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TESIS DOCTORAL

APLICACIÓN DE LOS ULTRASONIDOS DE POTENCIA EN LA ETAPA DE BATIDO DE LAS PASTAS DE ACEITUNAS. EFECTO SOBRE EL RENDIMIENTO DEL PROCESO Y CARACTERÍSTICAS DEL ACEITE PRODUCIDO

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Aspirante al grado de Doctor

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HIGH POWER ULTRASOUNDS APPLICATION DURING THE OLIVE PASTE MALAXATION STEP. EFFECT ON PROCESS OIL YIELD AND OLIVE OIL CHARACTERISTICS.

PhD. Thesis

Mohamed Aymen Bejaoui

Jaén, May 2016

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Resumen

Resumen

El objetivo principal de esta Tesis Doctoral ha sido el estudio de la integración de la tecnología de los ultrasonidos de potencia en la mejora de la etapa de batido durante el proceso de elaboración de los aceites de oliva vírgenes. En este sentido se han desarrollado trabajos preliminares a nivel de laboratorio del pretratamiento continuo por ultrasonidos de potencia de las pastas de aceitunas previo a la operación de batido. Estos ensayos mostraron que este tratamiento permite un calentamiento instantáneo de la pasta de aceituna y la mejora del rendimiento. La eficacia de este tratamiento depende de las características del fruto usado, potencia de trabajo y caudal de pasta aplicado. En relación con la calidad del aceite de oliva no se ha observado efecto negativo de esta técnica o una aceleración de los procesos oxidativos. Este tratamiento permite obtener aceites con unos mayores contenidos en pigmentos y tocoferoles mientras que se obtienen aceites con un menor contenido en compuestos fenólicos.

Atendiendo a los resultados obtenidos en trabajos previos, se ha desarrollado un prototipo para la aplicación en continuo de los ultrasonidos de potencia usando tres frecuencias de trabajo diferentes (20, 40 y 80 kHz). Se ha empleado el prototipo como apoyo o alternativa a la operación de batido, obteniéndose un calentamiento inmediato y una mejora de los rendimientos industriales. El tratamiento de las pastas a 40 kHz, sin batido, mostró rendimientos equivalentes a los obtenidos con el batido convencional. Los tratamientos a 40 y 80 kHz, sin batido posterior, permitieron obtener aceites con un perfil sensorial más equilibrado que el batido convencional.

Por último, se ha integrado el prototipo en línea en un proceso de elaboración de aceites de oliva vírgenes a nivel de mini planta. El tratamiento de las pastas de aceitunas con ultrasonidos a una frecuencia de 40 kHz, y sin batido, dio lugar a unos rendimientos equivalentes a los producidos por el batido convencional y aceites con características similares a los obtenidos en los trabajos a nivel de laboratorio, permitiendo además un ahorro en el consumo energético.

Summary

The aim of the present PhD thesis is the study of the High Power Ultrasounds implementation to enhance the malaxation step of the Virgin Olive Oil elaboration process. In this sense, preliminary works were developed at laboratory scale for the continuous pretreatment of olive paste by High Power Ultrasounds before malaxation. These tests allowed the instantaneous heating of olive paste and the improvement of the oil yield. The High Power Ultrasounds efficiency depended on the fruit characteristics, ultrasounds power and the olive paste flow rate. Regarding to the virgin olive oil quality, negative effects or acceleration of oxidative processes were not detected. Ultrasound treatments gave oil with higher pigments and tocopherols content, whereas the phenolic compounds showed a decrease.

Based on the previous works, a prototype for the continuous High Power Ultrasound application was developed working at three frequencies (20 kHz, 40 kHz and 80 kHz). It was tested as an aid or alternative to the malaxation, obtaining an immediate heating of olive paste and oil yields improvement. The treatment of olive paste at 40 kHz without kneading gave equivalent oil yields to those obtained by conventional malaxation. The treatments at 40 kHz and 80 kHz without kneading gave olive oil with more equilibrated sensorial profile than conventional malaxation.

Finally, the prototype was implemented at mini plant scale as alternative to malaxation. The ultrasounds treatment at 40 kHz without gave oil yields equivalent to those obtained by conventional malaxation and virgin olive oil with characteristics similar to those obtained at laboratory scale, allowing in consequence energy saving.

Capítulo 1. Introducción general

Índice

1. EL ACEITE DE OLIVA VIRGEN

1.1. Definición

El aceite de oliva es definido por el Consejo Oleícola Internacional (2015) como el aceite procedente únicamente del fruto del olivo (*Olea europaea* L.), y extraído mediante procedimientos mecánicos, con exclusión de los aceites obtenidos por disolventes o por procedimientos de reesterificación y de toda mezcla con aceites de otra naturaleza.

El cultivo del olivo existe desde miles de años en los países del sur de Europa, Norte de África y Oriente Medio. En este área de la Cuenca Mediterránea se produce el 95 % del total del aceite de oliva a nivel mundial, alcanzando por los países productores una gran importancia económica (Barjol, 2013). España es el mayor productor mundial de aceite de oliva con una superficie de cultivo de 2,4 millones de hectáreas, el 28 % de la superficie mundial, y una producción del 38-45 % del aceite de oliva (Barjol, 2013). En Andalucía se produce en torno al 80 % del aceite español, siendo la provincia de Jaén la zona de mayor producción (Figura 1.1).

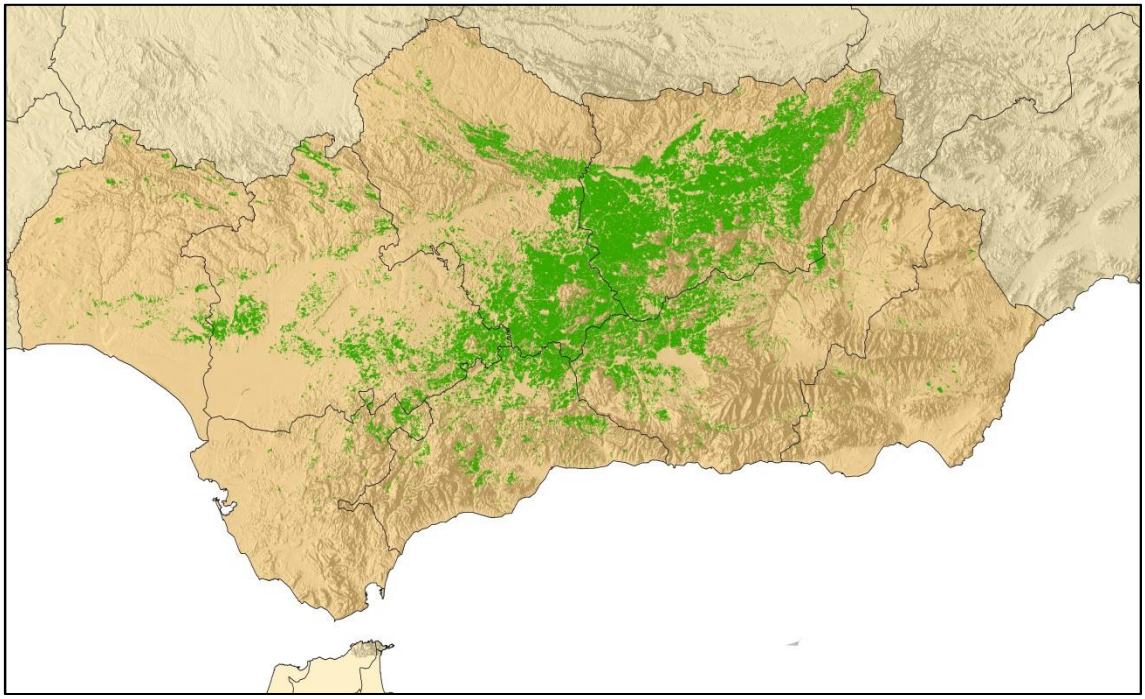

Figura 1.1. Distribución del olivar en Andalucía

(Cortesía de Manuel Perujo)

1.2. Calidad del aceite de oliva virgen

La calidad del aceite de oliva se encuentra representada por el conjunto de propiedades o atributos que el posee y que determina el grado de aceptación del consumidor respecto a un determinado uso. Existen, por tanto, diferentes aproximaciones a la calidad del aceite de oliva en función del fin al que se destina, concluyéndose que la calidad no es única. Uceda et al. (2008) proponen cuatro tipos de calidad para el aceite:

- Calidad reglamentada: Está determinada por los reglamentos Europeo (CE/299/2013, 2015) y del COI (Consejo Oleícola Internacional, 2015). Según estos reglamentos, se fija la categoría comercial del aceite en función de las características fisicoquímicas, sensoriales y de composición. Se dividen en parámetros de calidad y de pureza.
- Calidad nutricional y terapéutica: El aceite de oliva virgen, comparando con otros tipos de grasas, muestra una serie de propiedades nutricionales que vienen definidas por su composición tanto en la fracción mayoritaria como en la minoritaria. En cuanto a la fracción minoritaria, entre los compuestos con propiedades bioactivas, destacan: compuestos fenólicos, tocoferoles y pigmentos gracias sus funciones antioxidantes. En la fracción mayoritaria destacan los ácidos grasos y en especial, el ácido oleico (Uceda et al., 2008; Aparicio y Harwood, 2013).
- Calidad culinaria: Está ligada, en parte, a los aspectos nutricionales y terapéuticos. En este campo se ha de diferenciar la utilización en crudo y su uso en fritura. En cuanto a su utilización en crudo, son los caracteres sensoriales lo fundamental a la hora de definir calidades. En el segundo aspecto, es decir, su uso en fritura, son los parámetros como la resistencia a la termooxidación, penetración de la grasa muy ligada al gasto del aceite, la vida útil en repetidas frituras, que naturalmente están relacionadas con la composición de los aceites, los que hay que determinar para evaluar esta calidad (Uceda et al., 2008, 2010).
- Calidad comercial: Es quizás la más difícil de precisar, en la que interviene diferentes parámetros subjetivos de carácter hedonístico o preferencial, como el color o los sabores más o menos suaves que varían según regiones o países de origen de los clientes. Asimismo, la vida útil del producto determina en gran medida este aspecto de la calidad (Uceda et al., 2008).

En la práctica, la totalidad de los aceites obtenidos en una almazara tendrán la consideración de aceites vírgenes. Dentro de éstos, y según sus características, tanto físico-químicas como organolépticas, pueden establecerse las siguientes categorías (CE/299/2013, 2015; Consejo Oleícola Internacional, 2015):

Aceite de oliva virgen extra: Es el zumo de la aceituna recolectada en su mejor momento de madurez y procesada adecuadamente con una acidez libre, expresada en ácido oleico, como máximo de 0,8 g por 100 g. Un contenido en ésteres etílicos inferior a 35 mg/kg. Tiene las características sensoriales que reproducen olores y sabores del fruto que procede, la aceituna. Con una mediana de los defectos igual a 0 y la mediana del atributo "frutado" superior a 0.

Aceite de oliva virgen: Es un aceite que puede presentar ligeras alteraciones, bien sea en sus índices analíticos, con una acidez libre, expresada en ácido oleico, como máximo de 2 g por 100 g de aceite, o en sus características sensoriales con la mediana de los defectos que es superior a 0 e inferior o igual a 2,5 y la del atributo "frutado" que es superior a 0. Estas alteraciones, sobre todo sensoriales, pueden ser prácticamente imperceptibles, pero deprecian la calidad del producto.

Estas dos primeras categorías son las únicas que tienen su origen directo de la almazara y que pueden encontrarse envasadas en el mercado.

Aceite de oliva virgen lampante: Es el aceite de oliva virgen que no es apto al consumo directamente, al presentar graves alteraciones en sus índices físico-químicos, con una acidez libre, expresada en ácido oleico, superior a 2 g por 100 g de aceite, o sensoriales con una a mediana de los defectos superior a 2,5, o bien la mediana de los defecto inferior o igual a 2,5 y la del atributo "frutado" igual a 0, y hay que someterle a un proceso de refino para utilizarlo en consumo.

Habría que destacar que la reglamentación especifica del Consejo Oleícola Internacional (2015) y contemplada por el CODEX alimentarius incluye otra categoría que es el aceite de oliva virgen corriente. Se trata de una categoría intermedia entre el aceite de oliva virgen y virgen lampante con una acidez libre, expresada en ácido oleico, inferior a 3,3 g por 100 y con una mediana de los defectos superior a 3,5 e inferior a 6.

1.3. Composición del aceite de oliva

Los compuestos químicos del aceite oliva pueden integrarse en dos fracciones. Una fracción mayoritaria, 98 a 99 % del peso total, formada por una mezcla de triglicéridos, y ácidos grasos libres. Y otra fracción minoritaria, aproximadamente entre 1 y 2 % del total, formada por una serie de compuestos que tienen una fuerte incidencia en la estabilidad, características nutricionales, sabor y aroma del aceite de oliva (Aparicio y Harwood, 2013; Barranco et al., 2008).

1.3.1. Componentes mayoritarios del aceite de oliva

Está formada por triglicéridos, diglicéridos (1,3 %), monoglicéridos (0,2 %) y algunos ácidos libres (Barranco et al., 2008; Aparicio y Harwood, 2013).

1.3.1.1. Ácidos grasos

El aceite de oliva contiene fundamentalmente ácido oleico (monoinsaturado) aunque contiene una cantidad moderada de ácidos grasos saturados (palmítico y esteárico) que no supera el 20 % en conjunto y una cantidad menor, en proporción, de poliinsaturados fundamentalmente ácidos linoleico y linolénico. La composición acídica del aceite de oliva se ve influenciada no solo por las condiciones edafoclimáticas (Fedeli, 1996), sino que también existe una elevada influencia varietal (Beltrán et al., 2004). La composición acídica del aceite de oliva y los límites porcentuales establecidos para su clasificación como aceite de oliva por el Consejo Oleícola Internacional (2015) se reflejan en la Tabla 1.1.

Tabla 1.1. Composición en ácidos grasos del aceite de oliva según Consejo oleícola internacional (2013).

Ácidos grasos	Nomenclatura	Límites C.O.I.
Mirístico	C 14:0	< 0,05
Palmítico	C 16:0	7,5-20,0
Palmitoleico	C 16:1	0,3-3,5
Margárico	C 17:0	< 0,3
Margaroleico	C 17:1	< 0,3
Estériaco	C 18:0	0,5-5,0
Oleico	C 18:1	55,0-83,0
Linoleico	C 18:2	3,5-21,0
Linolénico	C 18:3	< 1,0
Aráquico	C 20:0	< 0,6
Eicosanoico	C 20:1	< 0,4
Behénico	C 22:0	< 0,2
Erúcico	C 22:1	No establecido
Lignocérico	C 24:0	< 0,2

Esta composición le confiere un gran interés desde el punto de vista químico y biológico, al ser los ácidos grasos monoinsaturados mucho más estables en los procesos oxidativos (Jiménez-Márquez et al., 1995) y desde la perspectiva nutricional. En este sentido muchos estudios científicos han mostrado los efectos beneficiosos del aceite de oliva como grasa monoinsaturada sobre la salud humana (Perona y Botham, 2013; Yaqoob, 2013; Zampelas y Kafatos, 2004) y constituye la base de una alegación nutricional del aceite de oliva (CE/432/2012).

1.3.1.2. Triglicéridos

Los triglicéridos están presentes en todos los tejidos vegetales y animales y constituyen la más importante reserva energética del organismo y es también una fuente de nutrientes esenciales. Son ésteres de la glicerina con tres ácidos grasos. Según la composición en ácidos grasos se podrían formar más de 70 triglicéridos en el aceite de oliva. Sin embargo, el número de ellos que realmente se encuentran en dicho aceite es mucho menor, sólo unos pocos se encuentran en porcentaje significativo: la trioleina, OOO, entre el 40 y 59 %, POO del 12 al 20 %, OOL entre el 12,5 y el 20 %, POL del 5,5 al 7 %, siendo P ácido palmítico, E ácido esteárico, O ácido oleico y L ácido linoleico (Boskou, 2006)

1.3.1.3. Mono- y di glicéridos

El aceite de oliva, además de contener mayoritariamente triglicéridos, también contiene glicéridos parciales. La presencia de mono- y di-acilglicéridos es debida, en parte, a una incompleta biosíntesis, pero principalmente a la hidrólisis del aceite. Pueden ser 1,2- diglicéridos procedentes de la síntesis incompleta de los triglicéridos, y 1,3-diglicéridos provenientes de la hidrólisis de los triglicéridos; la relación entre ambos sirve para establecer las condiciones de almacenamiento y la edad de ciertos aceites. En el aceite de oliva virgen, las concentraciones de diglicéridos varían entre 1,0 y 2,8 %. Los monoglicéridos están presentes en cantidades mucho menores (menos del 0,25 %) (Boskou, 2006; Barranco et al., 2008).

1.3.2. Fracción minoritaria del aceite de oliva

La fracción minoritaria del aceite de oliva virgen representa entre el 1 y el 2 % del peso del aceite de oliva. Esta fracción incluye una gran variedad de compuestos

químicos aunque en peso supone una pequeña parte de la composición del aceite de oliva virgen.

La fracción de componentes menores en el aceite de oliva virgen puede ser dividida en dos grupos (Boskou, 2006). El primer grupo consta de ésteres de esteroles, fosfolípidos, ceras y esteres metílicos y etílicos. El segundo grupo incluye clases de compuestos que no están químicamente relacionados con los ácidos grasos, que son hidrocarburos, alcoholes alifáticos, esteroles libres, tocoferoles, clorofilas, carotenoides y compuestos fenólicos polares.

1.3.2.1. Esteroles

Los esteroles son importantes constituyentes no glicerídicos que se relacionan con la calidad del aceite de oliva y se utilizan para comprobar su autenticidad (Consejo Oleícola Internacional, 2015). Sus concentraciones en el aceite de oliva son próximas a 2600 mg/kg dependiendo de la variedad de aceituna. Los esteroles que predominan en el aceite de oliva son β-sitosterol (75 - 90%), Δ5-avenasterol (5 - 36%) y campesterol (3%). El contenido de estos compuestos se ve afectado por factores agronómicos (Kyçyk et al., 2016), variedad, sistema de extracción y refinación del aceite de oliva virgen. También estos compuestos despertaron un interés particular por sus características nutricionales y saludables: efecto anti-inflamatorio, antimicrobiano, prevención del cáncer y capacidad de reducir el colesterol y LDL (Kritchevsky y Chen, 2005).

1.3.2.2. Fosfolípidos

El aceite de oliva virgen recién producido puede contener cantidades pequeñas de fosfolípidos, entre 40 y 135 mg/kg. Los principales fosfolípidos encontrados en el aceite de oliva son: fosfatidilcolina, fosfatidiletanolamina, fosfatidilinositol y fosfatidilserina. El ácido graso predominante en la estructura de los fosfolípidos es el ácido oleico, y el patrón de ácidos grasos es similar al de los triglicéridos (Barranco et al., 2008; Aparicio y Harwood, 2013).

1.3.2.3. Ceras

Las ceras son esteres de alcoholes grasos con ácidos grasos. En el aceite de oliva están caracterizadas por un número elevado de átomos de carbono; en consecuencia, las

ceras pueden tener hasta 58 átomos de carbono, lo que influye en sus propiedades físicas. Las ceras más frecuentes en el aceite de oliva son: C 40, C 42, C 44 y C 46 (Consejo Oleícola Internacional, 2015; CE/299/2013, 2015). El contenido en ceras es muy bajo, no supera los 35 mg/100 g. Las ceras se encuentran en la piel de los frutos y tienen como misión evitar la pérdida de agua del mesocarpio. Por eso, son más abundantes en los aceites de orujo, al obtenerse de los restos de la piel y pulpa por extracción con disolventes (Aparicio et al., 2013).

1.3.2.4. Hidrocarburos

El aceite de oliva contiene diferentes tipos de hidrocarburos: terpénicos, esteroideos, y policíclicos aromáticos. Los hidrocarburos terpénicos son los hidrocarburos mayoritarios encontrados en el aceite de oliva virgen (Beltrán et al., 2015; De Leonardis et al., 1998); el escualeno es el principal hidrocarburo terpénico y es un precursor bioquímico de la ruta biosintética del colesterol y de todas las hormonas esteroides. Este compuesto tiene un potencial anti-cancerígeno (Ghanbari et al., 2012), y de incremento del HDL (Gabás-Rivera et al., 2014). También está caracterizado por una actividad antioxidante (Cherif et al., 2013).

El otro hidrocarburo terpénico es el β-caroteno, un terpenoide de cuarenta átomos de carbono. Es un precursor de la vitamina A, y es el principal caroteno presente en el aceite. Los hidrocarburos esteroides y aromáticos no se encuentran de forma natural y procede del proceso de refinación en el caso de los esteroides o de contaminación externa para los aromáticos (Aparicio y Harwood, 2013).

1.3.2.5. Tocoferoles

Los tocoferoles son un grupo de antioxidantes lipofílicos que juegan un papel importante en la prevención contra la peroxidación de los lípidos. Son los compuestos responsables de la actividad de la vitamina E del aceite, que es sintetizada de forma exclusiva por las plantas y cuyo aporte es considerado esencial en la dieta humana. Son relativamente abundantes en el aceite de oliva, entre 100 y 400 mg/kg (Uceda et al., 2008). En el aceite de oliva se ha descrito la presencia de α, β, y γ tocoferoles, siendo el α el predominante con más del 90 %. Su composición y contenido varía en función de la variedad y de la época de recolección (Beltrán et al., 2010).

Los tocoferoles contribuyen de forma importante a la estabilidad del aceite de oliva (Kiritsakis y Osman, 1995; Baldioli et al., 1996). Los tocoferoles están considerados como la principal fracción antioxidante liposoluble del cuerpo, y se encuentra en las lipoproteínas, especialmente en las LDL. Se encuentra en las membranas celulares, tanto en el interior como en el exterior de la célula, mejorando la protección de ésta frente al ataque de radicales libres. La vitamina E mejora la función inmunitaria y puede intervenir en la reparación de las membranas dañadas (Ghanbari et al., 2012).

1.3.2.6. Pigmentos

El aceite de oliva es un producto que presenta un color entre el verde-amarillo hasta el dorado, dependiendo de la variedad y del estado de madurez del fruto. Los pigmentos que proporcionan el color al aceite de oliva pertenecen principalmente a dos familias: clorofilas y carotenoides.

a) Clorofilas y feofitinas

Las clorofilas están presentes en el aceite de oliva con diferentes niveles y principalmente como sus productos de degradación, las feofitinas. El contenido en clorofilas y sus derivados dependen principalmente del estado de madurez de las aceitunas reduciéndose conforme avanza este (Gómez-Rico et al., 2007). Se encuentran dos tipos de clorofila: la clorofila a y b que son las responsables del color verde de los aceites. Su contenido en el aceite de oliva varía entre 1 y 20 mg/kg. Además de sus funciones como pigmentos, las clorofilas actúan como pro-oxidantes en presencia de la luz y como antioxidantes en la oscuridad lo que les confieren un papel importante en la conservación del aceite de oliva (Gómez-Rico et al., 2007).

b) Carotenoides

Son los pigmentos responsables de la coloración amarillenta que presentan los aceites de oliva; el carotenoide más simple es el licopeno y el más importante el β-caroteno (Minguez-Mosquera et al., 1991; Aparicio-Ruiz et al., 2009). Este último ejerce una protección importante contra la foto-oxidación y juega el papel de desactivador del oxígeno singlete producido por la clorofila en presencia de luz (Bradley y Min, 1992).

1.3.2.7. Compuestos fenólicos

La fracción fenólica del aceite de oliva consiste en una mezcla heterogénea de componentes, que están presentes directamente en el mesocarpio de las aceitunas o bien se encuentra en forma de precursores metabólicos (Barranco et al., 2008; Aparicio y Harwood, 2013). En aceite de oliva virgen, están presentes en formas libres o esterificadas en cantidades diferentes. Esta fracción fenólica de los aceites de oliva virgen se puede diferenciar los siguientes grupos de compuestos (Bendini et al., 2007):

- Alcoholes fenólicos (hidroxitirosol o 3,4-dihidroxifenil etanol, tirosol o p-hidroxifenil etanol y 3,4-dihidroxifenil etanol glucósido).

- Ácidos fenólicos (Ácidos benzoicos: ácido benzoico, p-hidroxibenzóico, protocatéquico, gálico, vanílico y siríngico; Ácidos cinámicos: ácido cinámico, cafeico, ferúlico, sinápico, p-cumárico y o-cumárico).

- Flavonoides (luteolina y aspigenina).

- Secoiridoides (derivados de la oleuropeina y ligustrosido: 3,4 DHPEA-EDA, 3,4 DHPEA-EA, p-HPEA-EDA y p-HPEA-EA). Estos compuestos se forman durante la extracción del aceite de oliva a partir de la hidrólisis de la oleuropeina y del ligustrosido mediante β-glucosidasa enzima endógena en las aceitunas (Romero-Segura et al., 2012).

- Lignanos (pinoresinol y acetoxipinoresinol).

La concentración de fenoles totales varía entre 50 y 500 mg/kg de aceite, pero se pueden encontrar aceites con contenidos de hasta 1500 mg/kg (Beltrán et al., 2000; Tsimidou, 2013). El contenido en fenoles depende de la variedad de aceituna, de las variables climatológicas y agronómicas del cultivo o campaña (Mohamed-Mousa et al., 1996), el grado de maduración del fruto, el área de producción, el sistema de extracción y conservación del aceite (Uceda et al., 2008; Tsimidou, 2013).

Los compuestos fenólicos del aceite de oliva virgen participan en gran parte de las propiedades antioxidantes y el valor biológico del aceite de oliva. La presencia de estos

compuestos es de gran importancia para la estabilidad de los aceites de oliva vírgenes ya que actúan como inhibidores de la autooxidación (Tsimidou, 2013). Los polifenoles están también implicados en los caracteres sensoriales y son responsables del amargor, picante y astringencia, atributos positivos de los aceites de oliva vírgenes (Beltrán et al., 2000, 2007; Carrasco-Pancorbo et al., 2004).

Estos compuestos suscitan un gran interés debido a sus efectos sobre la salud humana y han sido aprobados por la Comisión Europea (CE/432/2012) en la alegaciones nutricionales de los aceites de oliva. En este sentido, se ha demostrado que los compuestos fenolicos tienen efectos beneficiosos sobre el sistema digestivo, la piel, los huesos, la circulación sanguínea, procesos de envejecimiento en general (Carrasco-Pancorbo et al., 2004), enfermedades cardiovasculares (Visioli et al., 2005; Covas et al., 2006; Rubio-Senent et al., 2015), y diferentes tipos de cáncer (Warleta et al., 2011; Fuccelli et al., 2014).

1.3.2.8. Compuestos volátiles y aromáticos

Los aceites de oliva presentan un aroma y flavor muy característico que se deben a un cierto número de sustancias aromáticas agradables así como a otros relacionados con los defectos sensoriales, Tabla 1.2. Se recogen algunos compuestos volátiles en relación con ciertas características sensoriales (Angerosa et al., 2004; Kalua et al., 2007; Morales et al., 2013; Sánchez-Ortiz et al., 2013).

La fracción volátil del aceite de oliva virgen se puede agrupar en: hidrocarburos alifáticos y aromáticos, alcoholes alifáticos, terpenos oxigenados, aldehídos, cetonas, derivados tiofénicos, ésteres de seis átomos de carbono (C6), y diversos compuestos de cinco átomos de carbono (C5) que son los principales responsables de las notas sensoriales verdes y frutadas propias del aceite de oliva virgen de alta calidad (Morales et al., 2013). Existen grandes diferencias en el contenido en compuestos volátiles en los aceites de oliva que se ven afectados por una serie de factores, tales como la variedad, el medio agrológico, condiciones de cultivo, maduración del fruto, método de elaboración y conservación del aceite.

Tabla 1.2. Compuestos volátiles identificados en los aceites de oliva (Morales et al., 2013)

Compuesto químico	Apreciación sensorial	Compuesto químico	Apreciación sensorial
Acetato de metilo	Verde	Z-2-Hexenal	Verde
Octeno	Verde	2-Metilbutan-1-ol	Miscelánea 3
Acetato de etilo	Indeseado	3-Metilbutanol	Indeseado
Butan-2-ona	Frutado	3-Metil-2-butenil acetato	Indeseado
3-Metilbutanal	Frutado maduro	Dodeceno	Indeseado
1,3-Hexadien-5-ino	Verde	Pentan-1-ol	Frutado
Alcohol	Frutado	Etenilbenceno	Frutado
Etilfurano	Miscelánea 4	Acetato de hexilo	Verde
Propanoato de etilo	Miscelánea 4	Cetona C8	Verde
Alcohol + Hidrocarburo	Miscelánea 3	Octan-2-ona	Frutado maduro
3-Pentanona	Verde	3-4-Metil-3-pentenilfurano	Frutado maduro
4-Metilpentan-2- ona	Verde	3-Hexenil acetato	Verde
Pent-1-en-3-ona	Dulce	Z-2-Penten-1-ol	Verde
2-Metilbut-2-enal	Indeseado	6-Metil-5-hepten-2- ona	Amargo
Hidrocarburo	Miscelánea 4	Nonan-2-ona	Miscelánea 4
Metilbenceno	Frutado maduro	Hexan-1-ol	Indeseado
2-Metilbut-3-enol	Indeseado	E-3-Hexen-1-ol	Miscelánea 2
Butil acetato	Miscelánea 4	Trideceno	Amargo
Hexanal	Dulce	Z-3-Hexen-1-ol	Verde
Hidrocarburo	Miscelánea 4	2-4-Hexadienal	Frutado maduro
2-Metilbutil propanoato	Miscelánea 2	E-2-Hexen-1-ol	Indeseado
2-Metil-1- propanol	Verde	Ácido acético	Indeseado
E-2-Pentenal	Verde	Metil-decanoato	Miscelánea 1
Alcohol	Indeseado	Hidrocarburo C11	Amargo
Z-2-Pentenal	Miscelánea 3	Hidrocarburo	Amargo-picante
Etilbenceno	Amargo	2-Metil-4-pentenal	Amargo-picante
E-3-Hexenal	Frutado maduro	1,2,4-Trimetilbenzeno	Indeseado
Z-3-Hexenal	Verde	4-Metil-1-penten-3-ol	Frutado maduro
1-Penten-3-ol	Indeseado	Alcohol C6	Frutado maduro
3-Metilbutil acetato	Amargo	Z-2-hexen-1-ol	Verde
Heptan-2-ona	Frutado maduro	2-Octenal	Verde
E-2-Hexenal	Amargo	Ácido propanoico	Miscelánea 1
		Hidrocarburo	Verde

2. PROCESO DE ELABORACIÓN DEL ACEITE DE OLIVA VIRGEN

El aceite de oliva virgen se extrae del fruto únicamente mediante procesos mecánicos (Consejo Oleícola Internacional, 2015). Tradicionalmente, el aceite de oliva se extraía mediante sistema de prensa incluyendo los molinos de empiedros, prensa mecánica y pozuelos de decantación (Hermoso et al., 1998; Alba, 2008). Debido a su coste de funcionamiento elevado, discontinuidad del proceso y los efectos negativos sobre el aceite de oliva, estos sistemas han sido abandonados y cambiados por otros continuos que se pueden dividir en 4 etapas (Figura 2.2): operaciones preliminares internas, preparación de las pastas de aceituna, separación del aceite de la pasta y almacenamiento del aceite extraído (Hermoso et al., 1998; Alba, 2008).

