%, is able to increase the antimicrobial, antioxidant and anti-inflammatory activity of the base honey, enhancing honey's bioactive properties if it is added at higher concentrations.

There are synergic antimicrobial activity between honeys and SPrEx.

Edible products made with honey and small amounts of SPrEx between 0.1% and 0.5% appeal to the consumers, improving the bioactive properties of the base honey what can help increase the profitability of beekeeping.

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References

Ahn, M. R., Kumazawa, S., Usui, Y., Nakamura, J., Matsuka, M., Zhu, F., & Nakayama, T. (2007). Antioxidant activity and constituents of propolis collected in various areas of China. *Food Chemistry, 101*, 1383–1392.

Al-Waili, N., Al-Ghamdi, A., Ansari, M. J., Al-Attal, Y., & Salom, K. (2012). Synergistic effects of honey and propolis toward drug multi-resistant *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* isolates in single and polymicrobial cultures. *International Journal of Medical Sciences, 9*(9), 793–800.

Banskota, A. H., Tezuka, Y., & Kadota, Sh. (2001). Recent progress in pharmacological research of propolis. *Phytotherapy Research, 15*, 561–571.

Beretta, G., Granata, P., Ferrero, M., Orioli, M., & Facino, R. M. (2005). Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytica Chimica Acta, 533*, 185–191.

Campos, J. F., dos Santos, U. P., Macorini, L. F. B., de Melo, A. M. M. F., Balestieri, J. B. P., Paredes-Gamero, E. J., ... dos Santos, E. L. (2014). Antimicrobial, antioxidant and cytotoxic activities of propolis from *Melipona orbignyi* (*Hymenoptera, Apidae*). *Food and Chemical Toxicology, 65*, 374–380.

Can, Z., Yildiz, O., Sahin, H., Turumtay, E. A., Silici, S., & Kolayli, S. (2015). An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles. *Food Chemistry, 180*, 133–134.

Duman, M., & Özpolat, E. (2015). Effects of water extract of propolis on fresh shibuta (*Barbus grypus*) fillets during chilled storage. *Food Chemistry, 189*, 80–85.

Escriche, I., Kadar, M., Juan-Borrás, M., & Domenech, E. (2014). Suitability of antioxidant capacity, flavonoids and phenolic acids for floral authentication of honey. Impact of industrial thermal treatment. *Food Chemistry, 142*, 135–143.

Feás, X., Pacheco, L., Iglesias, A., & Estevinho, L. M. (2014). Use of propolis in the sanitization of lettuce. *International Journal of Molecular Sciences, 15*, 12243–12257.

Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M., & Estevinho, L. M. (2009). Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry, 114*, 1438–1443.

Ferreres, F., Lopes, G., Gil-Izquierdo, A., Andrade, P. B., Sousa, C., Mouga, T., & Valentão, P. (2012). Phlorotannin extracts form fucales characterized by HPLC–DAD-ESI-MS^n: Approaches to hyaluronidase inhibitory capacity and antioxidant properties. *Marine Drugs, 10*, 2766–2781.

Fidaleo, M., Zuorro, A., & Lavecchia, R. (2011). Antimicrobial activity of some Italian honeys against pathogenic bacteria. *Chemical Engineering Transactions, 24*, 1015–1020.

Gómez-Caravaca, A. M., Gómez-Romero, M., Arráez-Román, D., Segura-Carretero, A., & Fernández-Gutierrez, A. (2006). Advances in the analysis of phenolic compounds in products derived from bees. *Journal of Pharmaceutical and Biomedical Analysis, 41*, 1220–1234.

Gutiérrez-Cortés, C., & Suarez Mahecha, H. (2014). Antimicrobial activity of propolis and its effect on the physicochemical and sensorial characteristics in sausages. *Vitae, 21*(2), 90–96.

Hadagali, M. D., & Chua, L. S. (2014). The anti-inflammatory and wound healing properties of honey. *European Food Research and Technology, 239*, 1003–1014.

Isla, M. I., Craig, A., Ordoñez, R., Zampini, C., Sayago, J., Bedascarrasabure, E., ... Maldonado, L. (2011). Physico chemical and bioactive properties of honeys from Northwestern Argentina. *LWT – Food Science and Technology, 44*, 1922–1930.

Juszczak, L., Galkowska, D., Ostrowska, M., & Socha, R. (in press). Antioxidant activity of honey supplemented with bee products. *Natural Product Research*. Doi: http://dx.doi.org/10.1080/14786419.2015.1057582.