2.1. Operaciones preliminares internas

Estas operaciones tienen lugar en el patio de la almazara donde los frutos, después de su recolección en el momento óptimo para obtener la máxima calidad (Uceda y Frias, 1975) y transporte hasta la fábrica, se someten a las operaciones de recepción, limpieza y lavado (Hermoso et al., 1998; Alba, 2008; Uceda et al., 2008; Di Giovacchino, 2013).

En el momento de su entrada en la almazara, las aceitunas deben ser clasificadas en función de su procedencia (Árbol o suelo), su calidad potencial (fruto sano o fruto dañado), con el fin de procesarlas por vías diferentes y con distintos tratamientos (Hermoso et al., 1998; Di Giovacchino et al., 2002; Alba, 2008; Di Giovacchino, 2013).

Tras su recolección, los frutos llevan una cantidad variable de material de diversa índole, dependiendo de su procedencia (hojas, ramas y en el caso del fruto del suelo restos de tierra y piedras), que es necesario eliminar. Así, en función de su procedencia el procesado debe ser diferente:

- Los frutos del árbol sólo deben someterse a un proceso de limpieza en el que se les eliminan todas las materias extrañas, menos densas que el fruto como hojas y tallos, por medio de limpiadoras basadas en corrientes de aire y cribas vibrantes. Sería conveniente evitar su lavado, pues se perdería extractabilidad en el proceso, además de que los aceites obtenidos, serían menos estables y fragantes (Uceda et al., 2008; Beltrán et al., 2016).

- Las aceitunas procedentes del suelo deberían ser sometidas, además de la limpieza, a un lavado en el que, por diferencia de densidad, se eliminarán en una corriente de agua los elementos más densos que suelen acompañar a los frutos procedentes del suelo como piedras, tierra, materiales metálicos, etc. (Beltrán et al., 2015).

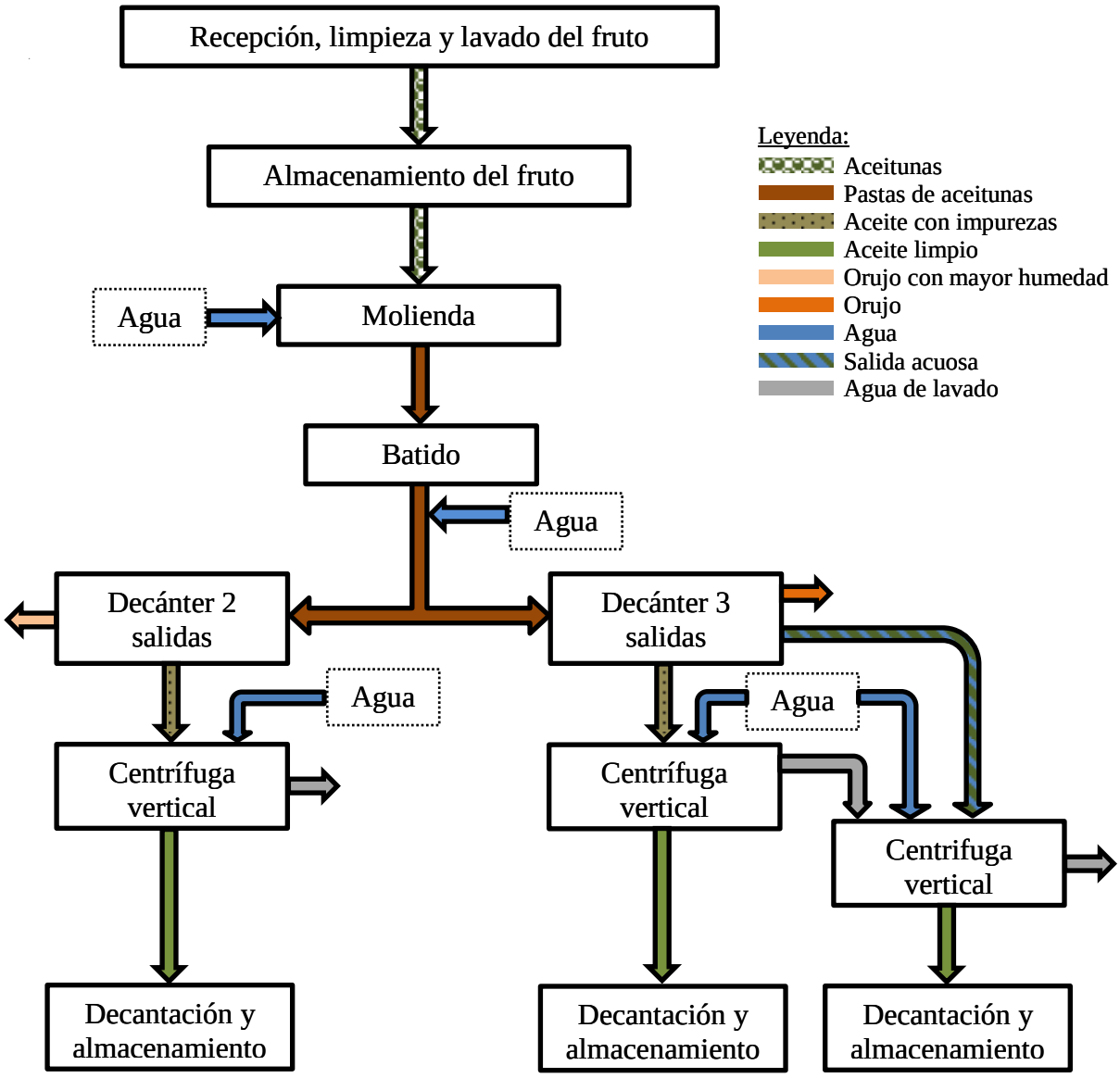

Figura 2.2. Diagrama de flujo en el sistema continúo de elaboración de los aceites de oliva vírgenes.

Después las aceitunas deberían molturarse siempre inmediatamente después de la recepción, sin embargo debido al desfase entre ambos procesos, debe almacenarse hasta su molturación. Este tiempo de espera debe ser inferior a las 24 h para garantizar la calidad final del aceite (Beltrán et al., 2016). El almacenamiento se produce en tolvas de

material inerte de calidad alimentaria principalmente de acero inoxidable (Hermoso et al., 1998).

2.2. Preparación de la pasta

En el proceso de elaboración del aceite de oliva virgen, la primera etapa es la preparación de la pasta que se divide fundamentalmente en dos operaciones la molienda y el batido. El objetivo de esta etapa es liberar y formar una fase continua de aceite (Di Giovacchino et al., 2002; Alba, 2008; Di Giovacchino, 2013).

2.2.1. Molienda

La molienda tiene por objeto la rotura de los tejidos del mesocarpio de la aceituna donde se aloja la materia oleosa en las vacuolas; estas gotas liberadas se ponen en contacto con otros componentes no glicéridos produciéndose la transferencia de compuestos entre las diferentes fases de la pasta producida. De esta forma muchos componentes pasan a las de la fase oleosa como pigmentos, ceras y alcoholes alifáticos del epicarpio (Hermoso et al., 1998: Di Giovacchino et al., 2002; Caponio et al., 2003; Alba, 2008).

La trituración puede conseguirse con estructuras derivadas de dos tecnologías distintas. En los procesos de extracción tradicionales (prensa), se utilizan los trituradores de piedra formados por una solera de granito sobre la que giran varias muelas también de granito. Mientras, que en los procesos continuos se utilizan trituradores metálicos que pueden dividirse en tres categorías: los de martillos, de discos dentados y de cilindros estriados (Di Giovacchino et al., 2002; Di Giovacchino, 2013; Leone et al., 2015b). Los molinos metálicos presentan algunas variables que permiten su regulación: el grado de molienda y la velocidad de giro de los martillos. La regulación de estas variables de la molienda determina el rendimiento del proceso, además de las características del aceite (Hermoso et al., 1998).

Los diferentes tipos de molinos no afectan la calidad reglamentaria del aceite de oliva virgen ni a la composición acídica, tocoferoles, escualeno ni la fracción digliceridica (Di Giovacchino et al., 2002; Allouche et al., 2010; Preziuso et al., 2010). Sin embargo, el uso de molinos de empiedros permite la obtención de aceites más ricos en sustancias volátiles, con menor contenido en polifenoles y, en consecuencia, menos amargos y picantes en comparación con los molinos de martillos (Di Giovacchino et al., 2002;

Alba, 2008; Inarejos-García et al., 2011; Di Giovacchino, 2013). Los aceites obtenidos tras el uso de molinos de martillos tienen mayor contenido en pigmentos clorofílicos y tocoferoles (Di Giovacchino et al., 2002; Caponio et al., 2003; Inarejos-García et al., 2011). Fregapane y Salvador (2013) encontraron que cuando se utiliza un tamaño de criba pequeño en el molino de martillo se facilita la extracción o liberación de los compuestos polares que incluyen los fenoles. El uso de los molinos metálicos permite obtener aceite con mayor percepción de los atributos de amargor y picante que los molinos tradicionales de piedra (Di Giovacchino et al., 2002; Di Giovacchino, 2013).

Esta etapa tiene una gran influencia en la composición de compuestos volátiles del aceite ya que estos compuestos derivados de la ruta de la lipoxigenasa empiezan a generarse que empieza en el momento de la rotura de los tejidos celulares y en contacto con las enzimas que interaccionan con el oxígeno (Sanchez-Ortiz et al., 2012).

2.2.2. Batido

En la operación de batido se pretende agrupar la fase oleosa para facilitar la separación posterior de las diferentes fases que constituyen la pasta. La pasta en la etapa de batido, está sometida a agitación suave y este proceso debe llevarse a cabo de forma que permita agrupar la mayor parte de las gotas de aceite sin provocar emulsiones que dificulten el proceso de extracción (Alba, 2008). Esta operación se desarrolla en las termo-batidoras que consisten en tanques cilíndricos o semicilíndricos con una camisa para la circulación de agua caliente, equipados con un eje vertical o horizontal. El eje está provisto de palas de diferentes diseños dependiendo de la marca. Durante el batido, se desarrollan procesos de transporte de cantidad de movimiento, transporte de energía y transporte de materia. La transferencia de calor en el interior del cuerpo de la batidora se atribuye a varios factores, como la conducción a lo largo de las paredes del tanque, la convección natural entre las zonas frías y calientes, el movimiento de la pasta debido al giro de las palas, la geometría de la batidora (volumen y ratios superficie de contacto y volumen) así como las características de la aceituna usada: variedad, índice de madurez etc.… (Ayr et al., 2015). En esta etapa comienzan una serie de actividades enzimáticas capaces de actuar sobre las más diversas y complejas estructuras moleculares originando compuestos más o menos solubles en el aceite. Así, la presencia de compuestos fenólicos en el aceite se asocia a la actividad de diversas enzimas como las ‘β-glucosidasas’, ‘Estearasas’ e ‘Hidrolasas’ que actúan sobre los glucósidos

secoiridoides (Romero-Segura et al., 2012; Servili et al., 2003a). La presencia de compuestos fenolicos y ácidos grasos oxidados se asocia a la acción de 'Oxidoreductasas' como 'Polifenoloxidasa', 'Peroxidasa' o 'Lipoxigenasa' (Sciancalepore, 1985; García-Rodríguez et al., 2011; Morales et al., 2013; Sánchez-Ortiz et al., 2013). Las variables que pueden ser reguladas en la etapa de batido con incidencia en el rendimiento del proceso y las características finales del aceite son:

- Velocidad de las palas móviles. Si es excesiva se favorecen las emulsiones y dificulta la posterior separación del aceite, debe reducirse en pastas difíciles. No tiene efecto en las características del aceite.

- Tiempo de batido. En general, está entre 45 a 60 minutos, puede alargarse en caso de pastas difíciles, para obtener rendimientos de aceite satisfactorios (Di Giovacchino et al., 2002; Alba, 2008; Aguilera et al., 2010; Clodoveo, 2012; Di Giovacchino, 2013). Amirante et al. (2001) mostraron que un batido en tiempo muy extendido puede tener un efecto negativo sobre el rendimiento. En relación con la calidad reglamentada del aceite de oliva el tiempo de batido no tiene una influencia significativa (Aguilera et al., 2010; Clodoveo, 2012). Aguilera et al. (2015) encontraron que cuando se alarga el tiempo de batido disminuye el contenido en compuestos fenólicos, estos resultados están de acuerdo con los obtenidos por (Di Giovacchino et al., 2002; Fregapane y Salvador, 2013). Aunque para otros autores (Angerosa et al., 2001; Kalua et al., 2007) no se encontraron diferencias. Otros compuestos influenciados por el tiempo de batido son los compuestos volátiles; un batido prolongado permite la acumulación de los compuestos C6 y C5, detectándose que se favorece la producción de hexanal (Angerosa et al., 2001; Fregapane y Salvador, 2013).

- Temperatura de batido. El aumento de la temperatura de batido permite disminuir la viscosidad del aceite favoreciendo el fenómeno de coalescencia y con ello una mejor extractabilidad del aceite (Ranalli et al., 2001; Aguilera et al., 2010). Para los parámetros de calidad reglamentada una temperatura alta provoca una intensificación de los procesos de oxidación primaria y secundaria y de hecho, un deterioro de la calidad del aceite debido al incremento de la actividad de las lipasas presentes en las pastas de aceitunas molida (Ranalli et al., 2001). En cuanto al contenido en fenoles,

algunos trabajos de investigación encontraron un aumento del contenido en polifenoles, amargor de los aceites y estabilidad al enranciamiento con el incremento de la temperatura de batido (Kalua et al., 2007; Fregapane y Salvador, 2013; Aguilera et al., 2015). La temperatura de batido superior a 25 ºC disminuye los componentes volátiles del aceite, desapareciendo su fragancia por el efecto inhibidor de la temperatura sobre la 'Lipoxigenasa' y la 'Hidroperóxido-liasa' que intervienen en la ruta de la 'Lipoxigenasa' (Angerosa et al., 2001). Se ha propuesto por otros autores como temperatura adecuada 30 ºC a 35 ºC en la pasta, mientras que una mayor temperatura afecta las características organolépticas (Amirante et al., 2001).

- Adición de coadyuvantes tecnológicos. Como se ha comentado anteriormente esta etapa tiene una gran influencia sobre el rendimiento. Para determinadas características del fruto se producen las llamadas `pastas difíciles´ en la que la fracción lipídica se emulsiona con el agua de vegetación y queda retenida en los microgeles constituyéndose una estructura coloidal en las pastas de aceituna (Aguilera et al., 2010; Caponio et al., 2016). El origen de las `pastas difíciles´ puede provenir de las características de las aceitunas, por ejemplo las pastas de aceitunas de la variedad 'Hojiblanca' (Sadkaoui et al., 2015) caracterizada por dar este tipo de pastas, o de malas prácticas durante la molienda y el batido (Hermoso et al., 1998). Para mejorar el rendimiento y evitar estos inconvenientes se pueden utilizar algunos coadyuvantes tecnológicos: agua, talco y arcilla caolinitica que son los únicos autorizados por la reglamentación Europea (CE/1513/2001, 2001; BOE, 2015):

  El uso del agua tibia para la mejora de los agotamientos ha sido propuesto desde décadas (Clodoveo, 2012), permite la fluidificación de las pastas y la mejora de los anillos de separación en el decánter (Hermoso et al., 1998). La adición de agua cambia los coeficientes de reparto de los compuestos minoritarios y varia su concentración en el producto final (Clodoveo, 2012).

  El otro coadyuvante más común es el talco natural micronizado, utilizado en los procesos de elaboración de los aceites de oliva a partir de las investigaciones realizadas en el Instituto de la Grasa (Alba, 1982). Se trata de un aditivo alimentario (E553b) autorizado por la Unión Europea (CE/1513/2001; BOE, 2015). Se utiliza para romper la emulsión del aceite y agua (Sadkaoui et al., 2015) lo que permite la

mejora del rendimiento de extracción (Aguilera et al., 2010; Clodoveo, 2012; Caponio et al., 2016). Las dosis de talco utilizadas son muy variables de 0,2 hasta 3 % (Aguilera et al., 2010; Clodoveo, 2012; Sadkaoui et al., 2015; Caponio et al., 2016). En relación con el efecto del talco sobre la calidad reglamentada del aceite de oliva no se ha observado deterioro (Aguilera et al., 2010; Clodoveo, 2012). El uso del talco aumenta el contenido en fenoles en el aceite de oliva (Aguilera et al., 2010; Clodoveo, 2012) así como el amargor y el picante. En cuanto a los compuestos volátiles no se han reportado cambios en su perfil y contenido en el aceite de oliva virgen (Caponio et al., 2016).

Otros coadyuvantes utilizados para la extracción del aceite de oliva virgen son: La Arcilla caolinítica autorizada recientemente para su uso como coadyuvante (BOE, 2015),y las enzimas pectinolíticas pueden ser utilizadas como coadyuvantes aunque no están autorizados por la Comunidad Europea (CE/1513/2001). Este coadyuvante se utiliza para la mejora de la extractabilidad y la modulación de los compuestos nutricionales y sensoriales del aceite de oliva (Clodoveo, 2012).

2.3. Separación sólido-líquido

Después del batido se obtiene una pasta formada por fases sólidas, que contienen trozos de hueso y otros restos de tejidos de aceituna, y fases líquidas, constituidas por aceite y agua de vegetación (Hermoso et al., 1998; Alba, 2008; Aparicio y Harwood, 2013). La separación de la fase oleosa de la pasta de aceituna se puede realizar mediante sistemas de prensa, percolación o centrifugación. En los sistemas de extracción continuos la separación de las fases de la pasta se produce mediante el uso de decánter de tres o dos salidas, siendo este último el método más usado hoy en día para la extracción del aceite de oliva virgen es España. Esta separación se realiza por acción de la fuerza centrífuga sobre la pasta de aceituna usando una elevada velocidad donde se separa las fases sólidas y liquidas (Figura 1.3), en función de sus diferentes densidades (Hermoso et al., 1998).

El decánter o centrifuga vertical consiste en un bol de forma troncocónica cilíndrica que puede girar a un régimen de 3000 a 3500 rpm, en cuyo interior se encuentra un sinfín adaptado a la forma del rotor que gira a un número de vueltas diferente, generalmente, menor que el del rotor, pero en el mismo sentido (Hermoso et al., 1998). Los dos

diferentes sistemas usados en las almazaras se diferencian en función del número de salidas:

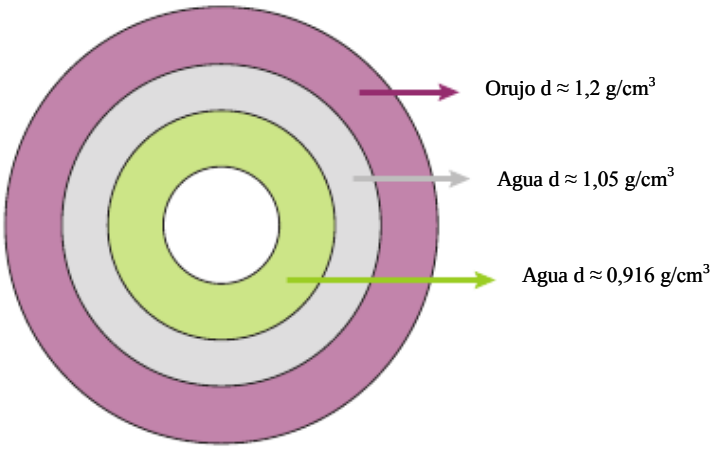

Figura 1.3. Anillos de separación de los componentes de la pasta de aceitunas sometida a centrifugación

Sistema de tres salidas (Figura 1.4): En este sistema la adición de agua es necesaria con la pasta batida para mantener la temperatura de trabajo, para disminuir la viscosidad de las fases liquidas y crear capas de líquidos de suficiente espesor para una adecuada separación de los tres anillos que se forman: agua, orujo húmedo y el aceite (Hermoso et al., 1998; Alba, 2008).

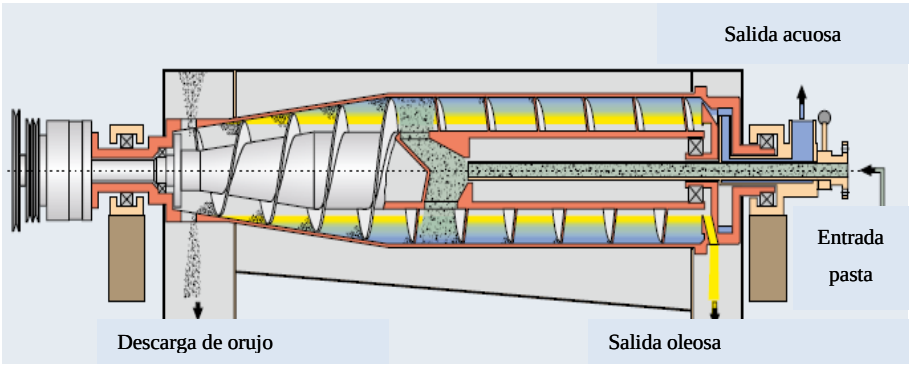

Figura 1.4. Esquema de funcionamiento del decánter de tres salidas

Sistema de dos salidas (Figura 1.5): En este sistema no se requiere, en principio, adición de agua, la pasta se inyecta dentro del decánter tal como se encuentra en el último recorrido de la batidora. Este sistema presenta dos salidas: una para el orujo (orujo muy

húmedo de 55 a 70 % humedad) y la segunda para el aceite (Hermoso et al., 1998; Alba, 2008).

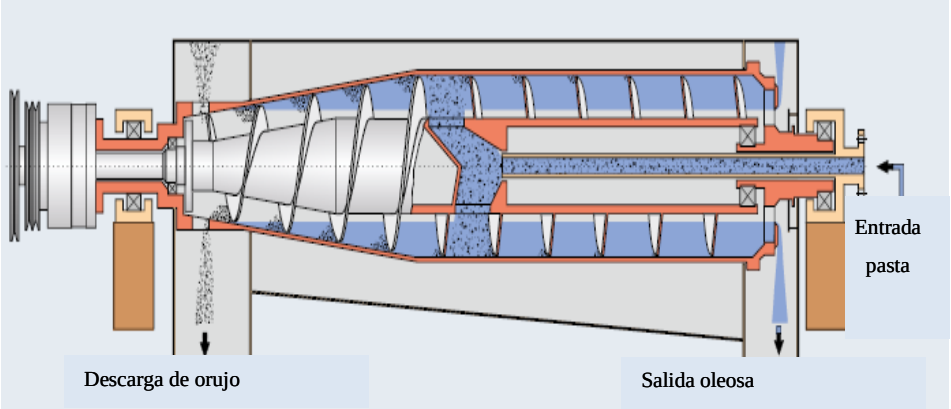

Figura 1.5. Esquema de funcionamiento del decánter de dos fases

La utilización de uno u otro sistema de separación incide sobre la composición del aceite de oliva. Así, en un sistema continuo de dos fases, la adición de agua es escasa lo que se traduce por un mayor contenido en compuestos fenolicos, mayor estabilidad y caracteres sensoriales más acusados en amargo y picante (Hermoso et al., 1998; Di Giovacchino et al., 2002; Alba, 2008; Di Giovacchino, 2013).

2.4. Clarificación del aceite

Tras su salida de la centrifuga horizontal el aceite tiene una cantidad variable de humedad e impurezas que tienen que ser eliminadas antes de su almacenamiento. Para limpiar el aceite de las impurezas es necesario someter a los líquidos a fuerzas centrífugas más elevadas que las conseguidas en las centrífugas horizontales. Dada la escasa diferencia de densidad entre los líquidos para obtener una separación eficiente se utilizan las centrífugas verticales, cuya velocidad de giro es mayor que la del decánter: 6000 - 7000 rpm (Hermoso et al., 1998; Alba, 2008; Di Giovacchino, 2013). En la Figura 1.6. se muestra, en esquema, una sección de una centrifuga vertical.

La clarificación con centrifuga vertical implica la adición de una cantidad variable de agua a una temperatura controlada. Por tanto, durante esta etapa es esencial controlar la temperatura y la cantidad del agua adicionada. Temperaturas elevadas pueden producir

en el aceite pérdida de características organolépticas, compuestos volátiles y un descenso del contenido en polifenoles y estabilidad (Jiménez-Márquez et al., 1995; Hermoso et al., 1998; Masella et al., 2009). Un caudal elevado de agua añadido produce un lavado más intenso del aceite, obteniéndose aceites menos amargos, picantes y menos estables (Hermoso et al., 1998).

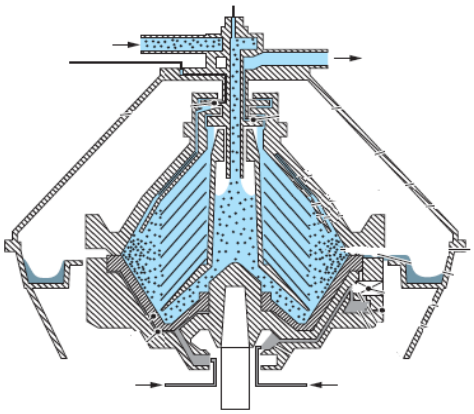

Figura 1.6. Esquema de una centrífuga vertical

Recientemente, y con el objetivo de eliminar los efluentes provocados por la adición de agua en la centrifuga vertical y sus efectos no deseados en el aceite como la reducción del contenido de fenoles y características organolépticas del aceite, surgió una tendencia de sustituir la centrifuga vertical por baterías de decantadores de fondo cónico. Estos sistemas para la decantación de los sólidos en suspensión y el agua se han diseñado con una configuración en estático o en dinámico (Bejaoui, 2011). Sin embargo se ha demostrado los efectos negativos de los sistemas de decantación estática en el aceite de oliva comparado con la centrifuga vertical con escasa adición de agua (5%).

2.5. Almacenamiento y conservación del aceite

Una vez obtenido, la calidad y las características del aceite de oliva virgen empiezan a deteriorarse debido a procesos continuos e irreversibles de oxidación e hidrólisis. Por tanto, el aceite debe ser almacenado de la forma más adecuada para garantizar que no se altere su composición, no se pierdan flavores naturales y se alargue su vida útil hasta su consumo (Alba, 2008; Uceda et al., 2008; Di Giovacchino, 2013).

Tras la etapa de clarificación, el aceite presenta pequeñas proporciones de agua y partículas en suspensión. Estos elementos, emulsionados en el aceite y ricos en

azúcares, terminan decantándose con el tiempo y fermentan rápidamente contaminando el aceite con olores y sabores extraños y deteriorando su calidad, por lo que es necesario retirarlos periódicamente mediante purgas periódicas (Hermoso et al., 1998).

La bodega ideal debe cumplir ciertos requisitos como que las paredes y techos sean aislantes para mantener una temperatura estable de 15 a 18 ºC que permita una buena decantación de los aceites y retarde los procesos de oxidación e hidrolisis; debe tener poca luminosidad, comodidad de acceso, fácil limpieza y no ser almacén de material auxiliar u otros materiales (Alba, 2008; Di Giovacchino, 2013). Los materiales de los depósitos deben ser inertes para evitar que reaccione con el aceite, impermeables, no absorban olores, permitan una fácil limpieza, aislantes de la luz y el aire, y resistentes a impactos y a la presión (Hermoso et al., 1998; Di Giovacchino, 2013).

Durante su almacenamiento el aceite de oliva virgen sufre un deterioro de su calidad reglamentada con un aumento de sus parámetros (grado de acidez, índice de peróxidos y absorción en UV) con el tiempo debido a los procesos oxidativos e hidrólisis (Tsimidou et al., 2005; Di Giovacchino, 2013). Los compuestos minoritarios (compuestos fenólicos, pigmentos y tocoferoles) se ven también afectados durante el almacenamiento reduciendo la estabilidad oxidativa del aceite (Morelló et al., 2004; Tsimidou et al., 2005). También, se ven reducidas la intensidad de los atributos positivos del aceite de oliva y los compuestos volátiles correspondientes, dando lugar a la generación de defectos relacionados con los procesos oxidativos y fermentativos que ocurren durante el almacenamiento (Angerosa et al., 2004; Kalua et al., 2007; Morales et al., 2013).

3. AVANCES EN LA ETAPA DE PREPARACIÓN DE LAS PASTAS DE ACEITUNA

La etapa de preparación de la pasta de aceituna es la etapa del proceso de elaboración del aceite de oliva virgen con mayor incidencia sobre el rendimiento de extracción y la calidad y composición del aceite de oliva debido al conjunto de reacciones químicas y bioquímicas que se desarrollan en esta operación (Hermoso et al., 1998; Alba, 2008; Clodoveo, 2012; Di Giovacchino, 2013). En esta etapa, la operación de batido de la pasta es la que menos desarrollo ha sufrido (Hermoso et al., 1998; Alba, 2008; Clodoveo, 2012; Di Giovacchino, 2013), por lo que las batidoras actuales presentan diferentes inconvenientes (Ayr et al., 2015):

- Ocupan el mayor espacio dentro de la sala de extracción en las almazaras, ya que es una etapa semicontinua en el proceso de elaboración continuo. Se llena el vaso de la batidora, se bate durante un cierto tiempo para separar después el aceite en los decánter. Requiere en la mayoría de las instalaciones de más de un cuerpo, para asegurar el funcionamiento continuo de todo el proceso y la alimentación en continuo del decánter.
- Calentamiento poco eficiente de la pasta de aceituna. La deficiente transferencia de calor de las paredes hasta el centro del cuerpo, de la batidora hace que se necesite entre 20 y 30 minutos para conseguir un calentamiento uniforme de la pasta lo que disminuye la eficacia del batido.
- La influencia negativa, que puede tener esta operación sobre la calidad, composición nutricional y características sensoriales del aceite de oliva virgen, debido a un deficiente movimiento de la pasta así como por su difícil limpieza.

En los últimos 10-15 años se han llevado a cabo cambios con objeto de modificar y mejorar la eficiencia de esta etapa del proceso y optimizar los rendimientos y características del aceite. Ayr et al. (2015) estudiaron cambios en la geometría para la mejora de la transferencia de energía dentro del cuerpo de batidora aplicando la mecánica de fluidos computacional. Además, en la última década nuevas técnicas están siendo introducidas en esta etapa para su mejora o en sustitución de la batidora:

- Control de atmósfera e inertización para evitar deterioro de la calidad del aceite y modelización de su composición (Di Giovacchino, 2013; Pastore et al., 2014;

Selvaggini et al., 2014). Durante el batido de la pasta, se desarrollan complejas reacciones química y bioquímicas en las que interviene la composición del espacio de cabeza de la batidora; en este sentido, una tendencia para el control del contenido de su composición ha sido introducida para la mejora del proceso por adición de gases inertes o aprovechando el CO_2 liberado de los tejidos vegetales con el objetivo de regular el contenido en O_2 (Parenti, 2006; Clodoveo, 2012; Servili et al., 2015). La inertización del espacio de cabeza puede permitir un batido largo para la mejora del rendimiento, en particular para las pastas difíciles, sin deteriorar la calidad del aceite. El contenido en O_2 en el espacio de cabeza influye negativamente sobre los compuestos fenólicos del aceite (Servili et al., 2003b, 2015; García-Rodríguez et al., 2011). Por otra parte, un cambio de la atmósfera del espacio de cabeza de las batidoras con una inertización permite aumentar el contenido de estos en los aceites (Parenti et al., 2006; Servili et al., 2008; Clodoveo, 2012). En relación con los compuestos volátiles, la disminución del contenidos en O_2 puede influir negativamente sobre la formación de estos compuestos debido a que la ruta de la LOX usa el O_2 como substrato (Kalua et al., 2007; Sánchez-Ortiz et al., 2013). En este sentido y de forma más reciente se han desarrollado nuevos diseños de batidora con control de inyección de oxígeno (Leone et al., 2014a; Tamborrino et al., 2014).

- Sistemas de calentamiento rápido de la pasta. Se basa en el calentamiento, en un intercambiador de calor tubular (Alfa Laval, Italia), de forma continua de la pasta de aceituna antes del batido (Esposto et al., 2013; Leone et al., 2015a). El uso de esta técnica permite acortar el tiempo de batido eliminando la etapa de precalentamiento en las batidoras sin afectar al rendimiento del proceso. El uso de los intercambiadores de calor en la preparación de la pasta no afecta a la calidad reglamentada del aceite. Sin embargo, se han descrito niveles más altos en compuestos fenólicos y volátiles del aceite. En la misma trayectoria, en Italia, el Grupo Pieralisi ha desarrollado un tipo de intercambiador de calor con sistemas de tornillo sin fin para mezcla y transporte de la pasta acoplado a un equipo de batido (Fiori et al., 2014).

- Aplicación de microondas: Los microondas son ondas electromagnéticas con frecuencias comprendidas entre 300 MHz y 300 GHz. El efecto principal de esta

técnica es térmico. Leone et al. (2014, 2015c) estudiaron la aplicación en continuo y a nivel industrial de esta tecnología. Los resultados mostraron que las microondas permiten reducir la etapa del batido con la eliminación del precalentamiento de la pasta, no observandose efecto en el rendimiento de los microondas comparando con el batido convencional; el equipo de microondas utilizado tiene una potencia de 24 kW. Clodoveo y Hachicha Hbaieb (2013) no encontraron que la aplicación de las microondas tuviese un efecto negativo sobre las características del aceite de oliva virgen.

- Tratamiento de la pasta por pulsos eléctricos: Esta tecnología se basa en la aplicación de pulsos cortos de alto voltaje con el objetivo de deteriorar la pared celular con la formación de poros permitiendo la liberación del contenido intracelular, además de un leve calentamiento del medio de aplicación. Abenoza et al. (2013); y Puértolas y Martínez-de-Marañón (2015) mostraron que el pretratamiento por pulsos eléctricos de la pasta antes del batido a nivel de planta piloto permite una mejora del rendimiento a temperatura más bajas de batido. En relación con el efecto sobre el aceite de oliva, estos autores observaron que no hay deterioro en la calidad del aceite, y un mayor contenido en compuestos fenólicos, fitoesteroles y tocoferoles.

4. APLICACIÓN DE LOS ULTRASONIDOS EN LA EXTRACCIÓN DE LOS ACEITES DE OLIVA VÍRGENES

En la industria alimentaria el uso de la tecnología de ultrasonidos de potencia es relativamente reciente, su uso se está extendiendo recientemente por sus efectos de mejorar los rendimientos de producción, conservar e incluso mejorar la calidad de los productos; su coste es reducido y tiene fácil mantenimiento (Patist y Bates, 2008).

4.1. Fundamento y mecanismos

Por ultrasonidos se conoce a aquellas ondas mecánicas situadas en una zona del espectro acústico que va desde los 16 kHz a los 10 MHz, aproximadamente, y que son inaudibles para el oído humano (Mason, 1998).

Este amplio campo de frecuencias (Figura 1.7) permite emplear los ultrasonidos con propósitos muy diferentes según las categorías en que estos se subdividen: los ultrasonidos de baja potencia o alta frecuencia (> 0,5 MHz) en los que las ondas ultrasónicas son utilizadas para obtener información sobre el medio que se propaga sin que se produzca alteraciones del mismo y los ultrasonidos de alta potencia o baja frecuencia (16 - 100 kHz) en los que las ondas acústicas producen efectos permanentes en el medio en el que se aplica.

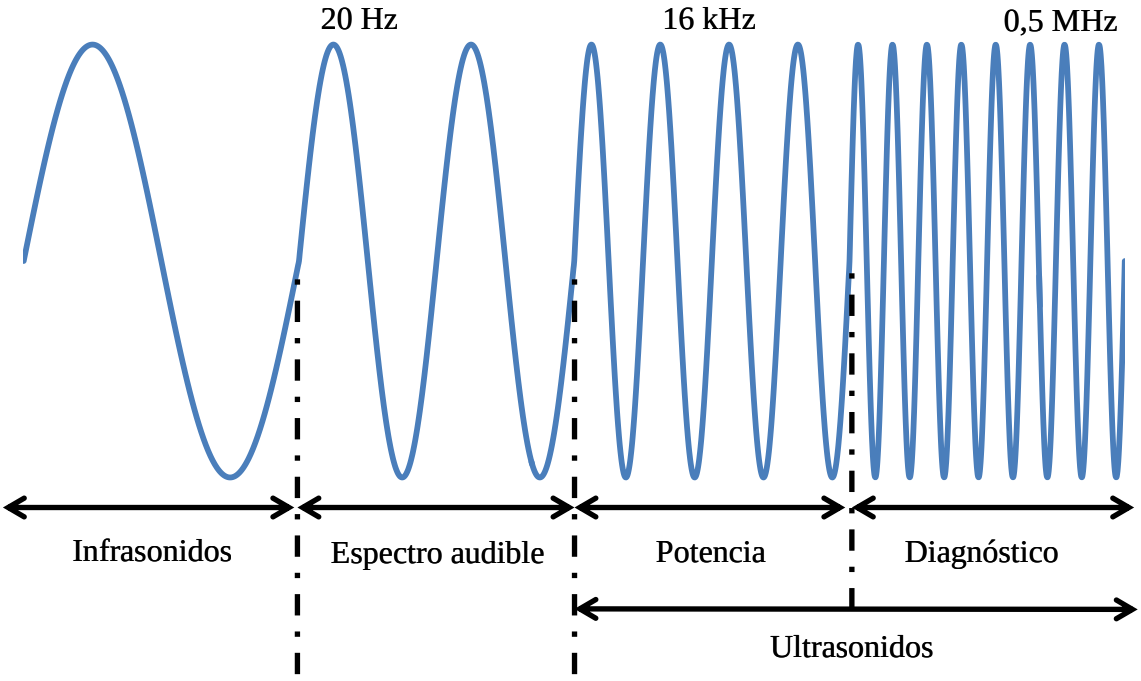

Figura 1.7. Espectro de sonidos

Las primeras son utilizadas tanto en medicina terapéutica (entre los 0,5 - 3 MHz) como en diagnóstico: ecografías, ensayos no destructivos, sensores, etc (entre 1 - 10 MHz); mientras que las segundas vienen desarrollando más recientemente sus aplicaciones para los más diversos sectores industriales (Mason, 1998; Mason et al., 1996; Kentish y Ashokkumar, 2011).

Una de las características de la aplicación de los ultrasonidos es que se produce una acción puramente mecánica sobre el medio aplicado, en forma de ciclos de expansión y compresión de manera alterna (Figura 1.8). Durante los ciclos de expansión (rarefacción), los ultrasonidos generan burbujas en el seno del medio o provocan el crecimiento de las ya existentes, mientras que en los ciclos de compresión estas disminuyen; cuando el crecimiento de la burbuja es tal que no permite absorber más energía, se produce una implosión violenta (Lorimer y Mason, 1987; Mason, 1998; Ashokkumar y Grieser, 1999).

Este fenómeno, conocido como cavitación se suele producir cuando el medio al que se aplican los ultrasonidos contiene líquido, como es el caso de la mayoría de los productos alimentarios, los cuales pueden considerarse constituidos generalmente, por una fase acuosa y una matriz de sustancias insolubles como proteínas, carbohidratos y grasas (Lorimer y Mason, 1987; Mason, 1998; Ashokkumar y Grieser, 1999). Esta cavitación puede ser estable, cuando hay una persistencia de los ciclos repetitivos de compresión y expansión de las burbujas antes la implosión de las burbujas, es conocida como cavitación estable (Kentish y Ashokkumar, 2011). O transitoria, cuando hay un número limitado de ciclos (generalmente 1 o 2) y donde las burbujas colapsan generando una violenta implosión (Mason y Lorimer, 2002). El predominio de uno u otro tipo de cavitación depende de diferentes factores (Mason y Lorimer, 2002; Kentish y Ashokkumar, 2011) como:

- *Presencia de gases disueltos en el medio líquido*.
- *Presión del medio*.
- *Intensidad de los ultrasonidos*.
- *Frecuencia aplicada*.
- *Temperatura del medio*.
- *Impurezas en el medio*.

En la cavitación estable se producen fenómenos de difusión de gases o líquidos, estos últimos forman las burbujas de cavitación, generándose su movimiento a través del

medio. Cuando estas burbujas de cavitación llegan a su talla critica provoca su implosión y genera de forma local del orden de 1800 ºK (Mason y Lorimer, 2002). En cuanto a la cavitación transitoria genera temperaturas altas, próximas a 5000 ºK, y presión de 1000 atmósfera causando fenómenos de erosión, emulsificación y sonoluminiscencia (Mason y Lorimer, 2002).

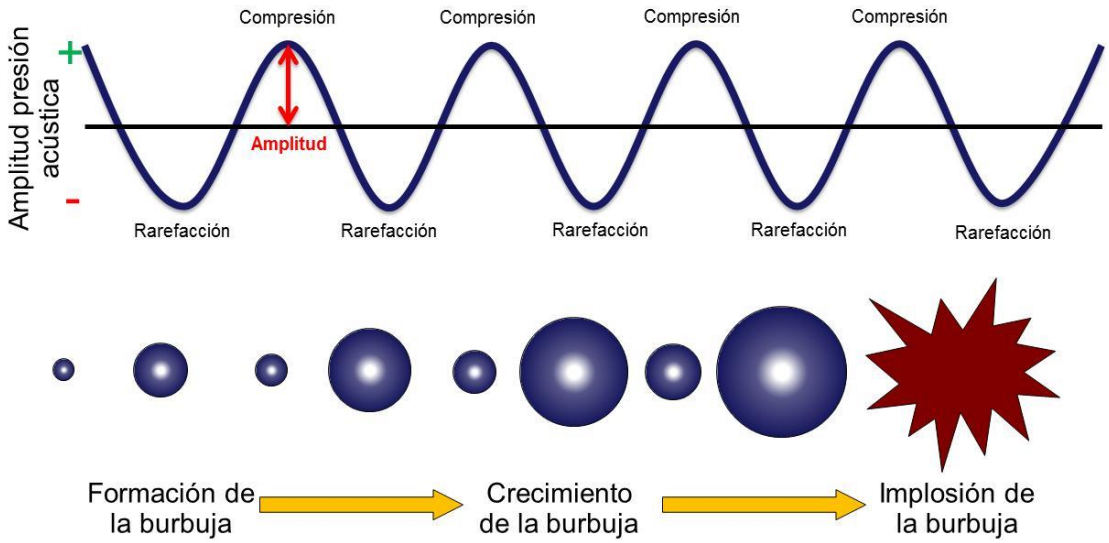

Figura 1.8. Representación del comportamiento de las burbujas de cavitación con la propagación de las ondas ultrasónicas

Se observan dos efectos con la aplicación de los ultrasonidos: físicos y químicos (Kentish y Ashokkumar, 2011; Moholkar et al., 2011). Los efectos físicos provocan grandes cambios en el seno del medio donde se propagan las ondas de ultrasonidos de potencia. Estos cambios se producen mediante diferentes mecanismos:

- *Microcorrientes* (‘Microstreaming’ en inglés). Se producen por la propagación de las ondas en un líquido. Se crea unas serie de movimientos oscilatorios de pequeña amplitud, generando fluctuaciones de la velocidad y presión del líquido (Kentish y Ashokkumar, 2011).
- *Efecto esponja*. Durante el paso de las ondas de ultrasonidos de potencia en un medio sólido los ciclos de compresión y rarefacción de la onda actúan de forma similar a las fases de compresión y expansión de una esponja (Bermúdez-Aguirre et al., 2011).

- *Microturbulencias o microconvección.* Se producen por el movimiento del fluido causado por los ciclos de compresión y rarefacción de las burbujas de cavitación. Este fenómeno está localizado muy cerca de las burbujas (Moholkar et al., 2011).
- *Ondas acústicas u ondas de choque.* Se forman durante la cavitación transitoria repetitiva y donde el colapso de las burbujas de cavitación genera condiciones extremas de alta presión (70-100 MPa), que se traducen en ondas de choque (Suslick, 1989). Este fenómeno puede provocar la rotura de las cadenas de polímeros o destrucción de la pared celular (Kentish y Ashokkumar, 2011).
- *'Microjets'*. Ocurren cuando la cavitación transitoria se produce en la proximidad de una superficie sólida, de la que resulta un gradiente de presión alrededor de las burbujas, y se traduce por una expulsión a presión de chorros ('jets') de fluidos a partir de las burbujas (Suslick, 1989; Kentish y Ashokkumar, 2011). Estos 'microjets' pueden causar la erosión de las superficies sólidas y la reducción del tamaño de partículas de los líquidos (Suslick, 1989; Kentish y Ashokkumar, 2011; Moholkar et al., 2011).
- *Calentamiento del medio*, es resultante de la disipación de la energía acústica generadas por los diferentes tipos de cavitación y sus parámetros citados previamente. Esto significa que la aplicación de los ultrasonidos siempre genera incremento de temperatura (Mason y Lorimer, 2002; Kentish y Ashokkumar, 2011). En la mayoría de los casos, este incremento es relativamente suave del orden de algunos grados Celsius pese a que de forma muy local la implosión de las burbujas de cavitación generan temperaturas muy altas. Este incremento es decisivo en la regulación de la potencia eléctrica aplicada para la aplicación deseada (Mason y Lorimer, 2002; Kentish y Ashokkumar, 2011;).

Además de los efectos físicos, la aplicación de los ultrasonidos también tiene unos efectos a nivel químico, los más destacados (Kentish y Ashokkumar, 2011) son la formación de radicales primarios (H• y OH•) como resultado de las altas temperaturas generadas dentro de las burbujas de cavitación a partir de vapor de agua. El radical OH• generado es altamente inestable que favorece los fenómenos de oxidación y puede afectar la calidad del producto. En este sentido, es conocido que un intenso tratamiento por ultrasonidos puede provocar defectos sensoriales. El radical H• actúa en la reducción de los iones metálicos. La formación de estos radicales se reduce con la aplicación de frecuencias más bajas (Kentish y Ashokkumar, 2011).

4.2. Aplicación en las industrias alimentarias

Los efectos físicos y químicos determinan en gran medida la aplicación de los ultrasonidos en diferentes ámbitos y en especial, en la industria alimentaria. En general, el objetivo de la introducción de la tecnología de los ultrasonidos de potencia en los procesos industriales alimentarios es la reducción del tiempo de proceso de los alimentos, el ahorro energético, así como la mejora de la vida útil y la calidad de los alimentos. Además, esta tecnología emergente tiene gran interés por sus características amigables con el medioambiente. Los ultrasonidos se pueden aplicar directamente en el producto, indirectamente a través de superficies o sumergido en baño (Chemat et al., 2011). Diferentes aplicaciones se están desarrollando en las industrias alimentarias:

Extracción asistida por ultrasonidos. De forma tradicional diferentes métodos se utilizan para la extracción de sustancias orgánicas y minerales de los alimentos como la extracción por Soxhlet, maceración, elución, destilación o prensado. Estas técnicas están caracterizadas por una baja eficiencia. Por ello, para mejorar la extracción se necesita: prolongar el calentamiento, utilizar altas temperaturas o usar grandes volúmenes de disolventes lo que requiere una etapa de concentración posterior. Los ultrasonidos de potencia se utilizan por sus efectos en la mejora de los fenómenos de transferencia de materias y la rotura de las paredes celulares (Chemat et al., 2011). Esta tecnología se aplica para la extracción de los aceites esenciales de plantas aromáticas como las hojas de menta (Shotipruk et al., 2001), la artemisia (Asfaw et al., 2005), la lavanda (Da-Porto et al., 2009), el ajo (Kimbaris et al., 2006) y las semillas de alcaravea (Chemat et al., 2004). También en la extracción de compuestos vegetales, antioxidantes y colorantes, para sus aplicaciones como aditivos, como los ácidos tartárico y málico de la uva y su semilla (Palma y Barroso, 2002), la capsaicina de la pimienta (Barbero et al., 2008), las antocianinas de las frambuesas (Chen et al., 2007), los compuestos fenólicos de las fresas (Herrera y Luque-de-Castro, 2005), y el licopeno de los tomates (Lianfu y Zelong, 2008).

Emulsificación y homogeneización. Durante la aplicación de los ultrasonidos de potencia el colapso de las burbujas de cavitación en el límite de la capa de dos fases líquidas inmiscibles da lugar a unas ondas de choque que permiten la formación de gotas de líquido finas y una emulsión muy estable (Patist y Bates, 2008). Por estas razones la emulsión ultrasónica está atrayendo gran interés para productos como zumos,

mayonesa, salsa de tomate, homogeneización de la leche y encapsulación de aromas (Chemat et al., 2011).

Cristalización y congelación. La aplicación de los ultrasonidos de potencia tiene influencia en la nucleación de los cristales y la formación de cristales de pequeño tamaño (Patist y Bates, 2008; Chemat et al., 2011; Delgado y Sun, 2011; Kiani et al., 2014; Mason et al., 2015). De esta forma se puede reducir los daños celulares durante la congelación de alimentos.

Modificación de la viscosidad. Dependiendo del tipo de tratamiento, los ultrasonidos de potencia pueden permitir disminuir la viscosidad de forma temporal o permanente con la disminución del peso molecular (Patist y Bates, 2008). También se puede incrementar la viscosidad, como en el caso de los purés vegetales facilitando la penetración del agua dentro de la red de fibras (Patist y Bates, 2008).

Preservación de los alimentos. Los ultrasonidos de potencia permiten inactivar los microorganismos, las esporas y las enzimas (Bermúdez-Aguirre et al., 2011; Chemat et al., 2011) presentes en los alimentos favoreciendo o mejorando su vida útil.

Separación. La aplicación de los ultrasonidos puede permitir la separación de las emulsiones en las fases de las que se componen (fase acuosa y fase lipídica); así mismo, permite la coalescencia de la fase lipídica (Gardner y Apfel, 1993; Pangu y Feke, 2004; Patist y Bates, 2008).

Otras aplicaciones. Los ultrasonidos de potencia se utilizan también para mejorar la eficiencia de la filtración, evitar la formación de espumas, desgasificación, cocción y corte (Patist y Bates, 2008; Bermúdez-Aguirre et al., 2011; Chemat et al., 2011; Feng et al., 2011) de los alimentos.

4.3. Aplicación de los ultrasonidos de potencia en el proceso de extracción del aceite de oliva virgen

La aplicación de esta tecnología en la etapa de batido de la pasta se traduce en una serie de fenómenos en el medio, similares a los que tienen lugar durante esta etapa en el proceso de extracción. Así, las microagitaciones, el efecto esponja, la transferencia de cantidad de movimiento, energía y materia son los principales fenómenos que podemos encontrar en la aplicación de esta técnica en un producto alimentario (Mason, 1998; Feng et al., 2011; Kentish y Ashokkumar, 2011), y que tienen su equivalencia en los

fenómenos de coalescencia, flujo de fluidos y transferencia de energía de la pasta durante la operación de batido.

Los primeros trabajos de aplicación de los ultrasonidos para la elaboración de los aceites de oliva vírgenes fueron llevado a cabo por Jiménez et al. (2006, 2007). Estos primeros trabajos consistieron en la aplicación de los ultrasonidos de potencia durante el batido de la pasta de aceituna de la variedad `Picual´ a nivel de laboratorio. Se evaluaron dos sistemas diferentes de aplicación de los ultrasonidos de potencia:

- De forma directa al comienzo del batido (4 y 8 minutos) con sonotrodos del tipo llamado `Horn´, con una frecuencia de 24 kHz y potencia de 105 W cm^{-2}.
- De forma indirecta durante todo el tiempo de batido con una frecuencia de 25 kHz, en baño con aplicación de los ultrasonidos a través del fondo del recipiente.

Los resultados mostraron que el tratamiento con ultrasonidos permitió un rápido calentamiento de la pasta comparando con el batido convencional. Además, se observó que este tratamiento mejoraba la extractabilidad del aceite de oliva con respecto al batido sin tratamiento ultrasónico. También se observó una diferencias entre ambos tipos de aplicación, así para la aceituna de mayor contenido en humedad el tratamiento directo fue el más eficiente, mientras que para la aceituna con menor contenido de humedad el tratamiento indirecto fue mejor. En cuanto al aceite, la calidad reglamentada (índice de acidez, índice de peróxidos, absorción en ultravioleta a 232 nm y 270 nm) no se vio alterada. Además, el tratamiento por ultrasonidos permitió aumentar el contenido en tocoferoles, clorofilas y carotenoides del aceite de oliva virgen. Por el contrario, el contenido en compuestos fenólicos y el amargor del aceite disminuye ligeramente con el tratamiento ultrasónico. En relación con las características sensoriales, los aceites obtenidos mediante la aplicación de los ultrasonidos mostraron una mayor intensidad de los atributos positivos sin que se aprecien defectos sensoriales. Por otra parte, se ha evaluado el contenido en compuestos volátiles del aceite, y los tratamientos tanto directo como indirecto mostraron una disminución del área total de los volátiles determinados por SPME; también se ha determinado la relación hexanal/*E*-2-hexenal como indicador de oxidación y aparición de defectos sensoriales como el rancio. Esta relación disminuye con el tratamiento ultrasónico lo que muestra que no aparece oxidación provocada por el tratamiento de ultrasonidos de potencia.

Otros trabajos más recientes dirigidos por Clodoveo et al. (2013a, 2013b) se han basado en la aplicación de los ultrasonidos en baño ultrasónico. La frecuencia aplicada fue de 35 kHz y 150 W de potencia, en el pretratamiento del fruto y después de la molienda en las pastas de aceitunas. La duración del tratamiento fue de 4 y 8 minutos. Los resultados mostraron que el tratamiento por ultrasonidos permite reducir de forma muy importante el tiempo de calentamiento de la pasta de aceituna comparado con el batido convencional. Esto permite reducir el tiempo de batido, aumentando la extractabilidad, tal y como se había observado por Jiménez et al. (2006, 2007), sin observar diferencia significativa entre métodos de aplicación aunque el tratamiento durante más tiempo ha sido más eficiente. Ninguno de los tratamientos evaluados afecta a los parámetros de calidad reglamentada de los aceites de oliva vírgenes. En relación con los componentes minoritarios del aceite se observó un incremento en las fracciones de tocoferoles y pigmentos con la aplicación de los ultrasonidos. En cuanto al contenido en fenoles, el tratamiento ultrasónico en la aceituna completa mostró un incremento en el contenido en compuestos fenólicos, mientras que en el tratamiento de las pastas se obtuvo una disminución en el contenido de estos.

Estos trabajos preliminares sobre el empleo de ultrasonidos de potencia en los proceso de extracción de aceites de oliva vírgenes muestran la viabilidad del uso de esta tecnología en la mejora de los rendimientos y la reducción del tiempo de la etapa de batido de las pastas. Los tratamientos, descritos en estos trabajos previos, se han desarrollado en `batch´, esta discontinuidad representa el mayor inconveniente de la etapa de batido de la pasta de aceituna. En este sentido la introducción de esta tecnología en el proceso de extracción de los aceites de oliva vírgenes necesita más investigación para desarrollo de nuevos sistemas continuos de tratamiento de la pasta.

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Capítulo 2. Objetivos e hipótesis

El objetivo general de este trabajo es profundizar en el estudio de aplicación de los ultrasonidos en la etapa de preparación de las pastas de aceitunas durante el proceso de elaboración de los aceites de oliva vírgenes, desarrollando un sistema para aplicación a nivel piloto de los ultrasonidos de potencia, con la finalidad de mejorar del proceso (rendimiento y calidad del aceite) y disminuir su coste energético.

Como hipótesis del trabajo se pretende encontrar una alternativa eficiente a la etapa de batido que conduzca a un mayor rendimiento y que mejore la calidad de los aceites de oliva vírgenes obtenidos. Esta alternativa propuesta se centra en la utilización de los ultrasonidos de potencia.

Esta tesis se ha centrado en 5 objetivos específicos:

- Examinar el efecto sobre el calentamiento de las pastas de aceitunas y de la eficiencia en el proceso de elaboración.
- Estudiar el efecto de este tratamiento sobre la extractabilidad del aceite de oliva.
- Analizar el efecto de los ultrasonidos de potencia sobre la calidad de los aceites de oliva obtenidos.
- Analizar el efecto de la aplicación de los ultrasonidos en la formación compuestos volátiles del aceite relacionados con los atributos positivos procedentes de la ruta enzimática de la ‘Lipoxygenasa (LOX)’ y otros relacionados con la formación de defectos provenientes de procesos oxidativos y malas prácticas durante esta etapa.
- Analizar el efecto del tratamiento de las pastas de aceitunas por ultrasonidos sobre el perfil fenólico de los aceites de oliva vírgenes.

Con la ejecución de éste trabajo se pretende realizar, mediante una serie de ensayos específicos, un estudio profundo del efecto del uso de los ultrasonidos sobre la extractabilidad de las pastas y sobre el producto final.

La primera etapa representa un ensayo a nivel de laboratorio, en el cual se trata de estudiar el efecto de los ultrasonidos en régimen dinámico en el proceso de elaboración de los aceites de oliva vírgenes empleando la sistemática descrita en los trabajos previos. Este estudio del efecto de la aplicación de los ultrasonidos esta concentrado sobre el proceso de extracción así como en la calidad y composición de los aceites de oliva vírgenes.

La segunda etapa consiste en el desarrollo de un prototipo para operar en continuo a nivel de mini planta. Con el objetivo de examinar la aplicación de los ultrasonidos en un régimen dinámico, con inyección continúa de las pastas de aceitunas y su efecto en la extractabilidad y las características físico-químicas y sensoriales de los aceites de oliva se ha utilizado el sistema 'Abencor'.

Finalmente se aborda el estudio de la integración del prototipo diseñado en el equipo de extracción ('Il Molinetto'. Pieralisi, España) para la elaboración de los aceites de oliva vírgenes. En esta etapa se analiza el efecto sobre la eficiencia del proceso así como la calidad y la composición de los aceites obtenidos.

Part 1: High power ultrasound application for virgin olive oil extraction at laboratory scale

Chapter 3. Continuous high power ultrasound treatment before malaxation, a laboratory scale approach: Effect on virgin olive oil quality criteria and yield.

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Short Communication

Continuous high power ultrasound treatment before malaxation, a laboratory scale approach: Effect on virgin olive oil quality criteria and yield

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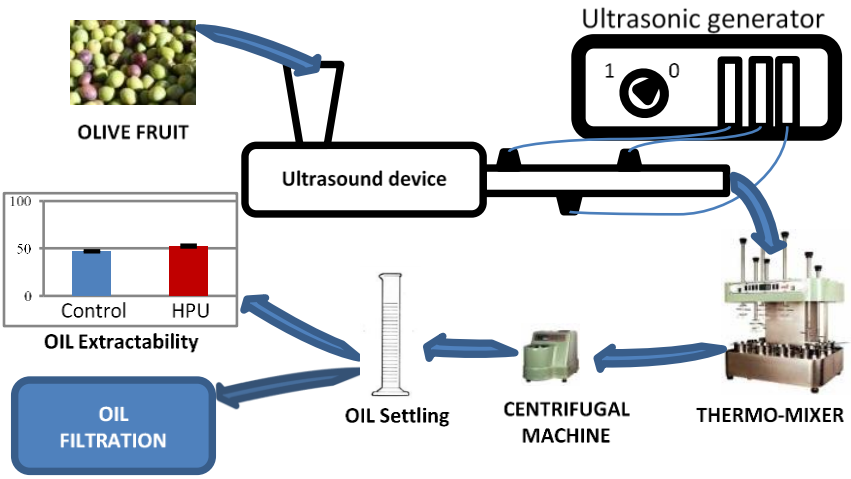
Abstract:

Continuous application of high power ultrasound (HPU) on olive paste previous to malaxation at laboratory scale for virgin olive oil (VOO) extraction and its effect on process yield and the VOO quality have been studied. For the VOO samples, we determined the quality indices (free acidity value, peroxide value, K_{232}, K_{270}), bitterness, volatile composition, phenolic content (total and biophenols), tocopherol content, and pigments. The application of ultrasound induced a quick heating of the paste from 20 to 28°C. The sonication treatment improved the industrial oil yield by 1% and the oil extractability by 5.74%. Moreover the HPU treatment did not cause changes in the quality indices, fatty acid composition and volatile aromatic compounds of the VOO. Furthermore the fat autoxidation process was not accelerated by this treatment. Meanwhile oil obtained from olive pastes treated with HPU showed higher content of tocopherol, chlorophylls and carotenoids whereas a reduction in phenolic content and bitterness index was observed.