Kalogeropoulos, N., Konteles, S. J., Troullidou, E., Mourtzinos, I., & Karathanos, V. (2009). Chemical composition, antioxidant activity and antimicrobial properties of propolis extract from Greece and Cyprus. *Food Chemistry, 116*, 452–461.

Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S., & Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluids. *Journal of Clinical Pathology, 54*, 356–361.

Kujumgiev, A., Tsvetkova, I., Serkedjieva, Y., Bankova, V., Christov, R., & Popov, S. (1999). Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. *Journal of Ethnopharmacology, 64*, 235–240.

Kuś, P. M., Congiu, F., Teper, D., Sroka, Z., Jerković, I., & Tuberoso, C. I. G. (2014). Antioxidant activity, color characteristics, total phenol content and general HPLC fingerprints of six Polish unifloral honey types. *LWT – Food Science and Technology, 55*, 124–130.

Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry, 91*, 571–577.

Miguel, M. G., Doughmi, O., Aazza, S., Antunes, D., & Lyoussi, B. (2014). Antioxidant, anti-inflammatory and acetylcholinesterase inhibitory activities of propolis from different regions of Morocco. *Food Science and Biotechnology, 23*(1), 313–322.

Moreira, L., Dias, L. G., Pereira, J. A., & Estevinho, L. (2008). Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal. *Food and Chemical Toxicology, 46*, 3482–3485.

Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A, 1054*, 95–111.

Pękal, A., & Pyrzynska, K. (2014). Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Analytical Methods, 7*, 1776–1782.

Pérez, E., Rodríguez-Malaver, A. J., & Vit, P. (2006). Antioxidant capacity of Venezuelan honey in wistar rat homogenates. *Journal of Medicinal Food, 9*(4), 510–516.

Sancho, M. T., Pascual-Maté, A., Rodríguez-Morales, E. G., Osés, S. M., Escriche, I., Periche, A., & Fernández-Muiño, M. A. (2015). Critical assessment of antioxidant-related parameters of honey. *International Journal of Food Science and Technology*. http://dx.doi.org/10.1111/ijfs.12988 (in press).

Serra-Bonvehí, J., & LaCalle-Gutiérrez, A. (2011). Antioxidant activity and total phenolics of propolis from the Basque Country (Northeastern Spain). *Journal of the American Oil Chemists' Society, 88*, 1387–1395.

Sherlock, O., Dolan, A., Athman, R., Power, A., Gethin, G., Cowman, S., & Humphreys, H. (2010). Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. *BMC Complementary and Alternative Medicine, 10*, 47.

Silici, S., & Kutluca, S. (2005). Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. *Journal of Ethnopharmacology, 99*, 69–73.

Silva, J. C., Rodrigues, S., Feás, X., & Estevinho, L. M. (2012). Antimicrobial activity, phenolic profile and role in the inflammation of propolis. *Food and Chemical Toxicology, 50*, 1790–1795.

Socha, R., Galkowska, D., Bugaj, M., & Juszczak, L. (2015). Phenolic composition and antioxidant activity of propolis from various regions of Poland. *Natural Product Research, 29*(5), 416–422.

Spinelli, S., Conte, A., Lecce, L., Incoronato, A. L., & del Nobile, M. A. (in press). Microencapsulated propolis to enhance the antioxidant properties of fresh fish burgers. *Journal of Food Process Engineering*. http://dx.doi.org/10.1111/jfpe.12183.

Stanislava, Z., Álvarez-Suarez, J. M., Novakovic, M., Pastor, F., Pezo, L., Battino, M., & Desanka, Z. (2013). Comparative analysis of antioxidant activity of honey of different floral sources using recently developed polarographic and various spectrophotometric assays. *Journal of Food Composition and Analysis, 30*, 13–18.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., & Pérez-Álvarez, J. A. (2008). Functional properties of honey, propolis, and royal jelly. *Journal of Food Science, 73*(9), R117–R124.

Voidarou, C., Alexopoulos, A., Plessas, S., Karapanoou, A., Mantzourani, I., Stavropoulou, E., ... Bezirtzoglou, E. (2011). Antibacterial activity of different honeys against pathogenic bacteria. *Anaerobe, 17*, 375–379.

Von der Ohe, W., Peresano-Oddo, L., Piana, M. L., Morlot, M., & Martin, P. (2004). Harmonized methods of melissopalynology. *Apidologie, 35*, S18–S25.

White, J. W. (1975). Composition of honey. In E. Crane (Ed.). *Honey, a comprehensive survey* (Vol. 5, pp. 157–206). London, UK: Heinemann.