Practical applications: The high power ultrasound (HPU) treatment of olive paste, before malaxation during the virgin olive oil (VOO) elaboration process, can improve the oil extraction yield without alteration of its quality parameters. The olive paste instantaneous heating observed with the HPU can allow the reduction of the malaxation step duration. The results obtained nowadays at laboratory scale are promising for the possibility of the continuous HPU treatment introduction in the olive oil elaboration process as aid or alternative to the malaxation. For this purpose further experiment at pilot and industrial scale are needed.

Graphical abstract:



Continuous application of high power ultrasound before malaxation and its effect on the oil extractability and virgin olive oil characteristics

Keywords: High Power Ultrasound / Malaxation / Olive oil elaboration / Olive paste

Abbreviations: HPU, high power ultrasounds; VOO, virgin olive oil

1. Introduction

Virgin olive oils (VOO) are extracted from healthy `*Olea europaea* L.´ fruit by exclusively using physical procedures. These procedures include fruit crushing, olive paste kneading, solid–liquid separation and finally a clarification by centrifugation and/or settling [1]. Kneading is a basic step in the VOO extraction procedures, is especially important to reach high and satisfactory extraction yield [2] and has several effects on the VOO quality parameters, nutritional and sensorial characteristics [3].

Hence a current trend is to enhance this procedure applying some emerging technologies such as fruit pitting before crushing, kneading under inert atmosphere and cold kneading. One of the technologies recently used is the pretreatment of olive paste by high power ultrasounds (HPU) [4, 5].

First works of HPU application during the virgin olive oil elaboration were carried out by Jimenez et al. [4, 5] and consisted in the ultrasound application during the kneading

of olive paste. HPU application gave a rapid heating of olive paste and the improvement of the olive oil extractability without any alteration of the virgin olive oil quality parameters (free acidity value, peroxide value, K_{270}, and K_{232}). Furthermore higher content of tocopherols, chlorophylls, and carotenoids were observed whereas bitterness and polyphenols lowered. Later Clodoveo et al. [6], applied HPU to the olive paste before malaxation and to the olives before crushing during washing. For both treatments a quick olive paste heating was obtained, improving the oil extractability and observing similar effects on the olive oil quality to those observed by Jimenez et al. [4, 5]. However attention should be paid in the non-continuous conditions used in these works in open conditions with atmosphere contact.

Therefore, the purpose of this paper was to study the HPU application under continuous conditions for olive paste pretreatment, previous to malaxation, at laboratory scale and its effect on oil extraction process yield and VOO composition and quality. This work is a previous step for the development of a HPU treatment device for pilot plant scale.

2. Materials and methods

2.1. Plant material

Fresh and healthy olives (*Olea europaea* L.) from cultivar "Picual" was picked on November 2012 from trees grown under irrigation in the experimental olive orchard of IFAPA Centro Venta del Llano in Mengibar, Jaén. The olives were processed immediately and were characterized measuring moisture and fat content.

2.2. Olive characterization

Fruit ripening index was determined according to the method the method proposed by Beltran et al. [7].

The olive moisture was determined by desiccation at 105 ºC of weighted crushed olives, the results were expressed in weight percentage. Fat content was measured using a nuclear magnetic resonance (NMR) fat analyzer (Minispec mq 20, Bruker Analytik Gmbh). The NMR was previously calibrated and validated with Soxhlet extractor [8]. The results were expressed as percent on fresh matter basis.

2.3. Experimental HPU treatment

The experiments were carried out at laboratory scale using a device for the HPU treatment, a thermomixer and a basket centrifuge from an Abencor system (MC2, Seville). The HPU device was composed by three units: a roller mill, a rectangular pipeline (Length: 40 cm, Width: 6 cm, Height: 2 cm) for olive paste transport equipped with three 40 kHz frequency piezoelectric transducers and a 150 W ultrasonic generator with an intensity regulator for transducers power supply. After the olive milling, olive paste was moved through the pipe by the own miller. The olive paste flow rate on the pipe was established by the olive fruit feed of the mill. As the olive paste flowed through the pipe two treatments were applied: HPU and control without HPU application.

Around 700 - 800 gr of the olive paste were taken at the exit of the HPU device for triplicate, for each treatment, and then, kneaded in the thermo-malaxer at 28 ºC for 30 minutes and centrifuged for 2 minutes. During the whole process olive paste temperature was monitored. After centrifugation, the liquid phases were recovered in a graduated cylinder and after settling, the volume of the oily phase was measured for oil yield (percentage of oil obtained from determined olives paste weight) and oil extractability (percentage of olive oil yield with respect to the total paste fat content) calculation .The oil was filtered and stored at -24 ºC until analyses.

2.4. VOO analytical determinations

2.4.1. Quality indices

Free acidity (% oleic acid), peroxide values (mEq O_2/kg VOO), and ultra-violet absorption at 232 and 270 nm (K_{232} and K_{270}) were measured following the analytical methods described in European Regulation EEC 2568/91 [9].

2.4.2. VOO compositions

Total phenol content: Phenolic compounds were extracted from oil dissolved in hexane with methanol:water (60:40) and the concentration was measured using Folin-Ciocalteau reagent and colorimetric measurement at 725 nm [10]. Results were expressed as mg of caffeic acid/kg VOO.

The bitterness index (K_{225}): was determined applying the method described by Gutierrez et al. [11]. The bitter compounds were eluted through the solid phase extraction column with methanol:water (50:50). The absorbance of the methanolic extract recovered was measured at 225 nm. The results were expressed as mg/kg of VOO.

Biophenol composition: The phenolic fraction was extracted in a methanol:water solution and the phenolic extract was analyzed by RP-HPLC as described by Beltrán et al. [12]. Quantification was performed at 280 nm using syringic acid as internal standard and the response factors determined by Mateos et al. [13]. The results were expressed as mg/kg of VOO.

Pigments content: Carotenoids and chlorophylls pigments were determined measuring the absorbance of olive oil weighed and dissolved in cyclohexane at 470 and 670 nm respectively as described by Mínguez et al. [14]. Results were expressed as mg/kg.

Tocopherol content: Tocopherol composition was analyzed by HPLC, applying the IUPAC method 2432 [15]. Results are expressed as mg/kg of VOO.

Fatty acid composition: The fatty acid methyl esters (FAMEs) were prepared as described by the European Union official method [9]. The results were expressed as relative area percent of the total.

VOO volatile compounds: Solid-phase microextraction (SPME) followed by GC-FID were used to analyze the volatile fraction of the virgin olive oil obtained. Compound identification was performed using a mass spectrometer (ISQ single quadrupole MS, Thermo Fisher Scientific, Austin, Texas, USA). Quantification was performed using individual calibration curves for each identified compound by adding known amounts of different compounds to deodorized olive oil [16]. Results were expressed as mg/kg VOO.

2.5. Data analysis

The results were expressed as mean ± standard deviation (n=3). Analysis of variance was applied, significant differences between treatments were determined applying Tukey's test $p < 0.05$ (Statistix 9.0 software, Analytical Software, USA).

3. Results and discussion

3.1. Effect on oil yield

Olives used in the experiments showed a ripening index of 1.48, the moisture was 59.84 % ± 0.78 and the fat content 17.21 % ± 0.90.

The High Power Ultrasound (HPU) pretreatment induced an instantaneous heating of the olive paste from 20 ºC (room temperature) to 28 ºC for a paste flow rate of 20 kg/h in the HPU device. It took around 20 minutes for the untreated olive paste in the thermo-malaxer as described by Jiménez et al. [4, 5].

The sonication treatment improved by 1 % the industrial oil yield and gave significant higher extractability 52.75 % ± 1.39 comparing with the untreated olive paste 46.83 % ± 0.93. This behavior can be explained because the HPU treated olive paste was kneaded all the time at 28 ºC whereas the untreated olive paste was malaxed only for 10 minutes at 28 ºC. Therefore this treatment may allow to reduce the malaxation time.

3.2. Effect on virgin olive oil quality indices and minor compounds

In general the virgin olive oils obtained were classified into the 'extra virgin olive oil' (EVOO) category according to the European Union Commission Regulation EEC 2568/91 [9] and the value of the quality parameters (free acidity value, peroxide value, K_{232}, K_{270}) were so far from the limits established by EEC 2568/91 (Table 1).

Table 1. Quality indices for VOO obtained from untreated and HPU treated olive paste of 'Picual' cultivar.

Analytical parameters	**Control**	**HPU**
Free Acidity (% oleic fatty acid)	0.12±0.01 a*	0.12±0.01 a
Peroxides Value (mEq O_2/kg)	3.30±0.07 b	3.98±0.08 a
K_{232}	0.11 ±0.01 a	0.10 ± 0.00 a
K_{270}	1.56 ± 0.10 a	1.40 ± 0.02a

*Significant differences in the same row are showed by different letters ($p<0.05$)

VOO did not show changes in the free acidity value, K_{232}, K_{270} parameters because of HPU treatment. For peroxide value the VOO from the HPU treated paste showed a slight increase comparing with that from untreated paste, although remained at very low levels. The fatty acid composition (Table 2) did not show significant changes for VOO from HPU treatment.

Table 2. Fatty acid composition (%) for VOO obtained from untreated (Control) and HPU treated (HPU) olive paste of 'Picual' cultivar.

Fatty acids	**Control**	**HPU**
$C_{16:0}$[φ]	13.78±0.06 a*	13.88±0.01 a
$C_{16:1}$	1.33±0.01 a	1.34±0.01 a
$C_{17:0}$	0.04±0.00 a	0.04±0.00 a
$C_{17:1}$	0.08±0.00 a	0.09±0.00 a
$C_{18:0}$	2.71±0.00 a	2.74±0.03 a
$C_{18:1}$	77.77±0.09 a	77.70±0.14 a
$C_{18:2}$	3.16±0.03 b	3.23±0.02 a
$C_{18:3}$	0.62±0.03 a	0.66±0.00 a
$C_{20:0}$	0.28±0.05 a	0.33±0.01 a
$C_{20:1}$	0.20±0.01 a	0.20±0.02 a
$C_{22:0}$	0.05±0.04 a	0.06±0.01 a

*Significant differences in the same row are showed by different letters ($p<0.05$).
[φ]Fatty acids composition: $C_{16:0}$ (Palmitic Acid), $C_{16:1}$ (Palmitoleic Acid), $C_{17:0}$ (Margaric acid), $C_{17:1}$ (Heptadecenoic acid), $C_{18:0}$ (Stearic Acid), $C_{18:1}$ (Oleic Acid), $C_{18:2}$ (Linoleic Acid), $C_{18:3}$ (Linolenic Acid), $C_{20:0}$ (Arachidic Acid), $C_{20:1}$ (Gadoleic Acid), $C_{22:0}$ (Behenic acid)

Composition of volatile fraction of VOOs obtained is presented in Table 3. Among the volatiles present in virgin olive oil, those C6 and C5 compounds from `Lipoxygenase´ pathway [16] were measured. The concentration of C5 compounds did not show differences between HPU treatment and control. However, the VOO from HPU treatment showed higher C6 alcohols content than the control, whereas the C6 aldehydes and the sum of C6 compounds was found higher for control oils.

Table 3. Volatile composition (mg/kg olive oil) for VOO obtained from untreated (Control) and HPU treated (HPU) olive paste of 'Picual' cultivar.

Volatile Compounds	Control	HPU
Hexanal	0.16±0.04 a*	0.06±0.00 a
E-3-hexenal	0.11±0.01 a	0.12±0.00 a
Z-3-hexenal	1.35±0.09 a	1.12±0.02 a
Z-2-hexenal	0.10±0.01 a	0.09±0.00 a
E-2-hexenal	0.96±0.01 b	1.03±0.01 a
Hexyl acetate	0.02±0.00 a	0.01±0.00 a
Z-3-hexenyl acetate	0.21±0.00 a	0.20±0.00 a
E-2-hexenyl acetate	0.01±0.00 a	0.01±0.00 a
Hexanol	0.03±0.00 a	0.04±0.00 a
E-3-hexenol	0.01±0.00 a	0.01±0.00 a
Z-3-hexenol	0.24±0.00 b	0.28±0.01 a
E-2-hexenol	0.04±0.00 a	0.04±0.00 a
Z-2-hexenol	0.01±0.00 a	0.01±0.00 a
∑ C6 aldehydes	2.68±0.04 a	2.43±0.02 b
∑ C6 alcohols	0.32±0.00 b	0.37±0.01 a
∑ C6 esters	0.24±0.00 a	0.23±0.00 a
∑ C6 compounds	3.24±0.05 a	3.03±0.03 b
∑ C5 alcohols	0.91±0.14 a	0.78±0.04 a
∑ C5 Carbonyl	0.98±0.09 a	0.92±0.02 a
∑ C5 compounds	1.89±0.22 a	1.70±0.06 a
Total	5.13±0.27 a	4.73±0.09 a
hexanal/nonanal	7.25±1.71 a	2.52±0.13 a
hexanal/E-2-hexenal	0.16±0.04 a	0.06±0.00 a

*Significant differences in the same row are showed by different letters ($p<0.05$)

So E-2-hexenal (responsible of bitter, almonds, green, green apple-like, fatty, bitter almond like and cut grass odor), and Z-3-hexenol (responsible of banana, leaf-like, green-fruity and pungent odor) [17] were observed at significant higher content in VOO from HPU treatment. This trend indicates that the HPU application did not inhibit the enzymes activities of the `Lipoxygenase´ pathway.

In order to discard any oxidative alteration there are some ratios between volatiles considered markers of VOO oxidative status as hexanal/nonanal [17] and hexanal/E-2-hexenal [5]. VOO obtained from HPU treated olive paste did not show significant differences for both ratios comparing with those from control. These results indicated that the sonication treatment applied in the experiments did not cause any alteration on the autoxidation state of VOO.

Tocopherols are natural antioxidants present in virgin olive oil that have been included in a health claim by European Union [18]. The continuous HPU treatment of olive paste allowed to obtain VOO with higher Total Tocopherols content and α-tocopherol concentration (Table 4). These results are similar to those described by Clodoveo et al. [6] in batch conditions.

Regards to chlorophylls and carotenoids, HPU treatment produced an increase of their content in the oil of 86 % and 39 %, respectively (Table 4).

The phenolic compounds are important minor compounds in the evaluation of the quality of the VOO. They are strongly related to the VOO shelf life because of their antioxidant ability, have bioactive activities and are also responsible of pungent and bitter sensory attributes [19]. Furthermore they have been included in a specific health claim for virgin olive oil by European Union [18]. Total phenols showed lower values in the VOO from HPU treatments [4, 5, 6] although significant differences were not obtained. Similar trend was observed for oil bitterness [4, 5].

In addition to the total phenol content, composition of phenolic fraction was determined (Table 4). A significant decrease of hydroxytyrosol, tyrosol, secoiridoid derivatives (dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), dialdehydic form of elenolic acid linked to tyrosol (ρ-HPEA-EDA), aldehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EA) and aldehydic form of elenolic acid linked to tyrosol (ρ-HPEA-EA)) and Pinoresinol was observed for HPU treatment. The sum of biophenols showed a decrease of 38% in VOO from HPU treated olive pastes. Clodoveo et al. [6] explained this reduction of VOO phenolics by the enhancement of the oxygen action for the non-enzymatic oxidation and its influence on the endogenous enzymes such as `Polyphenol oxidase´, `Peroxidase´ and `β-

glucosidase´, as well as the orientation of the phenolics, with hydrophilic properties, to the air-oil interface.

Table 4. Minor compounds for VOO obtained from untreated (Control) and HPU treated (HPU) olive paste of 'Picual' cultivar. Effect on the nutritional parameters of the HPU pretreatment.

Nutritional parameters		Control	HPU
Pigments (mg/kg)	Carotenoid	1.96±0.06 b*	2.73±0.04 a
	Chlorophyll	1.83±0.09 b	3.40±0.04 a
Tocopherols (mg/kg)	α	305±2 b	316±0 a
	β	1±0 a	1±0 a
	γ	28±1 a	29±0 a
	TOTAL	334±3 b	346±0 a
Bitterness		0.27±0.01 a	0.27±0.01 a
Total Phenols content (mg/kg)		374±10 a	362±11 a
Phenolic Compounds (mg/kg)	Hydroxytyrosol	1.91±0.10 a	0.82±0.01 b
	Tyrosol	2.58±0.02 a	1.47±0.09 b
	Vainillic Acid	0.41±0.08 a	0.20±0.06 a
	Coumaric acid	0.60±0.04 a	0.52±0.01 a
	Ferulic Acid	0.84±0.37 a	0.42±0.06 a
	3,4-DHPEA-EDA	108.92±2.37 a	66.61±0.84 b
	p-HPEA-EDA	94.20±3.81 a	62.62±0.29 b
	Pinoresinol	1.90±0.11 a	1.39±0.11 b
	3,4-DHPEA-EA	43.83±1.83 a	24.18±0.84 b
	p-HPEA-EA	4.45±0.56 a	1.68±0.42 b
	Total	259.64±8.93 a	159.92±1.74 b

* Significant differences in the same row are showed by different letters ($p<0.05$)

4. Conclusions

The continuous HPU pretreatment of olive paste before malaxation showed a quick-heating to 28 ºC of olive paste comparing with the untreated olive paste that takes 20 minutes to reach the same temperature. The sonication pretreatment improved the industrial yield and extractability of VOO. No alteration of the VOO quality parameters

and volatile aromatic compounds were observed with the HPU pretreatment. The ultrasound treatments gave oils with significant higher contents of tocopherols, carotenoids and chlorophylls whereas phenolics and bitterness decreased. These results of olive paste HPU pretreatment, using a laboratory device integrated in the `Abencor´ extraction system, shown that it may be possible to introduce this technique in future works using a similar device at pilot plant scale.

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Chapter 4. Continuous conditioning of olive paste by high power ultrasounds: Response surface methodology to predict temperature and its effect on oil yield and virgin olive oil characteristics

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Continuous conditioning of olive paste by high power ultrasounds: Response surface methodology to predict temperature and its effect on oil yield and virgin olive oil characteristics

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Abstract

The purpose of this work is the study of the continuous conditioning of olive paste using High Power Ultrasounds (HPU) previous to malaxation, and its effect on extraction yield and Virgin Olive Oil (VOO) characteristics. For this purpose a laboratory scale device for HPU treatment experiments was developed.

The HPU induced an instantaneous heating of olive paste until 28 ºC whereas it took 20 to 30 minutes in the traditional malaxation condition. The temperature increase depended on olive characteristics, olive paste flow rate and HPU intensity. A response surface model was carried out to predict the olive paste temperature for the variables identified previously. The sonication treatment improved the oil yield and gave higher extractability than conventional malaxation. The HPU treatment did not cause alteration on VOO quality indexes and composition. Olive oil volatile compounds related with oxidation mechanisms showed lower concentration for VOOs from HPU treated olive paste.

Key words: High Power Ultrasound; Malaxation; Olive Paste Heating; Virgin Olive Oil; Industrial Yield.

Abbreviation: Virgin olive oil (VOO), High Power Ultrasounds (HPU), Nuclear Magnetic Resonance (NMR), Response surface methodology (RSM), Paste flow rate in the pipeline (Q), High Power Ultrasounds intensity (W), Fruit temperature (OT), Olive moisture (OM), Olive fat content (OF), Industrial yield (IY), Oil extractability index (OE)

1. Introduction

The olive paste malaxation is a fundamental step of the Virgin olive oil (VOO) extraction procedures, since let to reach high and satisfactory extraction yield (Aguilera, Beltran, Sanchez-Villasclaras, Uceda, & Jimenez, 2010). Also, it has several effects on the VOO quality parameters, nutritional and sensorial characteristics (Aguilera, Jimenez, Sanchez-Villasclaras, Uceda, & Beltran, 2015). The main objective of kneading is the coalescence of oil drops, formed during olive crushing, to form the oily continuous phase. This phenomenon depends on olive paste rheological characteristics, kneading operating conditions (time and temperature) and addition of technological coadjuvants. Previous studies showed that to obtain higher oil yield it was necessary to perform the olive paste kneading at higher temperature and prolonged time (Aguilera et al., 2010).

However, in order to avoid any undesirable changes in oil composition and quality, it was recommended malaxation at softer conditions than 28 ºC and 60 minutes. The main factor leading to increase the kneading time is because heating up olive paste to 28 ºC requires from 15 to 20 minutes in the thermo-malaxer (Jiménez, Beltrán, & Uceda, 2007). The olive paste heating in the malaxer takes place from the wall to the central axe. This energy transfer is strongly influenced by the characteristics of paste (rheological properties and composition which depend on the olive varieties and the fruit ripeness) and operating conditions (geometry of malaxer, blade rotation speed and olive paste residence time in the malaxer).

Recently High Power Ultrasounds (HPU) were proposed for olive paste preparation (Jiménez et al., 2007; Jiménez Márquez, Beltrán Maza, Uceda Ojeda, & Aguilera Herrera, 2006). Ultrasounds are defined as the sound waves frequencies, not audible for human ear, from 20 Hz up to 20 kHz (Mason, 1998). These ultrasonic waves are generally classified in power ultrasounds (20 kHz to 1 MHz) and diagnostic ultrasounds (higher than 1 MHz). There are two principal effects resulting of high power ultrasounds (HPU) propagation through a medium: physical and chemical (Mason, Paniwnyk, & Lorimer, 1996). The main physical effects of HPU application was the mechanical movement generated by high and low pressure cycles. The resulting mechanical and shear forces help to increase mass transfer and can also break the cell walls (Ashokkumar, 2015). The power ultrasounds are applied in various areas in the

food industry as surface cleaning, production of emulsion, acceleration of chemical reactions, extraction of aromas, marinating, drying and dehydration, microbial and enzyme inactivation, extraction of bioactive compounds, oil and protein extraction and some other applications (Chemat, Zill-e-Huma, & Khan, 2011; Mason et al., 1996; Patist & Bates, 2008).

First works on HPU application to the virgin olive oil extraction process were carried out by Jiménez et al. (2007; 2006) and consisted in the ultrasounds application, under discontinuous conditions, during the olive paste malaxation describing two major effects: a rapid heating of olive paste and the improvement of the oil extractability. However, the effect of HPU treatment on virgin olive oil quality parameters (free acidity value, peroxide value, K_{270} and K_{232}) was not significant. Nevertheless higher concentration of tocopherols, chlorophylls and carotenoids were observed in the oils from ultrasound treatment, whereas lower levels of bitterness and polyphenols were obtained. Most recently Clodoveo, Durante, and La Notte (2013), reported two different HPU treatments: at kneading, as olive paste pretreatment, and to the whole olives before crushing during washing. For both treatments, a quick olive paste heating and higher oil extractability were described. Oil quality parameters were not affect as observed (Jiménez et al., 2007; Jiménez Márquez et al., 2006). All these previous experiments were applied in batch conditions, under non continuous process, and performed in open conditions with atmosphere contact.

The aim of this manuscript was studying and modeling the olive paste heating using HPU continuous conditioning, previous to malaxation, at laboratory scale. Response surface methodology was applied for optimization and prediction of the olive paste heating and how it was affected by the variables tested. Furthermore, the effect of HPU on process yield and VOO quality and composition was determined. This study is part of the previous works to the development of a HPU treatment device at pilot plant scale.

2. Materials and methods

2.1. Plant material

Fresh and healthy olives (*Olea europaea* L.) of the olive cultivar `*Picual´* were harvested from trees grown under irrigation in the experimental olive orchard of IFAPA

Centro Venta del Llano in Mengibar, Jaén. Four harvesting dates were used, from November to February, at different olive ripening stages. Olive fruit characteristics (moisture and fat content), maturity index (Beltran, Uceda, Jimenez, & Aguilera, 2003) and harvesting dates are shown in Table 1.

Table 1 Effect of high power ultrasound treatment on olive paste temperature

Date	Olive paste composition			$\Delta T^{(1)}$ (ºC)	
	MI[2]	Fat (%)	Moisture (%)	Test	HPU
09/11	2.7	15.61	51.54	2.5	15.3
10/12	3.8	24.74	48.56	3.6	23.2
15/01	4.6	24.45	44.29	3.4	11.6
10/02	5.2	21.63	49.72	5.4	16

[1] ΔT temperature increase for treated and untreated paste

[2] MI: Olive fruit maturity index

2.1.1. Olive moisture

The olive fruit was crushed and the milled paste was desiccated at 105 ºC after weighted, these two later operations were repeated until obtain constant weight. The results were expressed in weight percentage.

2.1.2. Olive fat content

Oil content was measured, for the dried olive paste obtained from the olive moisture determination, using a Nuclear Magnetic Resonance (NMR) fat analyzer Minispec mq 20 (Bruker Analytik Gmbh). The NMR was previously calibrated and validated with Soxhlet extractor. The results were expressed on weight percent (on a fresh matter basis).

2.2. VOO extraction and HPU treatment apparatus

A laboratory scale device for HPU treatment was implemented as described in Fig. 1. This device was composed by three units: a rollers mill, a rectangular pipeline for olive paste transport equipped with three 40 kHz frequency piezoelectric transducers and a 150 W ultrasonic generator with an intensity regulator to power the transducers.

The olives were added in the roller mill and then flowed through the pipe until the output where the olive paste was collected for processing. The olive paste flow rate through the device was established by the olive fruit feed rate in the mill and measured for each conditions. After olive paste flowed through the device, it was taken at the output and VOO was extracted immediately at laboratory scale using the thermo-malaxer and the centrifuge of an Abencor system (MC2, Seville). Around 700 - 800 g of the olive paste were kneaded in the thermo-malaxer for the experimental conditions described for each experiment. After kneading, the olive paste was immediately centrifuged to separate oily must from solids. The liquid phases were recovered in a laboratory graduated cylinder and after settling, the volume of the oily phase was measured. The oil was filtered and stored at -24 ºC until analyses.

2.3. Experimental

2.3.1. HPU treatment for olive paste heating

In order to test the HPU device ability for olive paste heating the experiments were carried as follows: the paste flowed through the pipeline when the piezoelectric transducers reached 60 ºC using 100 % of the generator intensity. The olive paste flow rate required to reach a paste temperature around 28 ºC was comprised between 20 and 25 kg h^{-1}. The variation of temperature between olive fruits and the paste (treated and untreated) was taken at the device output (Fig. 1). The temperatures were measured for the olive fruit before device entrance and the olive paste after treatment through the device (Fig. 1). For these experiments the olives harvested at the four harvesting dates were used.

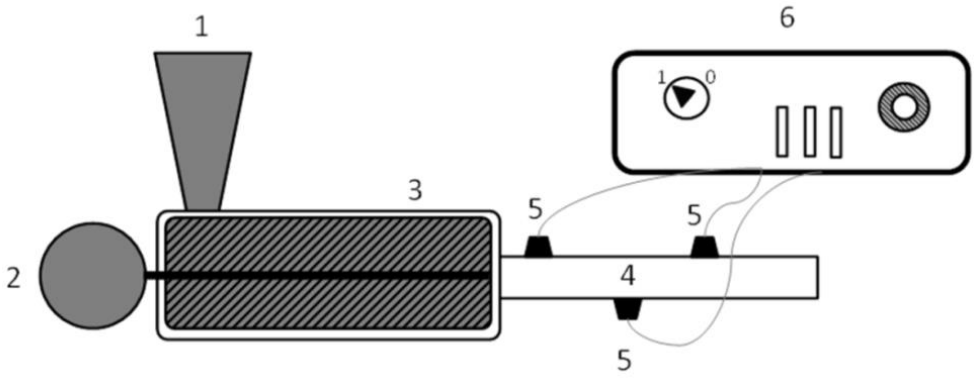

Fig. 1. High Power Ultrasound device for olive paste continuous treatment composed of: (1) Olives reception hopper, (2) mill motor, (3) rollers mill, (4) rectangular pipeline, (5) piezoelectric transducers and (6) ultrasonic generator.

2.3.2. Response surface methodology of olive paste heating

Because olive fruit characteristics may affect the HPU efficiency to increase the olive paste temperature a response surface methodology (RSM) was used to determine the effect of the olive fruit characteristics. The RSM consist in a collection of mathematical and statistical techniques useful for modeling and analysis of problems in which a response is influenced by several variables and the objective is to predict the relationship between the factor and the response to optimize this latter (Montgomery, 2005).

In this study, a Historical Data Design, using the software Design Expert (STAT-EASE®SOFTWARE, TRAINING, AND CONSULTING FOR DOE Design-Expert®, Version 8.0.1, Minneapolis), was performed to study the different factors affecting the olive paste heating with HPU treatment. Historical design is used to evaluate the previously obtained experimental results, this design allows importing the already exists data and without limitation of design factor number (Chen, Liu, Sun, & Huo, 2015; Kockal & Ozturan, 2011). The design consisted of 1 central point, 4 axial points and 4 corner points for all the harvesting date employed, 32 run were finally considered. The RSM model was built considering five numerical factors and simulation ranks showed in Table 2, where the independent variable with their high, middle and low levels were coded respectively 1, 0 and -1.

Table 2 Independent process variables and experimental design level used

Variables	Unit	Code	-1	0	1
Olive paste flow	kg h^{-1}	Q	16.9	95.8	95.8
HPU intensity	%	W	0	100	100
Fruit temperature before crushing	ºC	OT	12.8	18.6	18.6
Olive moisture	% weight/weight	OM	44.29	51.59	51.59
Olive fat content	% weight/weight	OF	15.61	24.75	24.75

These variables from HPU device were: paste flow rate in the pipeline (Q), HPU intensity (W) and some olive fruit characteristics such as fruit temperature before crushing (OT), olive moisture (OM) and olive fat content (OF). The experiment consisted in to measure the OPT after the HPU treatment (at the output of the HPU device) varying the Q and W for the four fruit harvesting dates, where the olives showed important differences in OT, OM and OF. Statistical validation of the model was established on the basis of Analysis of variance (ANOVA) and the 3D response graphics.

2.3.3. Effect of HPU on oil yield and extractability

Ultrasound treated (at 100 % power and 20 to 25 kg h^{-1} flow) and control untreated olive pastes were collected from the device output and then, malaxed at temperature lower than 28 ºC (European commission, 2012) for 30 minutes in the `Abencor´ system. Oil extracted, by duplicate, was measured for the industrial yield determination. The experiment was carried to the four harvesting dates.

The process industrial yield (IY) was defined as the percentage of oil obtained using the ABENCOR laboratory oil extraction system from 700 to 800 g of olives paste. The volume of olive oil was measured on graduated cylinder and the oil yield was calculated using the olive oil density of 0.915 kg/L Eq. (1). Results were expressed as percent.

$$IY = \left[\frac{(OV \times 0.915)}{WOP}\right] \times 100 \quad (1)$$

Where: OV : oil volume obtained from ABENCOR system (L)

WOP : weight of olive fruit paste (kg)

Furthermore, HPU application was tested as alternative or aid to olive paste malaxation. For this, the olive fruits collected for the last harvest date were used. Using the HPU application conditions described above (piezoelectric transducers reached 60 ºC using 100 % of the generator intensity), the olive pastes were taken from the device and kneaded at different malaxation times (0, 10, 20, 30 and 40 minutes) in the `Abencor´ thermo-malaxer at temperature below 28 ºC. As control treatment, olives were processed through the HPU device without sonication. The olive paste temperature was monitored and oil extractability was measured.

The oil extractability index (OE) was defined as the percentage of olive oil extracted with respect to the olive fat content (OF) (Beltran et al., 2003). Results Eq. (2) were expressed as percentage (%).

$$OE = \left({}^{IY}/_{OF}\right) \times 100 \quad (2)$$

2.3.4. Effect of HPU on oil quality and composition

In order to determine the impact of the olive paste conditioning with HPU on the VOO quality and characteristics the oil, from the four harvesting date of the industrial yield determination, was recovered, filtered and stored at -24 ºC until carrying out the next determinations:

2.3.4.1. Oil quality indices

Free acidity, peroxide values, and ultra-violet absorption at 232 and 270 nm (K_{232} and K_{270}) were measured following the analytical methods described in European Regulation EEC 2568/91 (European commission, 2013). Free acidity was expressed as percent of oleic acid, peroxide values were expressed as milli-equivalents of active oxygen per kilogram of oil (mEq O_2/kg); K_{232} and K_{270} extinction coefficients were calculated from absorption at 232 and 270 nm, respectively.

2.3.4.2. Oil minor compounds

2.3.4.2.1. Total phenol content

Phenolic compounds were extracted from an oil-in-hexane solution with methanol:water and their concentration was measured using Folin-Ciocalteau reagent and colorimetric measurement at 725 nm (Vázquez-Roncero, Janer del Valle, & Janer del Valle, 1973). Results were expressed as mg/kg of caffeic acid.

2.3.4.2.2. Pigment content

Carotenoid and chlorophyllic pigments were determined measuring the absorbance of olive oil weighed and dissolved in cyclohexane at 470 and 670 nm as described by Minguez-Mosquera, Rejano-Navarro, Gandul-Rojas, Sanchez-Gomez, and Garrido-Fernandez (1991). The results were expressed as mg/kg.

2.3.4.2.3. Phenolic composition

The Phenolic Compounds were extracted with methanol/water and the extracts were analyzed by RP-HPLC (Beltrán, Jiménez, Aguilera, & Uceda, 2000). Phenolic compounds were quantified at 280 nm using syringic acid as internal standard and the response factors determined by Mateos et al. (2001). The results were expressed as mg/kg.

2.3.4.2.4. VOO volatile compounds

Volatile compounds were analyzed by HS-SPME and GC-FID (Jiménez et al., 2007). Volatile compounds were identified by comparison of their retention times with those of pure standard substances. The results were expressed as mg/kg.

2.4. Data analysis

In the study of process efficiency of the HPU application and he VOO characteristics, the results were expressed as the mean ± standard deviation, the difference between treatments were determined by ANOVA with significance level of 0.05 was applied and performed using the Statistix 9 Data analysis software (Analytical Software, Tallahassee, Florida, USA).

3. Results and discussion

3.1. HPU treatment for olive paste heating

The olive paste preparation step, as described in previous sections, is an important step in the VOO elaboration process since an optimal kneading allows to get better industrial yields and modulating the VOO characteristics. Malaxation temperature plays a key role on both aspects. Higher temperature causes VOO viscosity reduction (Gila, Jiménez, Beltrán, & Romero, 2015), better oil droplet coalescence and then, formation of a continuous oily phase.

The continuous application of HPU showed two principal effects on the olive paste, a rapid increase of olive paste temperature and the improvement of VOO extractability as described for laboratory scale conditions (Jiménez et al., 2007). Table 1 showed the olive paste temperature variation after treatments and the extraction oil yield obtained for the four fruit harvesting dates.

The olive paste without HPU treatment displayed a slight temperature increase after fruit crushing, between 2.5 ºC and 5.4 ºC. This increase can be explained by the friction phenomenon induced in the crusher of the device.

HPU treatment produced an instantaneous temperature raise in the olive paste, achieving higher values than that untreated paste (from 11.6 ºC to 23.2 ºC). This heating effect allowed to get quickly an olive paste temperature around 28 ºC (European commission, 2012), from an olive fruit temperature ranging between 10 ºC and 15 ºC, depending on the harvesting date.

Therefore, HPU treatment gave a faster (<3 minutes) and efficient olive paste heating. This technology let to reduce the malaxation time the process time with the elimination of the olive paste preheating time in the conventional malaxer. As for other new techniques introduced for olive paste heating, like flash heating (Esposto et al., 2013) and microwave treatment (Leone, Tamborrino, Romaniello, Zagaria, & Sabella, 2014), the device proposed in this work let its integration in series between the crusher and the malaxer. This aspect confirms the viability of HPU treatment for its application in an industrial mill. The other aspects making possible the use of HPU at larger scale are the high energy efficiency of the current systems (ultrasonic generators and sonotrodes),

good installation adjustability, competitive energy costs, low maintenance costs and environmental friendly (Patist & Bates, 2008).

3.2. Response surface methodology modeling of HPU olive paste heating

The olive paste heating modeling using the HPU device was performed by RSM considering the olive paste temperature as response. The process variables studied for RSM were Q, W, OT, OM and OF (Table 2).

According to the statistical approach adopted, different models were obtained depending on the factors affecting to the paste heating. In Table 3 are shown the results for the RSM models obtained.

Table 3 Model statistical summary of responses for selection of suitable predictive model

RSM model factor					Response for olive paste temperature		
Olive paste flow	Ultrasound power	Olives temperature	Olives moisture content	Olives fat conten	RSM model	R^2-Adjusted	R^2-Predicted
X	X	---	---	---	Lineal	0.3084	0.2711
X	X	X	---	---	Quadratic	0.4089	0.3522
X	X	---	X	---	Cubic	0.9360	0.8611
X	X	---	---	X	Cubic	0.9221	0.8707
X	X	X	X	---	Quadratic	0.7984	0.6823
X	X	X	---	X	2FI	0.8412	0.8122
X	X	---	X	X	2FI	0.9035	0.8482
X	X	X	X	X	2FI	0.9332	0.8846

R^2-Adjusted= adjusted multiple correlation coefficient
R^2-Predicted= predicted residual sum of squares

These RSM models were performed using the two principal factors, Q and W, and the combination of the factors. The response of the RSM models was the olive paste temperature after treatment. The goodness of the model fit was determined by the Predictive-R^2, where the model having the highest value of Predictive-R^2 was considered as the best.

The results of Predictive-R^2 showed as olive paste flow rate and HPU intensity were not good enough to predict the olive paste temperature OPT (Predictive-R^2< 0.5), since olive fat content had a considerable effect on its prediction (Predictive–R^2 = 0.8707). The best fit was achieved by a two factor interaction (2FI) RSM model including all the process parameters (Q, W, OT, OM and OF) that gave a Predictive–R^2 of 0.8846 and Adjusted–R^2 of 0.9332.

Ideally, the model error (residuals) will consist of normally distributed random variation from the experimental process. Fig. 2 shows the normal probability plots of the residuals for the OPT, which is a diagnostic tool to assess the model validity. The points narrowly scattered around a straight line indicates that the residuals follow a normal distribution and the derived OPT model will not be improved by a change in the transformation, and thus, the experiments are valid for the design of experiment work.

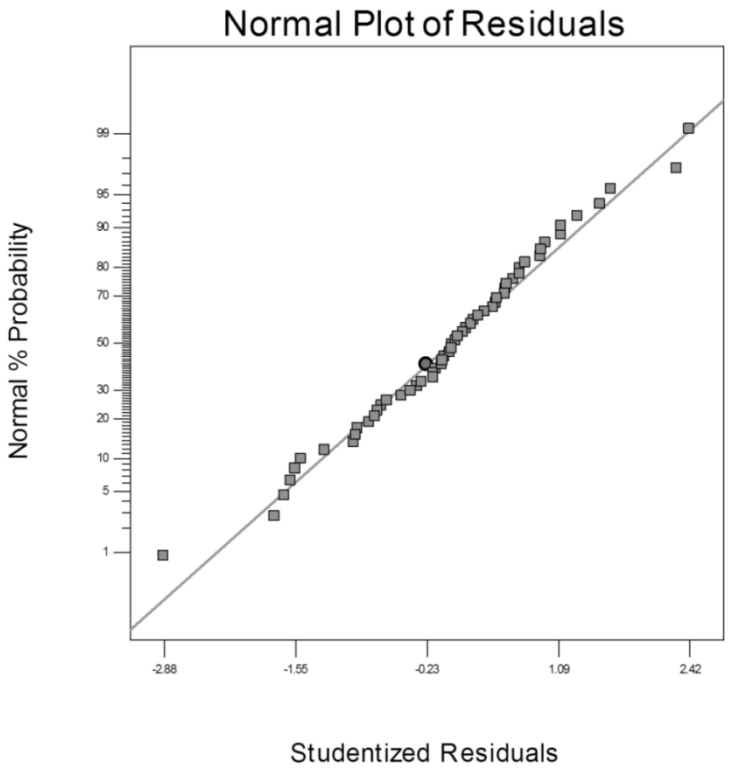

Fig. 2. Normal probability plots of residuals for the OPT

The analysis of variance of the 2FI RSM model (Table 4) showed that the regression model was significant for a p-value<0.0001. The Model F-value of 52.21 involves that the model is significant. There is only a 0.01% chance that the F-Value occurs due to noise. The significance of each model term was determined based on P-values less than 0.05. The significant terms of the model were Q, W and OT and the interaction terms Q*W, Q*OM, O*OF, W*OM, W*OF, OT*OF and OM*OF (Table 4), the rest of terms were not considered in the model. The equation of the 2 FI model Eq. (3), generated by the software, was as follows:

$$OPT = 21.27 - 2.45\,Q + 4.02\,W + 6.48\,OT - 1.48\,(Q \times W) + 1.35\,(Q \times OM) + 1.20\,(Q \times OF) - 2.10(W \times OM) - 4.17\,(W \times OF) - 7.93\,(OT \times OF) - 5.01\,(OM \times OF) \quad (3)$$

Table 4 Analysis of variance table for measured response of 2FI model

Source	Sum of squares	Degrees of freedom	Mean square	F-value	P-value
Model	789.82	15	52.65	52.21	<0.0001
Q	26.5247	1	26.5247	26.30092	< 0.0001
W	68.96633	1	68.96633	68.38449	< 0.0001
OT	5.565744	1	5.565744	5.518788	0.0238
OM	3.59609	1	3.59609	3.565751	0.0663
OF	0.029272	1	0.029272	0.029025	0.8656
Q W	20.44575	1	20.44575	20.27326	< 0.0001
Q OT	0.747439	1	0.747439	0.741133	0.3944
Q OM	6.430999	1	6.430999	6.376743	0.0156
Q OF	5.098799	1	5.098799	5.055783	0.0301
W OT	2.634767	1	2.634767	2.612538	0.1139
W OM	10.79137	1	10.79137	10.70033	0.0022
W OF	53.84781	1	53.84781	53.39352	< 0.0001
OT OM	3.106057	1	3.106057	3.079852	0.0869
OT OF	9.18	1	9.18	9.11	0.0044
OM OF	10.81	1	10.81	10.72	0.0022

Fig. 3 shows the RSM graphic of Eq. 3 as a function of ultrasound intensity and olive paste flow rate. The graphics showed how olive fruit characteristics affected to the olive paste flow and HPU intensity required to achieve that recommended temperature for an

efficient olive paste malaxation at 28 ºC. Olive fruit characteristics showed important differences between harvesting dates. The olive paste dry matter content remained constant during fruit ripening whereas moisture and oil content varied during maturation, achieving a great influence on HPU effectiveness.

In the early `veraison´ ripening stage, the olive moisture was higher and the fat content lower (Fig. 3a). Ultrasound waves diffusion within the paste was improved by its moisture and helped to its heating. However olive fruit with low moisture, as occurring during fruit ripening, lowered the ultrasound wave diffusion producing a less efficient olive paste heating (Fig. 3b, 3c). The olive paste heating by HPU can be explained by the cavitation phenomenon generated by the ultrasonic waves where the energy of expansion and reduction of the vapor bubbles, formed during the cavitation phenomenon, is dissipated in the medium. In this way, during the experiment the propagation of energy was aided by the water present in the olive paste.

Furthermore, for each fruit ripening stage the olive paste temperature variation was regulated by the paste flow rate and the intensity of HPU applied. A low olive paste flow and 100% of HPU intensity gave a paste temperature over than 28 ºC, whereas for higher paste flow rate the temperature raise was lower. Therefore, for early `veraison´ olives, a better heating was registered with higher paste flow rate than for ripe fruits because of the greater moisture that helped to ultrasound wave propagation (Fig. 3).

In order to verify the model, a comparison between the predicted olive paste temperature from the model and the temperature measured in the experiments was performed. The experiments consisted in continuous HPU treatment of olive paste, using olive fruits of the last harvesting date, through the device varying the olive paste flow; the olive paste temperature was measured continuously during 15 minutes at the output of the device. Experimental temperatures were compared with those predicted by the model using the variables included in the experiment. F-test with $p < 0.05$ was applied to determine the level of precision of the model.

The results for comparison of predicted versus experimental temperatures were presented in Fig. 4. The curves of the predicted and experimental temperatures showed the same shape depending on the olive paste flow rate.

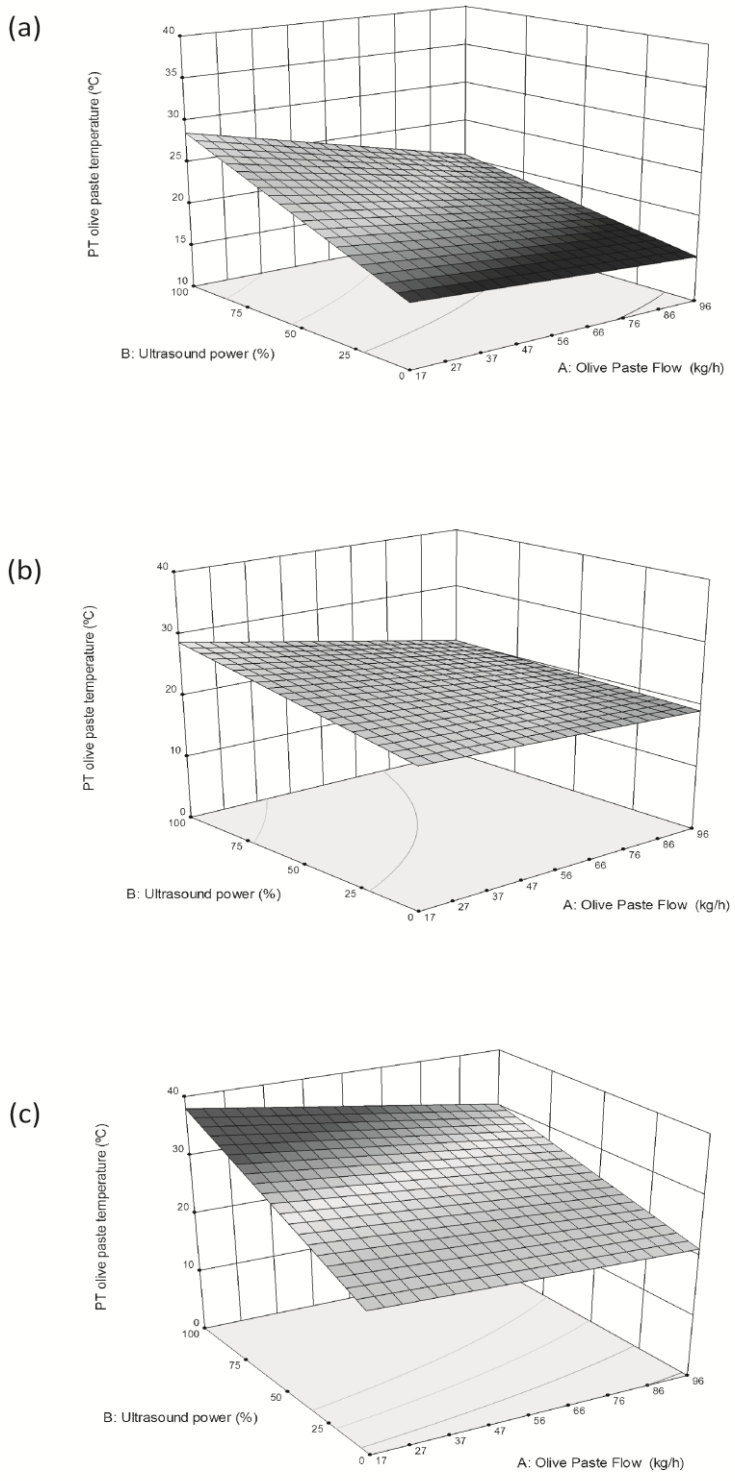

Fig. 3. 3D contour plots showing the effect of olive fruit composition to the olive paste temperature with olive fruit temperature 15.5 ºC, (a) OM=51.59% and OF=15.73%, (b) OM=47.74% and OF=20.06%, (c) OM=44.29% and OF=24.50%.

The F-test applied demonstrated that there were not significant differences between both of them, being $F_{experiment} = 2.179$ lower than $F_{theoretical} = 2.9338$, for a significance level $\alpha = 0.01$. Thus, the predicted values for paste temperature were adequately precise with respect to those obtained in the experiment. This model shows that the HPU treatment can be easily scaled to a device for industrial mill and its automatization using sensors and actuators.

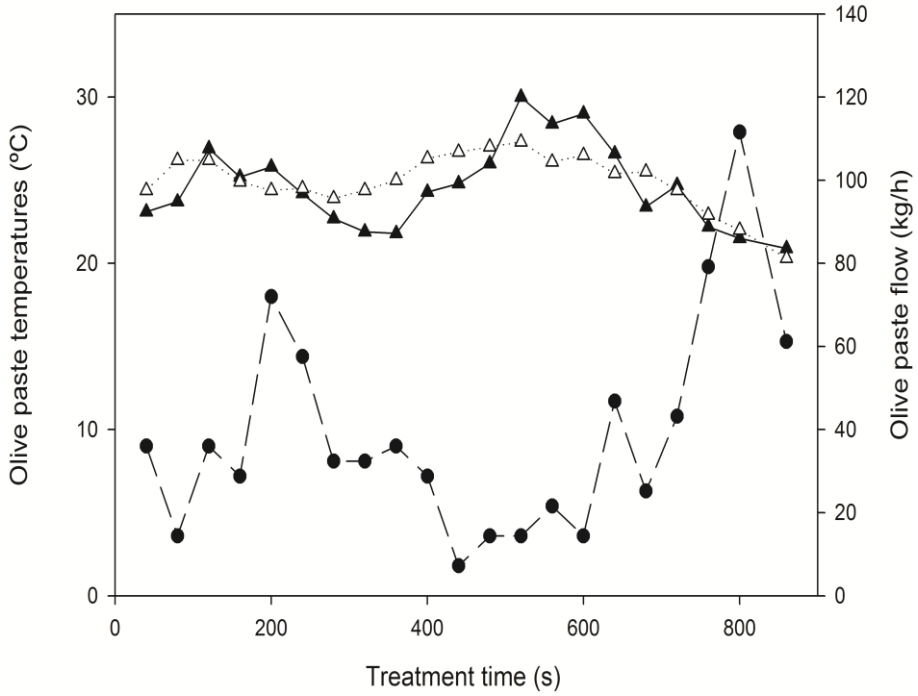

Fig. 4. Model adequacy checking using experimental olive paste temperature (...Δ...) versus predicted temperature (–▲–) taken continuously during 15 minutes of treatment, of 100% HPU intensity in function of Olive paste flow (--●--).

3.3. Effect of HPU continuous application on the oil extraction yield

For the evaluation of the HPU effect on oil yield, experiment with the four fruit harvesting dates were carried out. The increase of temperature registered for the HPU treated paste allowed an effective kneading since the olive paste could be malaxed for more time at the optimal temperature (Table 1). Previous works on HPU application during the olive paste kneading gave higher oil extraction yield (Clodoveo et al., 2013; Jiménez et al., 2007; Jiménez Márquez et al., 2006).

Therefore, the extraction process efficiency was enhanced because of the paste heating. The increase of oil yield varied from 0.81 % to 2.02 % for unripe and overripe olive fruits respectively (Table 5), obtaining significant differences between treatments only for the last harvesting date.

Table 5 Effect of high power ultrasound treatment, before malaxing, on process yield

Date	Yield efficiency (%)[(1)]	
	Test	HPU
09/11	11.06±0.41* a	12.47±1.97 a
10/12	16.88±0.04 a	17.69±0.33 a
15/01	17.50±0.47 a	18.71±0.31 a
10/02	9.21±0.18 b	11.23±0.20 a

* Mean value ± standard deviation

Means in the same row with different letter are significantly different according to ANOVA at significance level of 0.05.

The oil extractability and the olive paste temperature for different malaxation times are shown in Fig. 5. Oil extractability was higher with HPU treatment for the malaxation time assayed (10, 20, 30 and 40 minutes). The higher extractability using HPU treatment can be explained because the olive paste achieved instantaneously, from the beginning of the malaxation, the optimal kneading temperature (28 ºC) whereas it took 20 minutes for the conventional malaxation without HPU treatment.

Furthermore oil extractability for HPU treated olive paste malaxed for 20 minutes was higher than that of the untreated olive paste malaxed for 40 minutes. According to these results, HPU treatment could allow reducing the malaxation time by half, avoiding the olive paste preheating.

The oil yield improvement by the HPU treatment may be explained by the cavitation phenomenon too, where the microbubbles collapse leads to the formation of `sponge effect´ (Feng, Barbosa-Cánovas, & Weiss, 2011) in the conditioned olive paste. The energy liberated improved the oil diffusion phenomenon and viscosity, hence produced a fluidity raise of oil through the microgels of the olive paste. Therefore HPU treatment allowed to get a better extractability yield comparing with conventional malaxation.

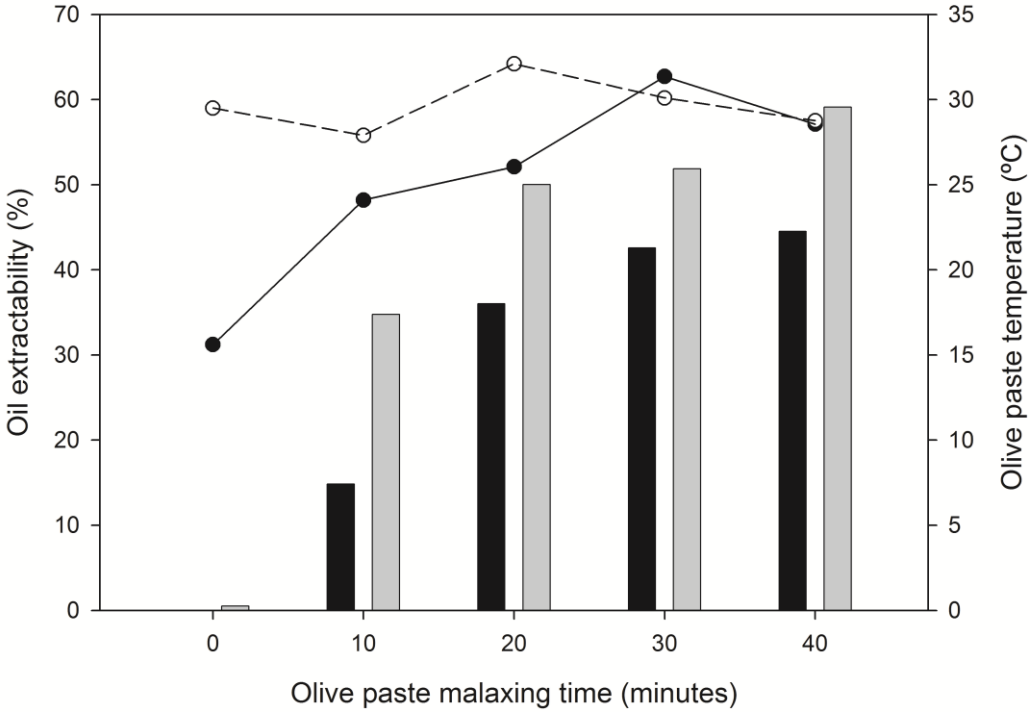

Fig. 5 Monitoring of Temperature of paste with HPU treatment (--○--) versus Temperature of paste without HPU treatment (—●—) and the Extractability for untreated olive paste (▮) against the olive paste treated with HPU (▮) Olive oil Extractability at different malaxation time (0, 10, 20, 30 and 40 minutes).

3.4. Effect of HPU continuous application on the VOO quality and composition

VOO quality indices and composition for the assays carried out on four harvesting dates are presented in Table 6a. All the oils were classified as `extra virgin´ for the parameters analyzed since the values obtained were into the limits established by the European Union Commission regulation (European commission, 2013).

Even although cavitation phenomenon can be registered during HPU treatments, all the parameters related with oxidative alteration (Peroxide value, K_{232} and K_{270}) did not change significantly.

The minor compounds concentration in the virgin olive oils obtained in the experiments are reported in Table 6a. HPU treatments showed an improvement of chlorophylls and carotenoids content during the harvesting dates studied, with significant differences only for carotenoids in December, whereas for chlorophylls in February. These results

were in agreement with results available in literature (Clodoveo et al., 2013; Jiménez et al., 2007).

Phenolics are related with stability, healthy and sensory properties of VOO. The ultrasounds treatment induced a decrease of total phenol content as described by Jiménez et al. (2007). These variations between treatments were only statically significant for the first harvesting date.

Furthermore, the study of the phenolic profile, (Table 6a) also showed a decrease of phenolics for the VOO obtained from HPU conditioned olive paste. The individual phenols identified were: hydroxytyrosol, tyrosol, dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), dialdehydic form of elenolic acid linked to tyrosol (ρ-HPEA-EDA), pinoresinol, aldehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EA) and aldehydic form of elenolic acid linked to tyrosol (ρ-HPEA-EA). The decrease of phenolic compounds was significant for the first and second harvesting dates, especially for the secoiridoids derivatives. This reduction of phenolics can be explained by the temperature increase and the possible effect of the sonication treatment on the endogenous enzyme of olive paste (β-glucosidase, Polyphenoloxidase and Peroxidase) (Clodoveo et al., 2013).

The main volatiles identified were Hexanal and (E)-2-Hexenal that contribute to green, apple bitter and almonds note (Angerosa et al., 2004). The HPU treatment did not affect significantly to their concentration. Hexanal can be also formed from autoxidation process, and then give the 'rancid' off-flavor (Kalua, Bedgood, Bishop, & Prenzler, 2006). Oxidation of olive oil could leads to a fast increase of Hexanal and the decrease of (E)-2-Hexenal. So a ratio of Hexanal/(E)-2-Hexenal can be considered as indicator of oil oxidation level (Jiménez et al., 2007). VOO from HPU treatments showed a lower value of Hexanal/(E)-2-Hexenal ratio compared with control VOOs, thus oxidation was not induced by sonication treatment. Other components indicating the oxidative degradation of VOO are the Octanol, 2,4-Heptadienal, Nonenal, Nonanal and Decanal (Kalua et al., 2007; Vichi, Pizzale, Conte, Buxaderas, & López-Tamames, 2003). In these experiments, VOO from HPU treatments showed a lower value for these compounds than control, confirming that sonication treatment did not alter the VOO oxidative status.

Table 6 a Quality indices and composition of olive oil from olive paste treated with HPU (HPUt) and untreated olive paste (Control) for four harvesting dates.

Analytical parameters		Sample	Sample harvesting date (+)			
			09-Nov	10-Dec	15-Jan	10-feb
Quality indices	Free acidity (%)	Control	0.12 ± 0.00 a	0.23 ± 0.00 a	0.23 ± 0.00 a	0.12 ± 0.00 a
		HPUt	0.12 ± 0.00 a	0.23 ± 0.00 a	0.18 ± 0.00 a	0.12 ± 0.00 a
	Peroxide value (meq O2/kg)	Control	2.17 ± 0.10 b	2.21 ± 0.10 a	1.35 ± 0.06 b	2.09 ± 0.44 a
		HPUt	3.31 ± 0.08 a	2.28 ± 0.05 a	1.61 ± 0.04 a	1.50 ± 0.16 a
	k232	Control	1.57 ± 0.02 a	1.74 ± 0.02 a	1.58 ± 0.02 a	1.53 ± 0.02 a
		HPUt	1.52 ± 0.06 a	1.79 ± 0.07 a	1.58 ± 0.06 a	1.56 ± 0.02 a
	k270	Control	0.14 ± 0.01 a	0.18 ± 0.01 a	0.14 ± 0.01 a	0.13 ± 0.00 a
		HPUt	0.13 ± 0.02 a	0.20 ± 0.03 a	0.14 ± 0.02 a	0.14 ± 0.01 a
VOO Composition	Total Polyphenols (mg/kg)	Control	501 ± 3 a	956 ± 5 a	663 ± 4 a	505 ± 4 a
		HPUt	389 ± 27 b	887 ± 61 a	622 ± 43 a	519 ± 14 a
	Carotenoids (mg/kg)	Control	3.5 ± 0.1 a	4.6 ± 0.2 b	4.8 ± 0.2 a	2.4 ± 0.1 a
		HPUt	4.9 ± 1.3 a	5.8 ± 0.2 a	4.8 ± 0.2 a	2.7 ± 0.3 a
	Chlorophylls (mg/kg)	Control	4.1 ± 0.4 a	3.9 ± 0.3 a	3.4 ± 0.3 a	1.1 ± 0.1 b
		HPUt	7.4 ± 3.7 a	5.2 ± 0.5 a	3.6 ± 0.3 a	1.7 ± 0.0 a
Phenolic compounds (mg/kg)	Hydroxytyrosol	Control	3 ± 0 a	10 ± 0 a	6 ± 0 a	6 ± 1 a
		HPUt	3 ± 1 a	2 ± 0 b	5 ± 1 a	2 ± 1 a
	Tyrosol	Control	3 ± 1 a	6 ± 2 a	3 ± 1 a	4 ± 1 a
		HPUt	3 ± 1 a	3 ± 1 a	4 ± 1 a	3 ± 1 a
	3,4-DHPEA-EDA	Control	181 ± 3 a	702 ± 11 a	526 ± 8 a	436 ± 13 a
		HPUt	139 ± 5 b	557 ± 20 b	536 ± 19 a	458 ± 5 a
	ρ-HPEA-EDA	Control	99 ± 5 a	234 ± 12 a	256 ± 13 a	157 ± 11 a
		HPUt	79 ± 3 b	205 ± 7 a	238 ± 9 a	179 ± 3 a
	Pinoresinol	Control	6 ± 1 a	18 ± 2 a	6 ± 1 a	3 ± 1 a
		HPUt	5 ± 1 a	11 ± 2 a	7 ± 1 a	2 ± 0 a
	3,4-DHPEA-EA	Control	46 ± 1 a	321 ± 5 b	137 ± 2 a	168 ± 6 a
		HPUt	45 ± 1 a	378 ± 6 a	138 ± 2 a	183 ± 3 a
	ρ-HPEA-EA	Control	13 ± 0 a	22 ± 0 a	7 ± 0 a	9 ± 1 a
		HPUt	13 ± 1 a	22 ± 0 a	10 ± 0 a	10 ± 1 a

Means in the same row with different letter are significantly different according to ANOVA at significance level of 0.05.
+ Nov=November; Dec=December; Jan=January; Feb=February

Table 6 b Quality indices and composition of olive oil from olive paste treated with HPU (HPUt) and untreated olive paste (Control) for four harvesting dates.

	Analytical parameters	Sample	Sample harvesting date (+)			
			09-Nov	10-Dec	15-Jan	10-feb
Volatile compounds (µg/kg)	PENTANAL	Control	4.21 ± 0.98 a	4.02 ± 0.81 a	4.44 ± 0.05 a	2.93 ± 0.21 a
		HPUt	6.12 ± 0.18 a	3.43 ± 0.54 a	4.20 ± 0.11 a	2.10 ± 0.79 a
	HEXANAL	Control	162.12 ± 4.14 a	38.61 ± 4.64 a	61.55 ± 0.88 a	36.89 ± 1.16 a
		HPUt	110.07 ± 1.75 b	40.96 ± 0.54 a	60.38 ± 0.63 a	34.59 ± 0.47 a
	(E)-2-Hexenal	Control	26.29 ± 0.75 a	7.00 ± 0.98 a	10.46 ± 0.02 a	10.51 ± 0.23 b
		HPUt	24.76 ± 0.31 a	8.37 ± 0.05 a	10.60 ± 0.34 a	12.46 ± 0.07 a
	HEXANAL/ (E)-2-Hexenal	Control	6.17 ± 0.02 a	5.52 ± 0.11 a	5.89 ± 0.07 a	3.51 ± 0.03 a
		HPUt	4.45 ± 0.01 b	4.90 ± 0.09 b	5.70 ± 0.12 a	2.78 ± 0.02 b
	(E)-2-Hexen-1-ol	Control	2.88 ± 0.05 a	1.23 ± 0.16 a	0.56 ± 0.00 a	0.61 ± 0.01 a
		HPUt	2.54 ± 0.06 b	1.40 ± 0.06 a	0.52 ± 0.02 a	0.50 ± 0.01 b
	OCTANOL	Control	3.86 ± 0.04 a	0.98 ± 0.13 a	2.23 ± 0.01 a	1.98 ± 0.04 a
		HPUt	3.74 ± 0.01 a	0.86 ± 0.00 a	2.10 ± 0.07 a	1.67 ± 0.04 b
	2,4-Heptadienal	Control	9.60 ± 0.05 a	8.37 ± 1.00 a	3.76 ± 0.03 a	1.61 ± 0.07 a
		HPUt	8.28 ± 0.00 b	8.60 ± 0.01 a	3.46 ± 0.10 a	1.52 ± 0.01 b
	Nonanal	Control	2.28 ± 0.30 a	2.01 ± 0.58 a	1.74 ± 0.32 a	5.43 ± 0.01 a
		HPUt	1.95 ± 0.29 a	1.91 ± 0.23 a	1.65 ± 0.18 a	4.26 ± 0.49 a
	Nonenal	Control	0.72 ± 0.01 a	0.58 ± 0.13 a	0.52 ± 0.01 a	0.51 ± 0.05 a
		HPUt	0.64 ± 0.00 b	0.52 ± 0.02 a	0.50 ± 0.02 a	0.44 ± 0.04 a
	Decanal	Control	11.18 ± 0.18 a	13.50 ± 0.11 b	9.45 ± 0.11 a	8.06 ± 0.05 a
		HPUt	11.33 ± 0.22 a	13.99 ± 0.08 a	8.96 ± 0.07 b	7.23 ± 0.01 b

Means in the same row with different letter are significantly different according to ANOVA at significance level of 0.05.
+ Nov=November; Dec=December; Jan=January; Feb=February

4. Conclusions

The continuous HPU conditioning of olive paste, allowed a rapid heating of olive paste until achieving the optimal malaxation temperature. The heating phenomenon induced by HPU was strongly related to the fruit characteristics and composition. The olive paste temperature control, through the device, was regulated by the paste flow rate and HPU intensity control. The effect of variables on paste temperature showed that the response behaved as a 2FI function. The coded equation and the 3D plots demonstrated as the main factor affecting to the OPT is the olive temperature before crushing, the flow rate through the device and the HPU intensity.

The HPU treatment leads to a significant improvement of the olive oil extractability and industrial yield. The HPU conditioning can reduce the malaxation step duration by half. This treatment did not cause alteration on VOO quality indexes and composition. Furthermore the HPU treatment tends to reduce the phenolic content of the oil. Regarding to the volatile compounds from oxidation mechanisms, the VOOs from HPU conditioned olive paste showed lower concentration.

In view of these results, the HPU treatment device proposed can be scaled for its application at industrial scale and can be an efficient tool to improve the malaxation of olive paste.

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Part 2: Design of a system for high power ultrasound application at pilot plant scale

Chapter 5. Fundaments and design

Contents

1. Introduction

High power ultrasound is a new technology considered safe and environmentally friendly in its application but is also efficient and economical. The high power ultrasound low frequency (16 kHz to 100 kHz) has a large number of applications in food processing (Mason et al., 1996; 2015; Ashokkumar, 2015). They can be applied to existing processes to reduce or eliminate chemicals, mechanical and/or heat application used in industrial food processing. Furthethermore, they can be used to improve existing process or to the development of new process steps (Mason et al., 1996; 2015; Ashokkumar, 2015). Most of the advances in this field was known as sonochemistry and has been carried out only at laboratory level with little work being scaled up to industrial scale (Patist and Bates, 2008).

During their application, the passage of ultrasound waves through a liquid medium can create cavitating conditions, when the intensity and frequency of irradiations are suitably adjusted. The ultrasound waves propagation induces a cyclic succession of expansion (rarefaction) and compression phases; when pressure amplitude exceeds the tensile strength of liquid in the rarefaction regions, small vapor-filled voids called cavitation bubbles are formed. Cavitation is the phenomena where bubbles or cavities are formed, grew, and subsequently collapsed very rapidly (typically in microseconds), releasing locally a large amount of energy (Mason et al., 1996). Cavitation occurs simultaneously at multiple locations in the medium and generates locally very high temperatures and pressures, also it generates local turbulences and an intense liquid circulation in the medium (Suslick, 1990). The acoustic cavitation can lead to a chemical changes commonly known as sonochemistry. The sonochemical reactions occur in a contactor, the sonochemical systems provide a method for a discrete energy input at the site of physical or chemical transformation, leading to significant process intensification.

Cavitation can be classified as transient or stable cavitation (Mason, 1998; Patist and Bates, 2008; Bermúdez-Aguirre et al., 2011) depending on: the maximum radius reached (resonant size), lifetime of the bubble in the liquid and the pattern of cavity collapse. Some variables must be considered to classify the cavitation type: the operating parameters and constitution of the medium. The type of cavitation also determine the energy requirements for the process; hence, it is important to identify the

controlling intensification mechanism for the specific application so that the collapse conditions can be suitably tailored, maximizing the desired effects while minimizing energy consumption.

The transient cavitation consists, in a large and quick variation of the bubble size to eventually becomes unstable after a number of cycles. In this case the size of a bubble increases drastically from tens to hundreds of times the equilibrium radius before its violent collapses during the compression cycle of ultrasonic wave. This phenomenon is generally obtained in gaseous or vapor-filled cavities produced for an ultrasonic intensity over 10 W/cm^2. In contrast, stable cavitation involves the formation of smaller bubbles containing gas (and a very small amount of vapor) that oscillate around the equilibrium size for many acoustic cycles at ultrasonic intensity in a range from 1 to 3 W/cm^2 (Patist and Bates, 2008; Bermúdez-Aguirre et al., 2011; Salazar et al., 2012; Gogate and Pandit, 2015).

The cavitation is the main sonochemical and for this reason its important to make it in consideration for system design. Gogate and Pandit (2015) described two principal aspects to consider defining the zone of influence in the system. First, the maximum size of cavitation bubbles reached before a violent collapse since it determines the magnitude of the pressure temperature pulse generated at the collapse time. Second, the persistance time of the cavitation bubble that determines the distance traveled by the cavity from the point where it was generated, it lets to measure the active volume of the device. Both aspects should be maximized and suitably adjusted for high power application in food processing.

Some process parameters should be considered in the application of high power ultrasound for food processing: frequency (f, or angular frequency $\omega = 2\pi f$), power amplitude (denoted by A or P), treatment temperature, treatment pressure and treatment time. These parameters have to be adjusted by the ultrasonic generation equipment and controlled by the user. This optimisation is based on results from experiments and considering the effects desired (Mason et al., 1996; Patist and Bates, 2008; Bermúdez-Aguirre et al., 2011; Salazar et al., 2012; Gogate and Pandit, 2015).

2. High power ultrasound parameters

2.1. Power and energy

The power represents the strength of the ultrasound treatment. This treatment is accomplished by passing an ultrasonic wave through the medium; therefore, the power of the treatment is determined by the amount of energy entering in the medium as well as the area of transducers used for transferring this energy, which determines the irradiation intensity (Bermúdez-Aguirre et al., 2011; Salazar et al., 2012; Gogate and Pandit, 2015).

Some studies of high power ultrasound treatment showed that the power causes greater alterations in the material, for these reasons it is important to set a maximum power limit depending on the properties of the medium considered (Bermúdez-Aguirre et al., 2011). Therefore, for a device design the researchers try to find the minimal power to process the food as desired and preserving the characteristics, nutritional composition and organoleptic properties of food (Patist and Bates, 2008; Mason et al., 2015).

The development of continuous sonochemical system involves understanding the hydrodynamic behavior of the medium since it depends on the power density. Furthermore, the choice of the equipment power rating, also determines the power dissipated in the bulk of medium and thereby the available power to create cavitation conditions. The power dissipation can be measured by a calorimetric approach based on the quantification of the changes in the medium temperature depending on the irradiation time and power level. Other important parameter for power dissipation is the use of a single or a number of ultrasound transducers.

The large scale operations may be limited by the dissipation of the entire power in a given system volume when using a single transducer because of the limitations of construction materials and the concentration of cavitational activity near to the application surface, requiring then more irradiating surfaces. The total area of ultrasound transducers should be distributed using multiple transducers. The number of transducers depends on the operating volume, dimensions of the transducer and the power applied (Gogate and Pandit, 2015).

All these factors depend on the type of HPU application in the process, their optimization need to be established by laboratory level investigation previous to

determine the operating conditions for a larger scale application, including larger areas of irradiation to ensure lower irradiation intensity (Gogate and Pandit, 2015). Establishing the adequate power input helps to optimize the operating costs for a physic-chemical system.

High power ultrasound intensity can be expressed as the actual power output per the application surface area of the sonotrode (W/cm^2), where the power input is the product of power output (kW) and the time of treatment. The sonication treatment time is directly related to the flow rate through the ultrasonic device (L/h or kg/h). Patist and Bates (2008) showed a general relationship between flow rate for a liquid medium and the energy for several ultrasonic applications (Figure 5.1).

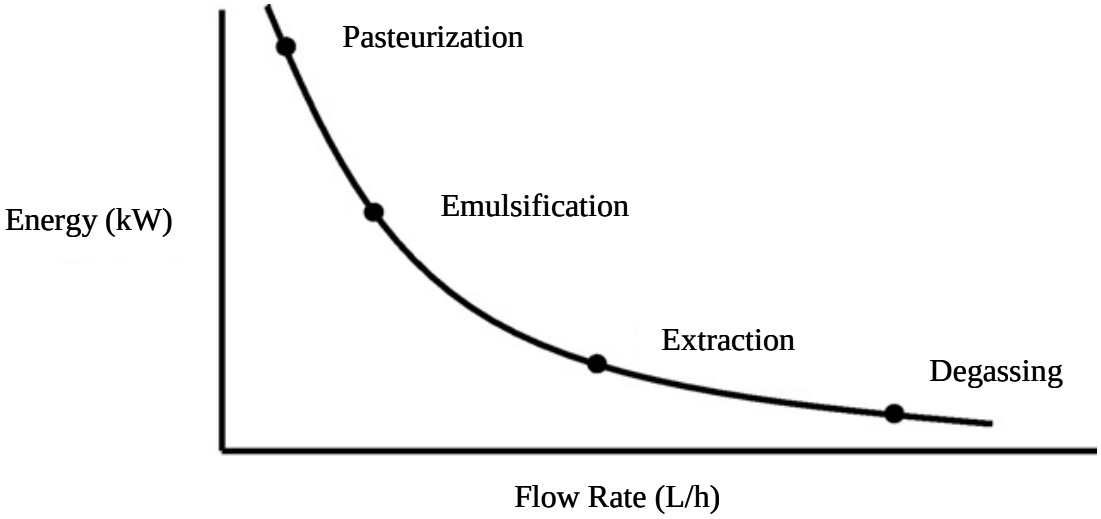

Figure 5.1. Generalized relationship of flow rate (L/h) vs. energy (kW) for several ultrasonic applications (Patist and Bates, 2008).

Power intensity is a way to measure the power transmitted to the medium from the sonotrode tip surface. It represents the power, P, distributed over a surface area A as shown in Equation [5.1] (Bermúdez-Aguirre et al., 2011):

$$P_i = {}^{P}\!/_{A} \text{ (W/m}^2\text{)} \qquad \text{[Eq 5.1]}$$

The power of ultrasonic waves can be represented by the vibrational amplitude. The vibrational amplitude is measured as the maximum displacement, in micrometers, of the vibrating tip of the sonotrode as it sends the acoustic waves into the medium. In general, vibrational amplitude is specified by the equipment manufacturer. Furthermore, the strength of ultrasonic treatment can be measured by the energy delivered to the medium. The energy can be expressed by the treatment time (s) and multiplied by their power

values to obtain the Joule energy applying the physics relationship shown in the Equation [5.2] (Gogate and Pandit, 2015):

$$1\,J = 1\,W \times s \qquad \text{[Eq 5.2]}$$

2.2. Frequency

As described previously, high power ultrasounds are accomplished with frequencies ranged between 16 kHz and 100 kHz. The propagation of an ultrasonic wave through a material can be described by its amplitude (A) and frequency (f). The amplitude is the maximum displacement of the medium from its equilibrium, and the frequency is the number of oscillations per second. Conversely, the period T is the time required for one complete cycle to occur, so that T = 1/f (Figure 5.2.). It is important to note that the frequency of a wave does not depend on the material characteristics and remains stable throughout the ultrasound wave propagation (Salazar et al., 2012).

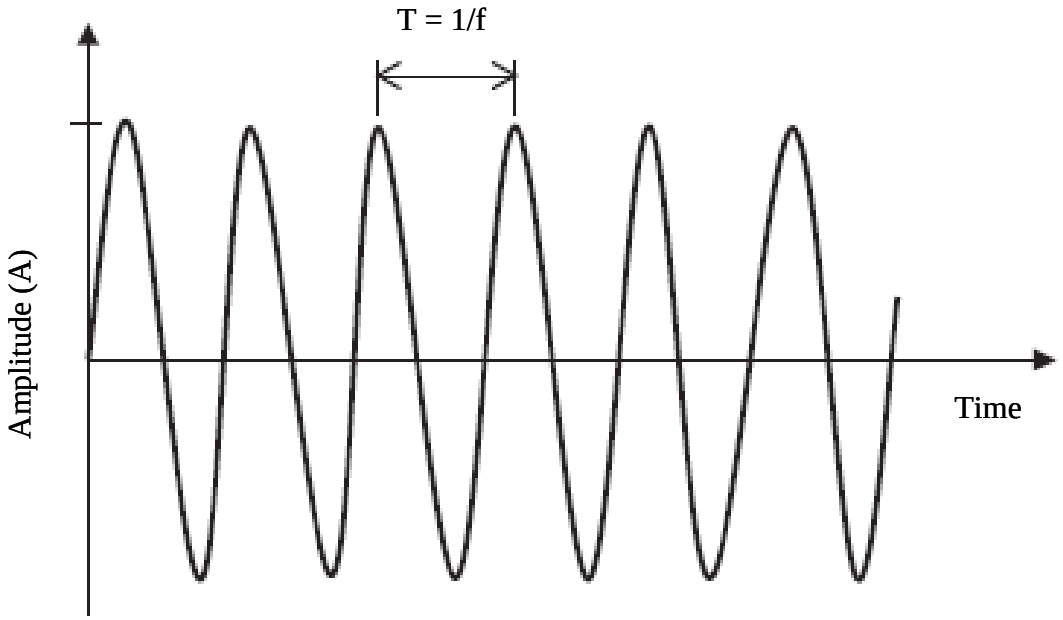

Figure 5.2. Ultrasound wave propagation characteristics

Lorimer and Mason (1987) showed that the ultrasound frequency is inversely proportional to the cavitation bubble size. Therefore, lower frequency ultrasounds are recommended for applications where intense physical and chemical effects are required because they generates large cavitation bubbles resulting in higher temperatures and pressures in the cavitation zone. The frequency used depends, mainly, on the amount of

power desired and the emitter (transducer) dimensions. The frequency choice, for an ultrasonic device, depends on the treatment and effect desired. However, multiple-frequency ultrasound operation is increasingly being employed to enhance overall cavitational activity and generating intensities suitable for chemical processing applications at higher energy efficiencies.

2.3. Effects of high-power sonication

The high power ultrasounds application causes some effect through the medium. They raise the temperature of the application medium, even if no external heat is applied. The ultrasonic waves propagation induces oscillations of the medium particles generating heat over time (Mason, 1998). The high power ultrasounds cause inertial cavitation in liquids, generating very high temperatures (up to 5,000 K) in small located areas in the medium due to the collapse of bubbles. This temperature raise increases the number of cavitation bubbles and the collapse is damped by the higher vapor pressure. In contrast, temperature affects the vapor pressure, surface tension, and viscosity of the liquid medium (Muthukumaran et al., 2006).

Patist and Bates (2008) described that the increase of the external pressure increases the cavitation threshold and thus the number of cavitation bubbles is reduced. On the other hand, increasing the external pressure will increase the pressure in the bubble at the moment of collapse resulting in a more rapid and violent collapse.

The medium properties (solid, liquid, and gas content) has a deep influence on the cavitation phenomena in terms of initiation of cavitation and size of initial nuclei including vapor pressure, viscosity, and surface tension (Mason, 1998). During their propagation over solids, the ultrasound waves induces a “sponge” effect, since condensations and rarefactions in the solid act similar to the squeezing and releasing of a sponge (Riera-Franco de Sarabia et al., 2000).

3. Overview of high power ultrasound equipments

The most commonly ultrasonic system used in the food processing industry is the electricity-driven sonication (Kiani et al., 2014), for this reason is the main equipment focused in this chapter.

The basic components of the High Power Ultrasound equipment are the power generator, that supplies electricity at the desired ultrasonic frequency to the transducer, that converts the electrical power to mechanical vibrations and the emitter, which physically sends the ultrasonic waves into the medium (Mason, 1996).

3.1. Electrical generator

The function of the power generator is to convert a standard electrical frequency (typically 50 - 60 Hz, depending on the country electrical grid) into high alternating frequency (over 16 kHz) required for power ultrasonic transmission through a series of oscillating, amplifying, and matching circuits. Most of the generators allow the power to be set only indirectly through voltage (V, measured in volt) and current (I, measured in amp) settings. The power (P expressed in watt) is the product of these two variables represented in Equation [5.3] (Bermúdez-Aguirre et al., 2011):

$$P = I \times V \qquad \text{[Eq 5.3]}$$

The generator is designed at a fixed frequency corresponding to the exact resonant frequency of the transducer. For multi-transducer system, only those transducers closest to the generator can operate at their maximum efficiency, what can lead to problems such as hot spots, standing waves… (Mason, 1998). To solve this problem another system was developed: the sweep frequency technology, where the frequency output of the generator is modulated around a central value and by sweeping, the frequency oscillates slightly above and below the central value. It allows to each transducer to operating on its resonant frequency and to achieve maximum efficiency (Fuchs, 1999). Furthermore, there are other power ultrasound generators for multiple frequency system combining the effects of 2 or more frequencies.

3.2. Transducer

The function of an ultrasonic transducer is to convert electrical energy into mechanical energy and vice versa at fixed ultrasonic frequencies (Mason, 1998). Ultrasound can be generated in different ways as described by Cochran (2012), including the electromagnetic techniques, the optical techniques, the capacitive techniques although the most common are the piezoelectric techniques.

The most important and critical part in an ultrasound system is the transducer. They are configured in different ways, chosen according to the specific application as: direct contact, delay line, dual element, immersion, angle beam, normal incidence shear wave. In food processing the most common transducer used is the piezoelectric transducer, because it is considered the most efficient ultrasonic transducer, achieving better than 95% efficiency (Feng et al., 2011).

The high power ultrasound application requires material generally operating in a structure in which multiple layers can function simultaneously, to keep the electrical impedance at optimal levels while operating at a low frequency in order to avoid any damage that in the transducers. The Langevin type transducers (Bermúdez-Aguirre et al., 2011; Salazar et al., 2012; Kiani et al., 2014) are the most common transducers used to generate high power ultrasonic waves (Figure 5.3).

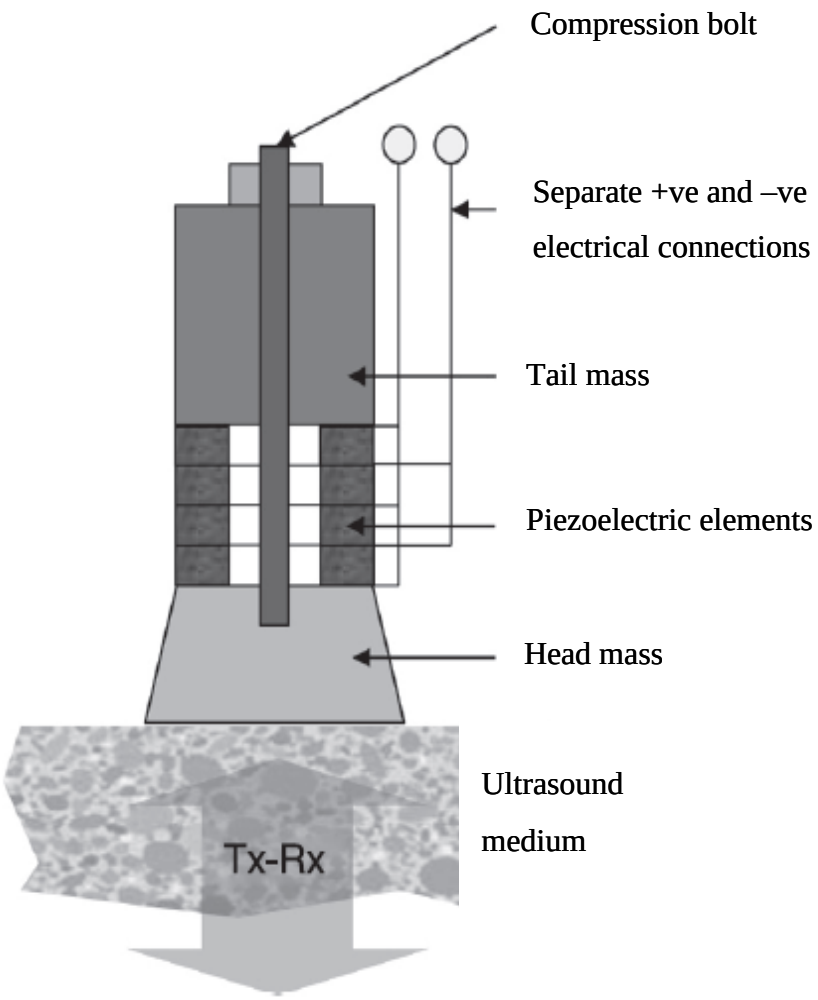

Figure 5.3. Schematic example of the Langevin transducer (Cochran, 2012)

Langevin type piezoelectric transducers are mounted by bolting head mass and tail mass on both ends of stacked piezoelectric ceramic. The piezoelectric ceramic components are often defined by the desired electrical impedance as well as by the ultrasonic performance of the device. Thus, a set of identical rings can be used, with alternating polarity and electrical connections so that they operate together rather than cancelling each other out. These are coupled to the tail mass, which is an inertial component, usually steel, chosen so that most of the motion is that of the light head mass. The head mass may be made of aluminium or titanium, if the ultrasonic load is water, for example, for underwater sonar or ultrasonic cleaning, or it may be of steel, for example if the transducer is to be used in drilling or cutting (Bermúdez-Aguirre et al., 2011; Cochran, 2012; Salazar et al., 2012; Kiani et al., 2014).

4. High power ultrasound systems

There are many types of ultrasonic systems currently in use, which vary according to the generator design, transducer type, the way how ultrasound is delivered to the process, the food process and the effects desired. Two types of ultrasound systems are commonly used in food industry: those using a horn as the sound emitter and others using a bath (Bermúdez-Aguirre et al., 2011; Salazar et al., 2012; Kiani et al., 2014).

Furthermore Gogate and Pandit (2015) described some key points have to be considered for an efficient large-scale configurations design of sonochemical system, and can be classified on its application field as:

- Chemical engineering: mass transfer, gas–liquid hydrodynamics, reaction engineering
- Material science: transducers capable of operating at conditions of high frequency and high power dissipation
- Acoustic effects

4.1. Probe systems

Probe systems or ultrasonic horn, are used for characterization experiments at laboratory scale. These are typically immersion-type systems with the transducer directly in contact with the liquid medium. It consists of a metal horn coupled to an

ultrasonic transducer. The purpose of the horn is to amplify the vibration produced by the transducer.

The main advantage of the horn system is its capacity to deliver large amounts of power directly to the reaction mixture through a small transducer area, although it is not distributed uniformly, achieving irradiation intensities of a several hundred of watts per square centimeter close to the transducer (Feng et al., 2011). The intensity decreases exponentially away from the horn depending on the maximum power input to the equipment and the operating frequency (Gogate and Pandit, 2015).

The Horn sonication systems resonate generally available at a fixed operating frequency, whereas the power dissipation can be regulated by varying the amplitude delivered to the transducer (Feng et al., 2011). The scaling-up for a large scale application of horn system has some restrictions: the lack of efficiency transmittance of the acoustic energy into a large volume, it leads to a poor distribution of the cavitational activity and the erosion and pitting of the horn tip in continuous operation at high-power dissipation. The erosion and pitting of the horn may contaminate the reaction medium (Salazar et al., 2012).

4.2. Ultrasonic baths

Ultrasonic baths are typically configured as a tank equipped with a transducers (either single or multiple depending on the capacity) bonded to it bottom. The active zone is plane above the transducers and hence multiple transducers could be installed to a better distribution of cavitational activity in the device. In this case, the position of the transducers can be mounted or modified to increase the active zone in the device. The tank is filled either with a coupling liquid, in which the reaction vessel is immersed, or directly with the mixture to be processed. In order to achieve a uniform distribution of the ultrasonic irradiation, it may be necessary to stir the mixture mechanically (Bermúdez-Aguirre et al., 2011; Salazar et al., 2012; Kiani et al., 2014).

The disadvantages of these equipments are the difficulties for temperature control of the reaction mixture and its reproducibility. Furthermore, this ultrasound equipment has a relatively low power density because undetermined transfer losses through the vessel walls and also due to the limitation of the transducers power to avoid cavitation damage to the tank walls (Salazar et al., 2012; Kiani et al., 2014; Gogate and Pandit, 2015).

This type of system are suitable for flow cell arrangements to large-scale operations because they provides flexibility in terms of continuous operation, also gives an option of arranging the transducers on the walls of the device can generate a standing wave. The system can be configured in hexagonal, cylindrical or rectangular giving an excellent distribution of cavitational activity. The number and location of the transducers also affect the hydrodynamic behavior and mixing characteristics in the device, which are important for physical processing applications (Gogate and Pandit, 2015).

5. Design of ultrasound device for olive oil extraction

The High power Ultrasound treatment for olive oil extraction was recently tested at laboratory scale. All the previous work, discussed in introduction, were carried-out at different ultrasounds frequencies: 24 kHz and 25 kHz by Jiménez et al. (2007) using ultrasound probe horn and ultrasound bath respectively and 35 kHz by Clodoveo et al. (2013) in an ultrasonic bath for the treatment of olive fruits before crushing and olive paste. Most recently Bejaoui et al. (2016a, 2016b) have investigated the application of HPU at 40 kHz at continuous flow condition through a rectangular tube and power of 150 W.

For designing a HPU prototype for the Virgin Olive Oil extraction, all the previous works were taken into consideration. The first aspect of the design was to choose the frequencies of the high power ultrasounds. Three frequencies were chosen: 20, 40 and 80 kHz. 20 and 40 kHz corresponding to frequencies previously studied for virgin olive oil extraction at laboratory level and 80 kHz to determine the effect of better resonance frequency on olive oil extraction yield and characteristics.

For the HPU treatment device, a food grade stainless-steel squared device was chosen with an indirect application of the high power ultrasounds since they provides the best result for olive oil extraction (Jiménez et al., 2007). The system was selected as ultrasonic bath with ultrasonic transducers bonded on the four walls of the device (Figure 5.4). The type of sonotrode chosen was the piezoelectric transducers since they were applied previously in the laboratory scale studies (Jiménez et al., 2007; Clodoveo et al., 2013; Bejaoui et al., 2016a; 2016b) and are the most common for high power ultrasound applications in food industries (Mason, 1998). Three different piezoelectric

transducers were mounted; each type delivered a fixed frequency respectively 20 kHz, 40 kHz and 80 kHz corresponding to the frequency chosen for the olive oil extraction as commented previously, each transducer delivered an intensity of 50 W. The area of application of each transducer, depending on its frequency, was 12.57 cm^2, 19.63 cm^2 and 28.27 cm^2 for 20 kHz, 40 kHz and 80 kHz, respectively. They delivered intensities of 3.98 W/cm^2 (20 kHz), 2.55 W/cm^2 (40 kHz) and 1.77 W/cm^2 (80 kHz), respectively.

The transducers were arranged and bounded process through the device longitude. The transducers were mounted in the four faces of the squared tube using a helical configuration for each transducer frequencies. To avoid any interferences or damages of the transducers, an axial (X) distance (Figure 5.4) of 8.75 cm (delivered by transducers supplier) was respected for each type of transducers. The generator used delivers 900 W, the intensity delivered by each transducer was 50 W and the number of 18 transducers was used for each frequency. Considering all these parameters and limitations, the system longitude was 1.60 m. The side of the squared tube section was fixed to 10 cm to allow bounding the different transducer type. The final volume of the system was 0.016 m^3 (16 L). The device used three different ultrasound generators, each one delivering a fixed frequency 20 kHz, 40 kHz or 80 kHz and 900 W of power. Hence the nominal volumetric energy density applied by the device was 56.25 W/L for each frequency treatment.

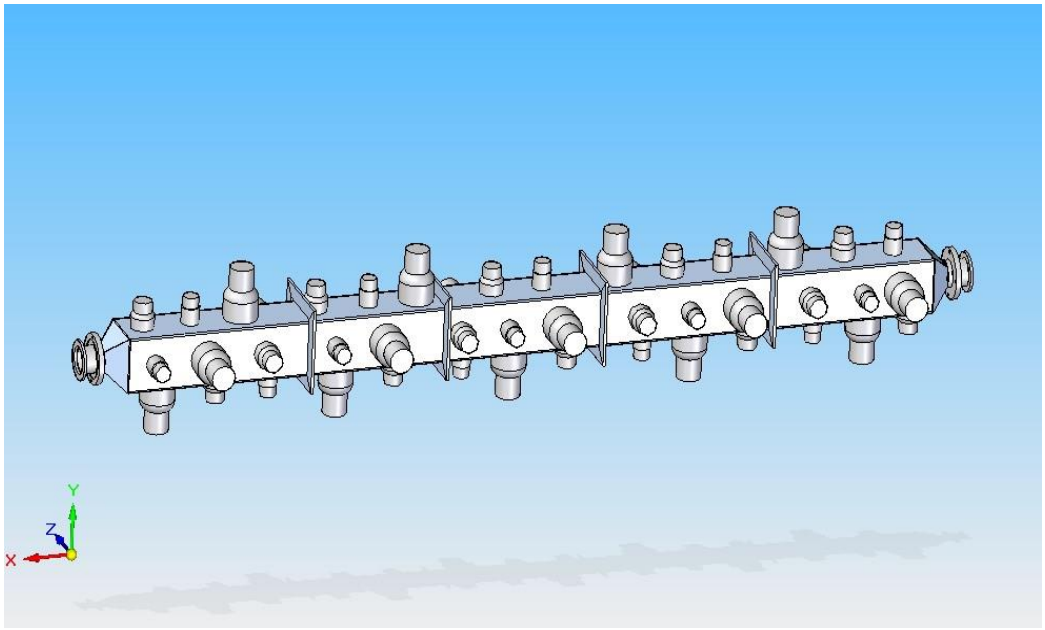

Figure 5.4. High power ultrasound device used for virgin olive oil extraction

The device was designed for its application at pilot plant scale to test the effect on the olive paste, oil yield and VOO characteristics. Furthermore, the technique was tested as help and/or substitution to the malaxation step before its introduction in the continuous industrial process at pilot plant scale. Figure 5.5 showed how the device should be mounted in the VOO extraction process. Where the device can be placed in two configurations:

- before malaxation as help of this step
- between crusher and the decanter (Horizontal Centrifugal Solid Bowl) as substitute to the malaxer.

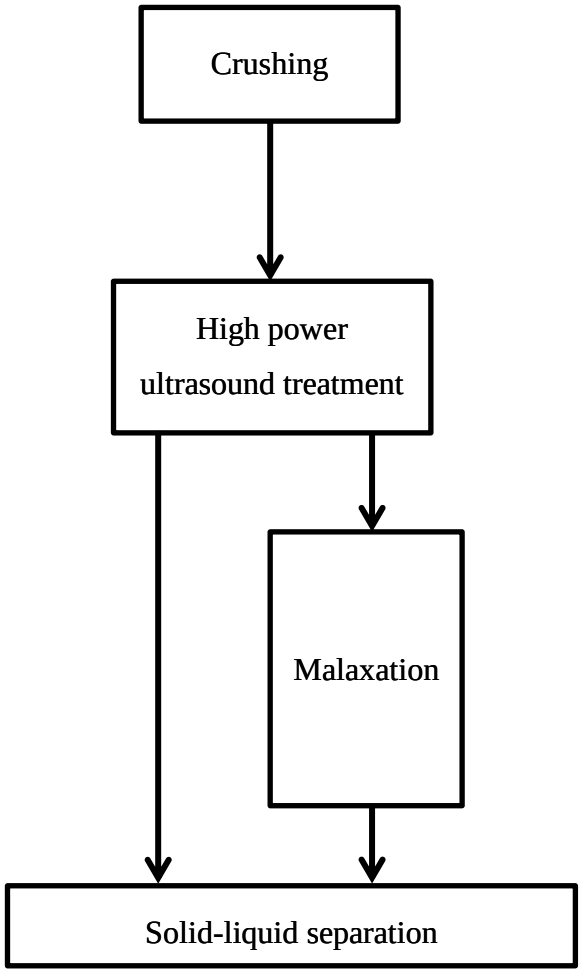

Figure 5.5. Flow diagram of high power ultrasound configuration for olive oil extraction

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Chapter 6. High power ultrasound frequency: Effect on the virgin olive oil yield and quality

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Contents

The high power ultrasound frequency: Effect on the virgin olive oil yield and quality.

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Abstract

In this work a pilot-scale device for continuous High Power Ultrasound (HPU) application was performed for its use at experimental plant level. The experiment consisted in the sonication treatment of olive paste at three frequencies 20, 40 and 80 kHz. After treatments the Virgin Olive Oil (VOO) was extracted at `ABENCOR´ laboratory scale extraction system. Two extraction conditions were compared, malaxation and centrifugation against direct paste centrifugation. The first effect observed of the HPU application on the olive paste for all the frequencies used when compared with the untreated paste. Regarding to the process efficiency, in general the application of HPU improve the process yield with respect to the untreated paste. HPU treatment at 40 kHz frequency followed by kneading produced higher olive oil extractability. With respect to the VOO quality and nutritional compounds no alteration was noted with the HPU treatment.

Key words: High Power Ultrasound, Frequency, Virgin Olive Oil, Oil Yield, Oil Quality.

Abbreviations: Virgin Olive Oil (VOO), High Power Ultrasound (HPU), Nuclear Magnetic Resonance (NMR), Industrial Yield (IY), Oil Volume (OV), Weight of Olive Paste (WOP), High Performance Liquid Chromatography (HPLC)

1. Introduction

The virgin olive oil (VOO) elaboration process has four principal steps. The first step is the olive fruit reception, cleaning and washing. Then the olive paste preparation, including the fruit crushing and the olive paste malaxation at a determined temperature for a specific time. After, the VOO is separated from pomace and vegetation water and finally the oil is clarified and stored (Aguilera et al., 2010; Hermoso et al., 1998; Uceda et al., 2006).

The olive paste malaxation is considered as an essential step to obtain higher VOO yield affecting deeply to VOO characteristics (Aguilera et al., 2015, 2010; Clodoveo et al., 2014a). This step is performed in the malaxer, this machine is formed by a semi cylindrical or cylindrical tank equipped with a heating jacket for warm water circulation and rotating axes (horizontal or vertical) with several blades (Aguilera et al., 2010; Ayr et al., 2015). The malaxation process consists in the continuous kneading of olive paste at a carefully monitored temperature. This phase is especially useful for achieving high and satisfactory yields of extraction. During malaxation the small oil droplets released during the milling merge into larger drops. It helps breaking the oil/water emulsions formed during the crushing operation allowing the coalescence of the oil and giving a continuous oily phase. In addition, this operation disrupts a proportion of the olive cells remaining uncrushed during the first step (crushing) allowing the recovery of a part of the not liberated oil.

The malaxing conditions; time, temperature, technological co-adjuvant addition and the atmosphere composition, have a deep influence on the oil extractability, oil quality and enzyme activities related to the oil nutritional and organoleptic properties (Clodoveo, 2013). Malaxation temperature lets to decrease the VOO viscosity (Gila et al., 2015), facilitating the oil droplet coalescence giving higher oil yield (Di Giovacchino et al., 2002; Hermoso et al., 1998). Therefore, longer malaxation time and higher temperatures improve the oil yield (Aguilera et al., 2010; Di Giovacchino et al., 2002; Hermoso et al., 1998). Also the Micronised Natural Talc addition lowered pomace oil content (Aguilera et al., 2010; Caponio et al., 2015).

Nevertheless, malaxation step cannot be considered a simple physical separation process since some complex bioprocess occur, affecting deeply to the final quality and composition of VOO (Clodoveo et al., 2014b). Considerable changes in the VOO have

been described because of the catalytic activity of fruit enzymes, chemical reactions and partition phenomena between oil and vegetation water (Aguilera et al., 2015; Angerosa et al., 2001; Artajo et al., 2006).

Some of the VOO minor compounds may be affected by malaxation conditions. Aguilera et al. (2015) showed that the phenol content increased with malaxation temperature whereas malaxation time did not show a considerable effect. Micronized talc addition produced a higher phenol, bitterness index and *o*-diphenols content.

However, the malaxer shows a low energetic efficiency for paste heating giving long malation time to obtain a satisfactory oil yield. To solve these limitations, some new technologies are being applied to malaxation step: controlled atmosphere malaxer (Tamborrino et al., 2014), flash thermal conditioning (Leone et al., 2015a), microwave treatment (Leone et al., 2015b), pulsed electric field (Abenoza et al., 2012) and Ultrasound treatments (Bejaoui et al., 2016a, 2016b; Clodoveo et al., 2013; Jiménez et al., 2007, 2006).

Power ultrasound refers to those with a frequency range from 20 kHz to around 1 MHz, which is close to the upper limit of the human hearing range (Mason, 1998). During the propagation of the ultrasound wave a cyclic succession of expansion (rarefaction) and compression phases is induced by mechanical vibration. When pressure amplitude exceeds the tensile strength of the liquid in the rarefaction regions, small vapor-filled voids called cavitation bubbles are formed (Chen, 2011). Cavitation is defined as the combined phenomena of formation, growth and subsequent collapse of microbubbles or cavities occurring over an extremely small interval of time, releasing large magnitudes of energy at the location of transformation (Suslick, 1990).

In the last decade some works were carried at laboratory scale for the improvement of malaxation by High Power Ultrasound (HPU) treatment or pretreatment. The first work of high-power ultrasound pretreatment on olive paste was carried by Jiménez et al. (2007), by direct and indirect application. The ultrasound treatment induced quick-heating of olive paste and improved the oil extractability. No changes on quality parameters were found whereas sonication gave VOO with lower bitterness and higher content of tocopherols, chlorophylls and carotenoids. Related to sensory characteristics, off-flavour volatiles were not detected in the oils from ultrasound treatments, showing higher intensity of positive organoleptic attributes than those untreated.

Clodoveo et al. (2013) tested ultrasound assisted virgin olive oil extraction. The sonication treatment was applied on olives submerged in a water bath (before the crushing) and on olive paste (after the crushing). The ultrasound technology provided a reduction of the malaxation improving the oil yields giving oil with higher minor compounds contents when the olives were submerged in the water bath.

Most recently Bejaoui et al. (2016a, 2016b) proposed a laboratory scale continuous device for HPU pretreatment of olive paste. The application of ultrasounds, as described for batch works, induced a quick heating of the paste. The sonication treatment improved the oil extractability by 5.74%. Moreover the HPU treatment did not cause changes in the quality indices, fatty acid composition and volatile aromatic compounds of the VOO. Furthermore oil autoxidation's mechanisms were not accelerated by this treatment. The VOO obtained from HPU treated olive paste showed higher content of tocopherol, chlorophylls and carotenoids whereas a reduction in phenolic content and bitterness index was observed too.

Previous works were carried-out at different ultrasounds frequencies: 24 kHz and 25 kHz (Jiménez et al., 2007), 35 kHz (Clodoveo et al., 2013) and 40 kHz (Bejaoui et al., 2016a, 2016b). And then, the results are difficult to compare. The aim of this work was studying the effect of three HPU frequencies (20, 40 and 80 kHz) applied `in line´ at semi-industrial scale on the oil yield and VOO characteristics. Furthermore, high power ultrasound technique was tested as aid or substitute of the olive paste malaxation step.

2. Materials and methods

2.1. Fruit material

Fruits, from `Picual´ olive cultivar *Olea europaea* L., were harvested in the 2013/2014 crop year. The olives were picked from mature trees grown under irrigation in the experimental orchard of IFAPA `Center Venta del Llano´ in Mengíbar, Spain. The olives were harvested by a mechanical shocker and transported immediately to the experimental oil mill of IFAPA for processing.

Olive fruit characteristics, maturity index, moisture and fat content, were determined. The maturity index was determined as proposed by Beltrán et al. (2003). The olive moisture was determined from the crushed fruit by desiccation at 105 ºC after weighted,

this two later operation was repeated until obtain constant weight (the results were expressed in weight percentage). Oil content was measured, for the dried olive paste using a Nuclear Magnetic Resonance (NMR) fat analyzer Minispec mq 20 (Bruker Analytik Gmbh). The NMR was previously calibrated and validated with Soxhlet extractor. The results were expressed on weight percent (on a fresh matter basis).

2.2. High power ultrasound device

The HPU treatment was carried using a pilot-device (Figure 1) for inline treatment of olive paste. The device consisted in a rectangular pipe of food quality stainless steel. The internal volume of the pipe was 0.016 m^3 being equipped with 54 utrasound transducers applying three different resonance frequency 20 kHz, 40 kHz and 80 kHz, 18 transducers per frequency. The transducers were mounted throughout the rectangular pipe on the 4 external faces. The transducers were powered by 3 ultrasound generators with a power of 900 W, each one generated a single frequency (20 kHz, 40 kHz or 80 kHz) and was connected to the corresponding transducers. The nominal volumetric energy density applied by the device was 56.25 W/L.

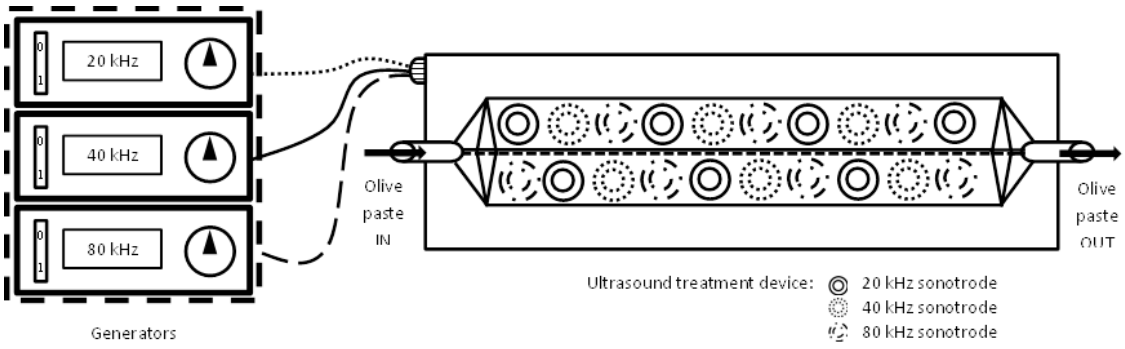

Figure 1. High Power Ultrasound device for olive paste treatment

2.3. Experimental setup

The experiments were performed in the `IFAPA´ oil mill at two different dates, 30 of January 2014 and 05 of February 2014, processing 500 kg of olives from homogeneous lots of 6000 kg for each date. The olives were milled in a hummer crusher (Pieralisi, Spain) equipped with a sieve of 7 mm. After crushing, the olive paste obtained was sent to an intermediate tank equipped with a pump to transport the olive paste to the ultrasound device.

The olive paste passed through the HPU device at a constant flow rate of 200 kg/h. Whereas the olive paste was flowing through the sonication device three different HPU treatments were applied: 20 kHz and 900 W, 40 kHz and 900 W and 80 kHz and 900 W. These HPU treatments were compared with a reference, passing the olive paste through the device without HPU application (Figure 2). During the experiments the olive paste temperature was monitored using a calibrated bimetal thermometer.

The paste from each treatment was collected at the HPU device exit, and then extracted at laboratory using an ``Abencor´´ system (Martínez et al., 1999). In order to determine if sonication could reduce or substitute the paste malaxation, the olive pastes of each treatment were centrifuged without malaxation (C) or malaxed at 27 ºC for 30 minutes and then centrifuged (M/C) (Figure 2).

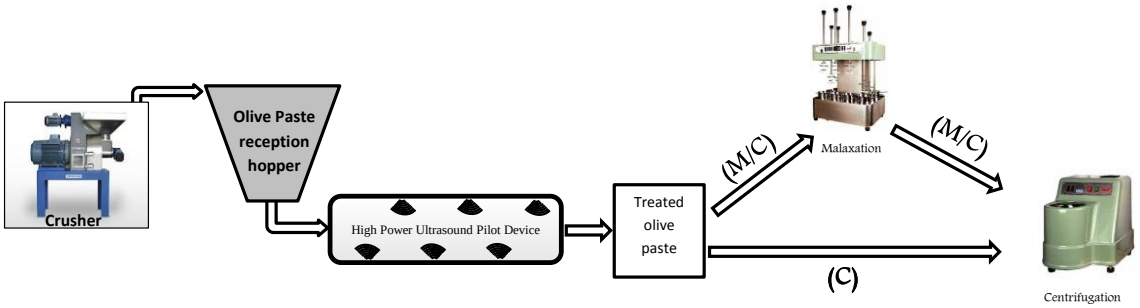

Figure 2. Flow diagram of High Power Ultrasound treatment experiments

After centrifugation, the liquid phases were recovered in a graduated cylinder. After settling, the oily phase volume was measured to determine the industrial yield. The industrial yield was calculated applying the Eq. (1). Results were expressed as percent.

$$IY = \left[\frac{(OV \times 0.915)}{WOP}\right] \times 100 \quad (1)$$

Where: OV: oil volume obtained from ABENCOR system (L)

WOP: weight of olive fruit paste (kg)

0.915: olive oil density (kg/L)

The oil was taken, filtered and stored at -24 ºC until analysis. All the HPU experiments for both extraction conditions (centrifuged or malaxed and centrifuged) were carried out for triplicate.

2.4. Olive oil quality parameters

Free acidity, peroxide values, ethyl esters and ultra-violet absorption at 232 and 270 nm (K_{232} and K_{270}) were measured as described in European Regulation EEC 2568/91 (European Commission, 2013). Free acidity was expressed as percent of oleic acid, peroxide values as milli-equivalents of active oxygen per kilogram of oil (mEq O_2/kg), K_{232} and K_{270} extinction coefficients were calculated from absorption at 232 and 270 nm respectively and ethyl esters were expressed as mg/kg olive oil (Beltran et al., 2015).

2.5. Oxidative stability

The oxidative stability was measured by accelerating the aging process of the oil by exposing it to heat and increased volumes of air using a Rancimat Model 743 (Metrohmn, Switzerland). 3 g of oil was weighed and incubated at 98°C, and air was bubbled through the oil at a flow rate of 12 L/h (Gutiérrez, 1989). The results were expressed as induction time in hours.

2.6. Olive oil composition

2.6.1. Total phenol content

Phenolic compounds were extracted from an oil-in-hexane solution with methanol:water and their concentration was measured using Folin-Ciocalteau reagent and colorimetric measurement at 725 nm (Vázquez-Roncero et al., 1973). Results were expressed as mg/kg of caffeic acid.

2.6.2. Bitterness index (K_{225})

The bitterness index was determined applying the method described by Gutiérrez Rosales et al. (1992). The bitter compounds were eluted through the solid phase extraction column with methanol:water (50:50). The absorbance of the methanolic extract recovered was measured at 225 nm. The results were expressed as mg/kg of VOO.

2.6.3. Tocopherol content

Tocopherol composition was analyzed by HPLC, applying the IUPAC method 2432 (IUPAC, 1992). Results are expressed as mg/kg of VOO.

2.6.4. Pigment content

Carotenoid and chlorophyllic pigments were determined measuring the absorbance of olive oil weighed and dissolved in cyclohexane at 470 and 670 nm as described by Minguez-Mosquera et al. (1991). The results were expressed as mg/kg.

2.7. Data analysis

The results were expressed as mean ± standard deviation (n = 3). Analysis of variance was applied, significant differences between treatments were determined applying Tukey's test $p < 0.05$ (Statistix 9.0 software, Analytical Software, USA).

3. Results and discussion

3.1. Effect of HPU treatment on paste temperature and oil yield

When HPU was applied the first effect observed was the temperature increase of olive paste (Table 1), although the fruit crushing gave a slight increase too (Bejaoui et al., 2016a). The HPU increased olive paste temperature between 1 ºC and 4.5 ºC for the first harvesting date and between 3 ºC and 5.6 ºC for the second harvesting date. The temperature raise was greater for the higher ultrasound frequencies. Furthermore, attention should be paid on how fruit characteristics affects to the olive paste heating by HPU since the olive paste was heated at higher temperature when fruit moisture was greater (Bejaoui et al., 2016a).

This HPU effect allowed an instantaneous olive paste heating till the kneading temperatures established (27 ºC), allowing kneading at this temperature for longer time than those untreated pastes. The fast olive paste heating let a more efficient kneading since the temperature increase allows the oil viscosity decrease (Gila et al., 2015), helping the coalescence phenomenon and then increasing the oil yield. Because the kneading temperature was achieved immediately, the malaxation was carried out for longer time at optimal temperature. It lets to obtain lower oil loses in the pomace Aguilera et al. (2010). Then, HPU can be considered as an effective heating procedure for olive pastes malaxation (Bejaoui et al., 2016a, 2016b; Jiménez et al., 2007).

Table 1. Fruit characteristics and olive paste temperature raise after olive crushing (ΔT0) and olive paste sonication at 20 kHz (ΔT1), 40 kHz (ΔT2) and 80 kHz (ΔT3) with respect to the untreated paste.

	01-30	02-05
Maturity index	4.32	5.36
Olive Moisture (% weight/weight)	42.31±0.41*	45.48±0.20
Olive Fat content (% weight/fresh weight)	27.05±0.40	22.13±0.16
ΔT0 (ºC)	2.7	2.3
ΔT1 (ºC)	1	3
ΔT2 (ºC)	4	4.6
ΔT3 (ºC)	4.5	5.6

* Mean value ± SD (n = 3)

Because HPU application involves physical effect other than heating, it was tested as an aid to malaxation to shorten it or as alternative to this operation. For this purpose, the olive pastes from each HPU treatment were malaxed and extracted or were centrifuged directly. Figure 3 showed the oil yields for the HPU device treatment applying 3 different frequencies and the reference without treatment, for both how the olive oil extraction procedure affect it. In general, the HPU treatments allowed to obtain a higher oil yield comparing with reference for both harvesting dates, these results were in agreement with previous works (Bejaoui et al., 2016a, 2016b; Jiménez et al., 2007).

The reference treatment (passing through the HPU device without sonication) with direct centrifugation showed an oil yield of 16.89 % and 13.73 % for the first and second harvesting date respectively (Figure 3). Although malaxation step is necessary, the oil released after fruit crushing by the mechanical effects during the olive paste pumping could be separated in the centrifuge. Obviously, when the same reference treatment was applied and the olive paste was malaxed and centrifuged the oil yield was higher as expected (Aguilera et al., 2010). Although HPU application improved the oil yield, differences between HPU frequencies were observed and for extraction conditions too. In general, when HPU was followed by malaxation and centrifugation the oil yields were higher.

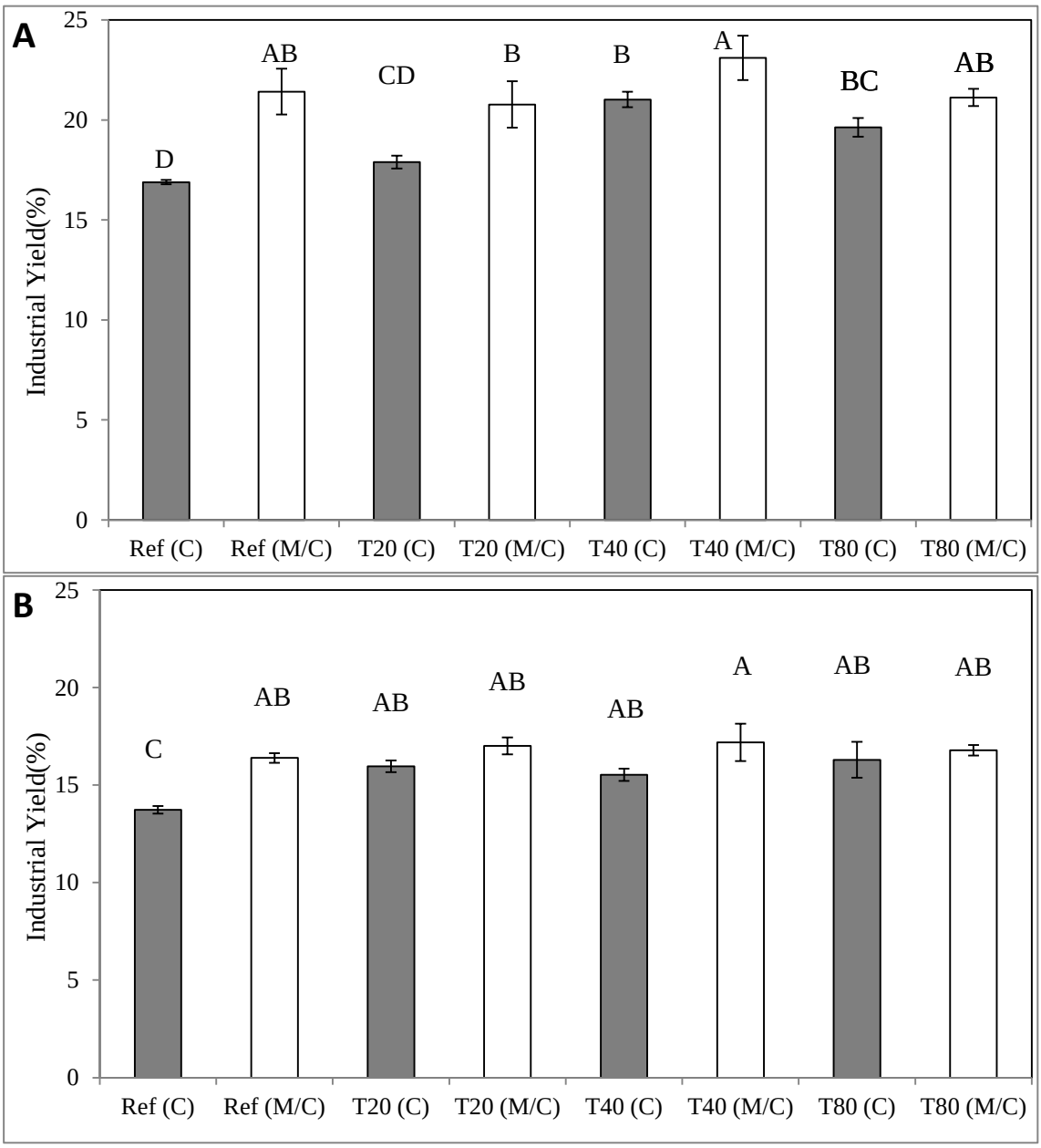

Figure 3: Oil yield obtained for malaxed (M/C) and not malaxed (C) olive pastes submitted to reference (Ref) and High Power Ultrasounds at 20 kHz (T20), 40 kHz (T40) and 80 kHz (T80) for two harvesting dates 30 January (A) and 05 February (B). Different letters represent significant differences at p = 0.05.

HPU application at 20 kHz and direct centrifugation for the first harvesting date (Figure 3a) gave a small increase of yield; although lower than the other two frequencies (40 and 80 kHz). For the second harvesting date, 20 kHz treatment showed similar oil yield than the other frequencies and conventional malaxation, however when HPU was applied at 20 kHz, and the olive pastes were malaxed and centrifuged, higher oil yields were observed.

Sonication treatment of olive paste at 40 kHz provided the highest oil yields between the olive pastes malaxed and centrifuged and those only centrifuged for the first harvesting date. Significant differences were obtained with respect to the corresponding reference treatments and to the other frequencies.

For the second harvesting date 40 kHz treatment with malaxation and centrifugation showed higher oil yield than reference, although differences were not founded respect to the other HPU frequencies. Application of HPU at 40 kHz, with direct centrifugation, showed the highest oil yield, however differences between HPU treatments with similar extraction conditions were not found.

The sonication treatment at 80 kHz without malaxation showed an increase of yield comparing with the reference directly centrifuged. This increase was higher for the second harvesting date. The HPU treatment at 80 kHz with malaxation and centrifugation gave a higher oil yield although significant differences were not detected for both harvesting dates.

Therefore, application of HPU at 40 kHz gave the best oil yields when the olive paste was malaxed and centrifuged. Thus, it may be considered as a malaxation aid since it may allow shortening this step and making it more efficient. But, other interesting results were obtained since application of HPU at 40 kHz and 80 kHz may be used without malaxation for olive paste preparation for both harvesting dates.

The increase of oil yield observed for HPU treatment without malaxation can be explained by the effects of the ultrasounds in a medium. The ultrasonic wave propagation in a solid produces a series of fast compressions and expansions in the medium. This movement can be compared to a sponge squeezed and released repeatedly, this phenomenon is known as the ‘‘sponge effect’’ (Feng et al., 2011). This phenomenon helps to the liquid to flow out of the cells, whereas the compressions and expansions of the medium can create microchannels helping the fluid movement through a medium.

The ‘‘sponge effect’’ described above can affect to the mass or heat transport in the medium improving some transport operations. During the HPU waves propagation the convective heat transfer coefficient can be improved in a similar way to the mechanical agitation (Cárcel et al., 2007; Feng et al., 2011). Furthermore, during the HPU treatment other effects can occur as the `ultrasonic agitation´, an increase of the turbulence and the

heat transfer. Furthermore, cavitation and microstirring generated by HPU can enhance the mass transfer in solid–liquid extraction processes allowing to obtain a better extraction yield. Also the propagation of ultrasound pressure waves and the resulting cavitation phenomena can induce the destruction of the cellular structures. Acoustic cavitation bubble collapse occurring at or in close vicinity to the surface of the cell membranes may cause microfractures or can punch holes through the cell wall (Feng et al., 2011). All these effects described above are similar to those observed during the olive pastes kneading in a traditional malaxer, and then, may explain the good results observed for the HPU treatment of olive paste at 40 kHz that were equivalent to the oil yield from the traditional extraction conditions (malaxation for 30 minutes at 27 ºC).

Another effect of the HPU helping increase the oil yield may be the hydrolysis of pectins from plant cell walls and their solubilization (Feng et al., 2011). The hydrolysis and solubilization of pectins induce the cell wall degradation and can break water-oil emulsions, since pectins play a major role in the water-oil activity (Sadkaoui et al., 2015).

3.2. Effect of HPU treatment on VOO quality parameters and oxidative state

According to the results showed in Table 2, all the oils were classified into the `extra virgin´ category for the quality parameters studied. Since the values were far below the limits fixed for extra virgin olive oil: free acidity ≤ 0.8 g oleic acid/100 g oil, peroxide value ≤ 20 meq O_2/kg, ethyl esters ≤ 35 mg/kg and ultra-violet absorption at 232 ≤ 2.5 and 270 nm ≤ 0.22 (European Commission, 2013).

Because of the acoustic mechanisms associated to HPU application a special interest was focused on those parameters related to the oil hydrolysis and oxidation. In general, the oil quality parameters analyzed did not show important differences between treatments for both harvesting dates, since slight differences were observed.

Ethyl esters content showed, for all the oils, values < 3 mg/kg and no effect of HPU treatment was observed. Therefore the values were too far from the limit of 35 mg/kg olive oil established by the European Regulation EEC 2568/91 for the crop year 2015/2016 (European Commission, 2013).

Table 2. Quality indices and oxidative state of VOO obtained for malaxed (M/C) and not malaxed (C) olive pastes submitted to reference (Ref) and High Power Ultrasounds at 20 kHz (T20), 40 kHz (T40) and 80 kHz (T80) for both harvesting dates.

	Free acidity (%)	Peroxide value (mEq O_2/kg)	Ethyl ester (mg/kg)	UV absorbance K_{232}	UV absorbance K_{270}	Oxidative stability (h)
Treatment of 30 January						
Ref (C)	0.17±0.00* a	5.97±0.18 ab	< 3	1.63±0.04 ab	0.16±0.03 a	175±8 a
Ref (M/C)	0.17±0.00 a	6.17±0.15 ab	< 3	1.59±0.02 b	0.13±0.01 a	159±4 a
T20 (C)	0.17±0.00 a	6.06±0.21 ab	< 3	1.60±0.02 ab	0.15±0.01 a	148±27 a
T20 (M/C)	0.17±0.00 a	6.40±0.53 a	< 3	1.62±0.02 ab	0.15±0.02 a	165±8 a
T40 (C)	0.17±0.00 a	6.27±0.20 a	< 3	1.64±0.03 ab	0.17±0.02 a	151±17 a
T40 (M/C)	0.17±0.00 a	5.87±0.42 ab	< 3	1.61±0.01 ab	0.13±0.01 a	163±12 a
T80 (C)	0.17±0.00 a	5.33±0.32 b	< 3	1.58±0.03 b	0.14±0.02 a	172±10 a
T80 (M/C)	0.17±0.00 a	6.16±0.07 ab	< 3	1.68±0.03 a	0.17±0.01 a	160±10 a
Treatment of 05 February						
Ref (C)	0.19±0.03 ab	3.98±0.28 a	< 3	1.74±0.05 a	0.18±0.03 a	159±10 a
Ref (M/C)	0.17±0.00 b	4.26±0.08 a	< 3	1.71±0.02 a	0.18±0.01 a	165±8 a
T20 (C)	0.17±0.00 b	3.68±0.23 a	< 3	1.69±0.03 a	0.17±0.02 a	165±8 a
T20 (M/C)	0.21±0.03 ab	4.31±0.09 a	< 3	1.67±0.01 a	0.16±0.01 a	165±6 a
T40 (C)	0.19±0.03 ab	4.21±0.41 a	< 3	1.70±0.03 a	0.17±0.01 a	169±11 a
T40 (M/C)	0.17±0.00 b	4.18±0.14 a	< 3	1.71±0.01 a	0.18±0.01 a	177±2 a
T80 (C)	0.17±0.00 b	3.90±0.39 a	< 3	1.72±0.01 a	0.18±0.01 a	179±5 a
T80 (M/C)	0.23±0.00 a	4.43±0.06 a	< 3	1.67±0.02 a	0.17±0.02 a	174±3 a

* Mean value ± SD (n = 3)
Different letters represent significant differences at p = 0.05.

Lipid oxidation is one of the most critical factors affecting the shelf life of VOO. The VOO acquire the resistance to autoxidation because of its monounsaturated fatty acid composition and minor compound with antioxidant activity. Therefore, knowing how the HPU treatment affects to the oxidative stability of the VOO has great interests. Table 2 showed the results of the oxidative stability of VOO obtained in the different experiments, there were not significant effect of the HPU treatment on the VOO oxidative stability. The values obtained were in accordance with those observed by (Mateos et al., 2006) going from 148 to 179 hours for both harvesting dates, confirming as HPU did not affect to VOO stability.

3.3. Effect of HPU treatment on VOO composition

No changes in the oil phenol content and bitterness index (K_{225}) were observed for the ultrasound treatments for the first harvesting date (Table 3) as observed by Bejaoui et al. (2015). Meanwhile for the second harvesting date a slight decrease of Total phenol content for the oils from malaxed and HPU treated pastes at 20 kHz and 40 kHz. For bitterness K_{225}, the lowest values were obtained for the oils from malaxed paste treated at 40 kHz.

The oil pigments increase with the paste malaxation (Criado et al., 2007). The malaxed paste after HPU treatment gave oils with higher chlorophyll and carotenoids content than the reference with malaxation (Table 3) as reported by Bejaoui et al. (2016, 2015) and Jiménez et al. (2007). The highest pigment increase was noted for the oils from olive pastes treated at 80 kHz and malaxed.

Regarding to tocopherols, three compounds were identified in the oils α, β and γ-tocopherol (Table 3). The major tocopherol in the virgin olive oil was the α-tocopherol (Beltrán et al., 2010). Non-significant differences between sonication treatments were observed for this compound, whereas the malaxation increase its content comparing with those oils from the directly centrifuged paste. β and γ-tocopherols were not affected by the treatments tested.

Table 3. VOO nutritional composition obtained for malaxed (M/C) and not malaxed (C) olive pastes submitted to reference (Ref) and High Power Ultrasounds at 20 kHz (T20), 40 kHz (T40) and 80 kHz (T80) for both harvesting dates.

	Total phenols (mg/kg)	K_{225}	Tocopherols (mg/kg)				Pigments (mg/kg)	
			α	β	γ	Total	Carotenoids	Chlorophyll
Treatment of 30 January								
Ref (C)	571±52* a	0.37±0.01 a	337±0 c	1±0 a	24±0 a	362±0 c	6.93±0.21 b	4.77±0.25 abc
Ref (M/C)	577±21 a	0.37±0.02 a	350±3 abc	1±0 a	24±0 a	375±3 ab	7.33±0.42 ab	5.00±0.87 abc
T20 (C)	521±17 a	0.37±0.01 a	336±2 c	1±0 a	23±1 a	360±2 c	7.07±0.21 b	5.10±0.50 abc
T20 (M/C)	546±17 a	0.37±0.03 a	351±0 ab	1±0 a	24±0 a	376±0 ab	7.27±0.06 ab	4.30±0.00 bc
T40 (C)	575±57 a	0.38±0.02 a	341±6 bc	1±0 a	23±1 a	365±7 bc	7.77±0.65 ab	5.77±0.85 ab
T40 (M/C)	553±62 a	0.39±0.02 a	343±10 abc	1±0 a	24±1 a	368±10 abc	6.67±0.84 b	3.97±0.75 c
T80 (C)	541±2 a	0.35±0.01 a	339±2 bc	1±0 a	24±0 a	364±2 bc	6.90±0.10 b	4.87±0.21 abc
T80 (M/C)	598±11 a	0.39±0.01 a	353±2 a	1±0 a	24±0 a	378±2 a	8.30±0.20 a	6.30±0.36 a
Treatment of 05 February								
Ref (C)	640±33 a	0.44±0.03 a	372±1 b	1±0 a	26±0 a	399±1 b	6.70±0.53 d	3.37±0.85 abc
Ref (M/C)	688±8 a	0.40±0.02 ab	398±2 a	1±0 a	26±0 a	425±2 a	7.53±0.12 bc	3.33±0.50 bc
T20 (C)	621±8 ab	0.41±0.02 ab	378±5 b	1±0 a	26±0 a	405±5 b	6.27±0.38 d	2.63±0.59 c
T20 (M/C)	558±21 b	0.36±0.02 bc	400±4 a	1±0 a	25±1 a	426±5 a	7.93±0.12 ab	3.93±0.21 ab
T40 (C)	643±26 a	0.41±0.01 ab	376±2 b	1±0 a	26±0 a	403±2 b	6.53±0.29 d	3.07±0.15 bc
T40 (M/C)	547±3 b	0.32±0.01 d	397±2 a	1±0 a	26±1 a	424±2 a	7.97±0.06 ab	3.97±0.06 ab
T80 (C)	650±29 a	0.36±0.02 bc	377±1 b	1±0 a	26±0 a	404±1 b	6.80±0.10 cd	3.30±0.10 bc
T80 (M/C)	652±50 a	0.43±0.03 a	403±1 a	1±0 a	25±1 a	429±1 a	8.47±0.25 a	4.60±0.36 a

* Mean value ± SD (n = 3)
Different letters represent significant differences at p = 0.05.

4. Conclusion

The High Power Ultrasound application to the olive paste allowed an instantaneous and homogeneous heating of olive paste, in continuous conditions, comparing with the traditional malaxation that need longer time. Therefore, it can be used to shorten the malaxation. HPU produced oil yield increase comparing with malaxation of untreated paste. The HPU treatment at 40 kHz and malaxation showed highest oil yields. For the HPU application at 40 and 80 kHz without malaxation gave a similar oil yields to conventional malaxation and then it may be used in olive paste preparation as a new technology to substitute the conventional malaxation.

Also the HPU frequency effect depend on the olive fruits characteristics particularly its moisture content. The ultrasound wave propagation favored the mass and heat transport in the medium, also help to destroy the cellular structures and the liberation of the oil

from cell vacuole. Therefore the sonication treatment can be applied as aid or substitute to olive paste malaxation allowing to shortcut considerably the elaboration process more specifically the malaxation step.

Regarding to the Virgin Olive Oil quality no deterioration or alteration on its oxidative state were observed with the HPU treatments and for the three different frequencies tested (20, 40 and 80 kHz), also for minor compounds no changes were noted. Furthermore, the malaxation of HPU treated olive paste did not show differences on the VOO quality and composition.

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Chapter 7. High power ultrasound frequency for olive paste conditioning: Effect on the virgin olive oil bioactive compounds and sensorial characteristics

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Contents

High power ultrasound frequency for olive paste conditioning: Effect on the virgin olive oil bioactive compounds and sensorial characteristics

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Abstract

High power utrasound (HPU) treatment of olive paste is used to enhance malaxation or as alternative to malaxer. Because previous laboratory scale experiments were conducted at different frequencies, its necessary to determine the best work frequency for future application at indutrial scale. For these reasons experiment of HPU treatment applying three differnet frequencies 20, 40 and 80 khz were carried out and compared with a reference without treatment. The virgin olive oil (VOO) were exracted after treatments by two procedures: HPU application and direct centrifugation and HPU application followed by malaxation and centrifugation. HPU treatment did not show ateration on the VOO fatty acid composition and phenolic compounds. The volatile compounds, related to the positive sensorial attribute, showed levels similar to those from conventional malaxation and those related to the off flavours were not observed. The HPU treatment gave Extra VOO with a more equilibrated sensorial profile.

Key words: High Power Ultrasound Frequency, Virgin Olive Oil, Fatty Acid, Phenolic compounds, Volatile Compounds, Sensory Characteristics.

Abbreviations: Virgin Olive Oil (VOO), High Power Ultrasound (HPU), Fatty Acid Methyl Esters (FAMEs), High Performance Liquid Chromatography (HPLC), Gas Chromatograph (GC).

1. Introduction

The virgin olive oil (VOO) represents one of the major components of the Mediterranean diet, this singular product has great organoleptic and bioactive properties. The main compounds involved in its nutritional effect are the fatty acids composition and phenolic compound as claimed by the European Commission (2012). The sensory characteristics are due to phenolics and volatiles (Aparicio and Harwood, 2013; Barranco Navero et al., 2008; Uceda et al., 2010). The extraction conditions have a deep influence on the nutritional and sensory characteristics of VOO and those compounds related to phenolics and volatiles.

The phenolic fraction of VOO has a great interest for its health-promoting proprieties since a specific health claim was regulated by European Commission (2012) and related to bitterness, pungency and astringency in VOO (Aparicio and Harwood, 2013). The phenolic compounds are released or formed during crushing and malaxation, their presence in the VOO depend on biochemical reaction involving enzymes, such as β-glucosidase (Romero-Segura et al., 2012), polyphenoloxidase and peroxidase (García-Rodríguez et al., 2011) and the partition coefficients between aqueous and oil phases. In the malaxation step, the phenolic compounds were affected mainly by the malaxation temperature (Aguilera et al., 2015; Angerosa et al., 2001; Clodoveo, 2013; Curtis M. Kalua et al., 2006; Servili et al., 2003).

The VOO volatile compounds, related to the organoleptic properties of VOO, are formed during the extraction and more precisely during the olive crushing and olive paste malaxation. The volatiles, related to positive sensory attribute, are formed through an enzymatic pathway known as `Lipoxygenase´ pathway involving different enzymes, Acyl hydrolase (AH), Lipoxygenase (LOX), Hydroperoxide lyase (HPL), Alcohol dehydrogenase (ADH), Alcohol acetyl transferase (AAT) from fatty acid (Linoleic acid and Linolenic acid) (Kalua et al., 2007; Salas and Sánchez, 1999; Sánchez-Ortiz et al., 2013). The malaxation time increased the content of C5 and C6 volatiles, and also promoted the Hexanal formation (Angerosa et al., 2001). Whereas malaxation temperature has a negative effect on volatile compounds formation (Angerosa et al., 2001; Salas and Sánchez, 1999).

Because the olive crushing some biochemical reactions are achieved, during paste malaxation are synthetized some nutritional and aroma compounds. Therefore, the

malaxation step variables influencing these compounds are: temperature, time, atmosphere composition and technological coadjuvants (Aguilera et al., 2015, 2010; Angerosa et al., 2004, 2001; Boselli et al., 2009; Clodoveo, 2012; Di Giovacchino, 2013; Di Giovacchino et al., 2002; Hermoso et al., 1998; Kalua et al., 2007; Curtis M Kalua et al., 2006; Sanchez-Ortiz et al., 2012).

Recently some new techniques were used to enhance the olive paste malaxation, among them application of the High Power Ultrasounds (Bejaoui et al., 2016a, 2016b; Clodoveo et al., 2013; Jiménez et al., 2007). The VOOs obtained from HPU treated olive paste showed lower phenolic content than those from untreated olive pastes (Bejaoui et al., 2016a, 2016b; Clodoveo et al., 2013; Jiménez et al., 2007). Jiménez et al. (2007) found that HPU decreased the bitterness of VOO comparing with the untreated olive pastes. Also, Bejaoui et al. (2016a, 2016b) showed as the sonication affected the phenolic compounds and more specially the secoiridoid derivatives.

The volatile compounds also were studied by Bejaoui et al. (2016a, 2016b) and Jiménez et al. (2007), describing as the sonication of olive paste did not induced autoxidation reactions, furthermore Jiménez et al. (2007) described a reduction of the Hexanal/E-2-hexenal ratio.

In relation with the sensory characteristics, the HPU treatment, as observed by Jiménez et al. (2007), showed higher intensity of positive attributes and lesser for negative characteristics than for conventional malaxation. Clodoveo et al. (2013) did not show appearance of negative sensorial characteristics and the oils obtained were less bitter than for conventional malaxation.

The variability in the ultrasound frequency and application conditions used in the previous works gave different effects on VOO nutritional and sensorial characteristics. The aim of this work was the study of effect of HPU application, at different frequencies as help or alternative to malaxation operation, on the VOO bioactive, volatile and sensory characteristics.

2. Materials and methods

2.1. Experimental procedure

The experiments were carried out using `Picual´ olive cultivar *Olea europaea* L. harvested in the 2013/2014 crop year at two different dates, showing different ripening stages 4.32 and 5.36 (Beltran et al., 2003) and different moisture 42.31 % and 45.48 %

respectively. The olives were picked from mature trees grown under irrigation in the experimental orchard of IFAPA `Center Venta del Llano´ in Mengíbar, Spain. The olives were harvested by a mechanical shocker and transported immediately to the experimental `IFAPA´ oil mill for processing (Figure 1).

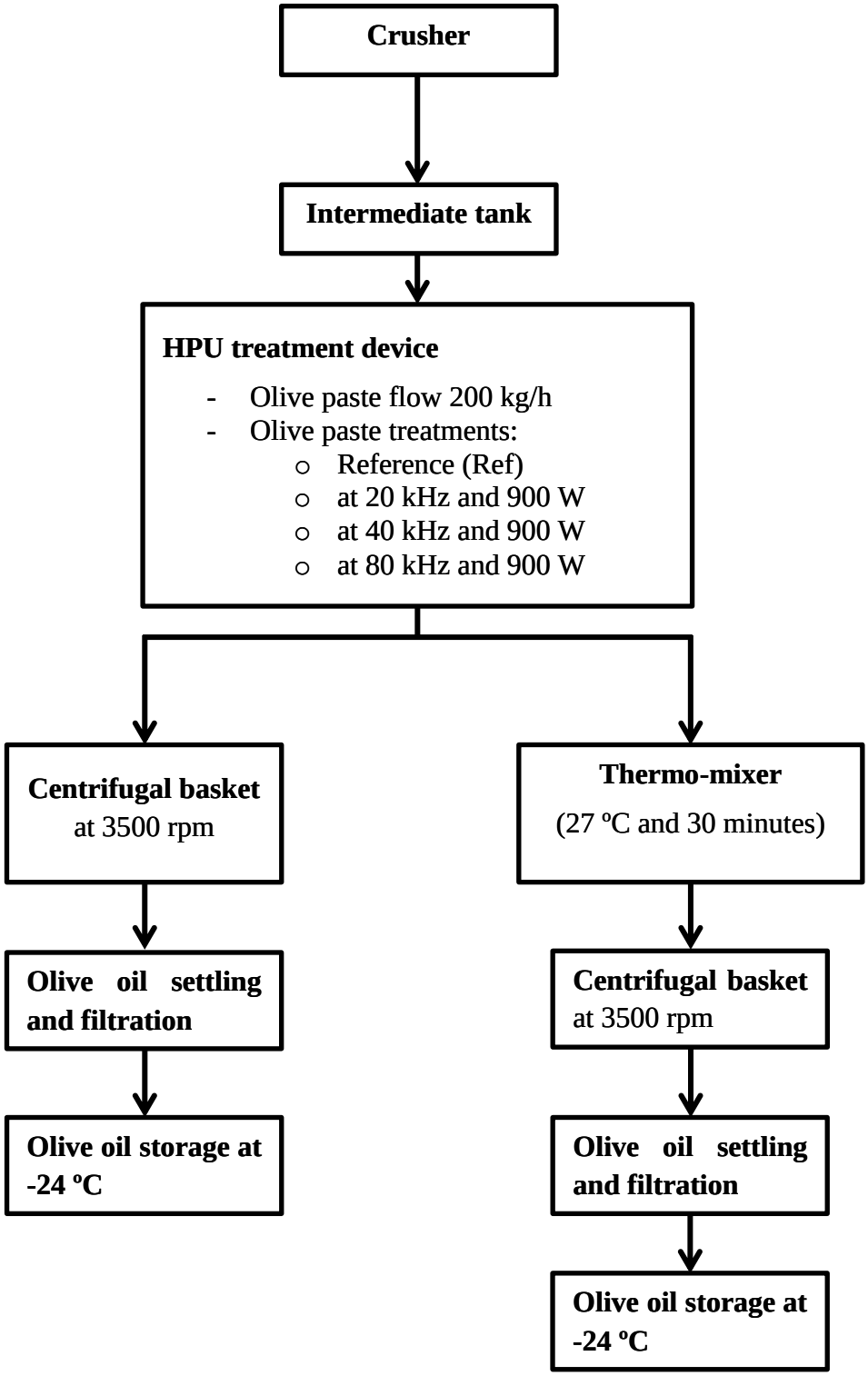

Figure1: Flow diagram of the olive oil extraction and experiment procedures

For the experiments 500 kg of olives was used from homogeneous lots of 6000 kg. Olives were milled, using a hammer crusher (Pieralisi, Spain) equipped with a 7 mm sieve. After crushing, the olive paste was sent to an intermediate stainless steel tank equipped with a pump to transport the olive paste to the ultrasound device. The olive pastes were passed through the HPU device at a constant flow rate of 200 kg/h. This device was built in a rectangular pipeline applying the ultrasound through its four faces. The device can apply three different HPU frequencies 20 kHz, 40 kHz and 80 kHz with power regulation. The olive pastes were subjected to three different HPU frequencies delivered by the device at 900 W. These treatments were compared with untreated olive paste passing through the device at 200 kg/h without sonication.

The olive paste from each treatment was collected at the HPU device exit and the olive oil was extracted at laboratory using an ``Abencor´´ system (Martínez et al., 1999). In order to test if olive paste sonication could help to improve or substitute the paste malaxation step, two different extraction procedures were tested. For malaxation aid the olive pastes from each HPU treatment were malaxed at 27 ºC for 30 minutes and then centrifuged (M/C), whereas to test HPU as malaxation alternative the olive pastes obtained were centrifuged at 3500 rpm without malaxation (C). Figure 1 shows the design and procedures carried out during experiments. For each HPU treatment and extraction conditions (centrifuged or malaxed and centrifuged) three olive pastes samples were taken for oil extraction. Finally, the liquid phases were recovered in a graduated cylinder. After settling, the oily phase was taken, filtered and stored at -24 ºC until analysis.

2.2. Fatty acid composition

The Fatty Acid Methyl Esters (FAMEs) were prepared as described by the EU official method (Comisión Europea, 2015). Then the FAMEs were analyzed by chromatographic separation using a Perkin-Elmer Autosystem gas chromatograph (Perkin-Elmer, Spain) equipped with an autosampler, a split/splitless injector, and a flame ionization detector (FID). The operational conditions were as described by Beltrán et al. (2004). A fused silica capillary column BPX70 (50 m length × 0.25 mm i.d. and 0.25 μm film thickness, SGE Scientific Pty. Ltd., Australia) was used. Helium was used as the carrier gas, and the oven temperature was maintained at 198 °C. The

injector and detector temperatures were 235 and 245 °C, respectively. The results were expressed as relative area percent of the total.

2.3. Phenolic compounds determination

The extraction of the phenolic compounds from the olive oil was carried as described by (Beltrán et al., 2000). An amount of 1.5 g olive oil was weighted and dissolved in 1 mL of hexane, a 1.25 ml methanol:water (60:40 v:v) and then the mixture was centrifuged at 4000 rpm during 6 minutes, this operation was repeated. After separation the methanol: water extract was filtered.

RP-HPLC Determination of Phenols (Beltrán et al., 2000): HPLC analysis was performed using a Hewlett Packard 1100 (Hewlett Packard, Spain) system equipped with an autosampler, quaternary pump, and a diode array detector. A reversed-phase C18 Pecosphere column (83 × 4.6 mm i.d., 3 μm particle size, Brown Lee Columns) was used with an injection volume of 20 μL and a flow rate of 0.45 mL/min. The mobile phase was a mixture of water/acetic acid (98:2 v/v) (solvent A) and methanol/acetic acid (98:2 v/v) (solvent B). The total run time was 70 min, the solvent gradient changed according to the following conditions: 90 % A–10 % B for 10 min, 80 % A–20 % B in 8 min then remained for 2 min, 60 % A–40 % B in 10 min, 50 % A–50 % B in 10 min, and 100 % B in 10 min until the end of the run.

The phenolic compounds were identified as described by Mateos et al. 2001 and using standards hydroxytyrosol and tyrosol from EXTRASYNTHESE, (Lyon, France) and vanillic acid, p-coumaric acid and ferulic acid from Sigma Aldrich (San Luis, USA). Phenolic compounds were quantified at 280 nm using syringic acid as internal standard and the response factors determined by Mateos et al. 2001. The results were expressed as mg/kg.

2.4. Volatile compounds determination

Volatile compounds in the olive oil samples were analyzed by HS-SPME as described by Sánchez-Ortiz et al. (2013). Olive oil samples were tempered at room temperature. Then, 1.000 ± 0.001 g of each oil sample was weighted and placed in a 10 mL glass vial capped with polytetrafluoroethylene septum (PTFE).

After the samples were left for 10 min at 40 °C on a heating magnetic platform agitation until reach the equilibration of the volatile compounds in the headspace. After this the

volatile substances were adsorbed using a 50/30 μm divinylbenzene–carboxen–polydimethylsiloxane fiber (DVB–CAR–PDMS). Desorption of volatile compounds trapped in the SPME fiber was performed directly into the gas chromatograph (GC) Varian 3800 (Varian, Walnut Creek, CA) GC instrument with a flame ionization detector (FID), and equipped with a SupelcoWax® 10 capillary column (30 m × 0.25 mm, 0.25 μm, Sigma-Aldrich Co. LLC). Helium was used as carrier gas at a flow rate of 0.9 mL/min, and the desorption temperature program included the following settings: injector and detector temperature of 250 °C; column held for 5 min at 40 °C and then ramped at 4 °C/min to 200 °C and held for 5 min.

Compound identification was performed using a mass spectrometer (ISQ single quadrupole MS, Thermo Fisher Scientific, Austin, Texas, USA). Quantification was performed using individual calibration curves for each identified compound by adding known amounts of different compounds to deodorized olive oil. Results were expressed as mg/kg VOO.

2.5. Sensory analysis

Sensory Analysis was performed by the panel of the Citoliva Foundation laboratory during a series of oil-tasting sessions, in accordance with the European Commission (2015), this determination was only carried out for the first harvesting date.

Based on these regulations, the tasters smelt and tasted each olive oil sample to evaluate its positive attributes according to the list of descriptors included in this method. The sensory profile of each VOO sample was expressed as the median value for each descriptor. Moreover, the tasters evaluated direct or retronasal aromatic olfactory sensations (fruity, green leaf/fresh-cut grass, apple, almond, artichoke), and other positive attributes; gustatory sensations (bitterness and sweetness); and tactile/kinesthetic sensations (pungency) according to International Olive Council (2015). The tasters had to rate the intensity of the different descriptors on a continuous 0-10 cm scale.

2.6. Data analysis

The results were expressed as mean ± standard deviation (n = 3). Analysis of variance was applied, significant differences between treatments were determined applying Tukey's test $p < 0.05$ (Statistix 9.0 software, Analytical Software, USA).

3. Results and discussion

3.1. Fatty acid composition

Olive oil is characterized by containing high proportion of monounsaturated fatty acids and more precisely the oleic acid and by a low content of saturated fatty acids. This fatty acid composition awakens interest in the health benefit of the VOO (Aparicio and Harwood, 2013; European Commission, 2012). Table 1 showed fatty acids composition (% m/m methyl esters) of the oils obtained were into the standard established (European Commission, 2015) for Extra Virgin Olive Oil.

Table 1. Fatty acid composition in percentage for VOO obtained for malaxed (M/C) and not malaxed (C) olive pastes submitted to reference (Ref) and High Power Ultrasounds at 20 kHz (T20), 40 kHz (T40) and 80 kHz (T80) for both harvesting dates.

	Ref (C)	Ref (M/C)	T20 (C)	T20 (M/C)	T40 (C)	T40 (M/C)	T80 (C)	T80 (M/C)
Trial 1								
C16:0	10.81 ± 0.08* a	10.84 ± 0.19 a	10.88 ± 0.05 a	10.90 ± 0.12 a	10.79 ± 0.05 a	10.80 ± 0.07 a	10.83 ± 0.12 a	10.89 ± 0.11 a
C16:1	0.84 ± 0.01 a	0.85 ± 0.02 a	0.84 ± 0.01 a	0.83 ± 0.00 a	0.83 ± 0.01 a	0.84 ± 0.02 a	0.83 ± 0.02 a	0.84 ± 0.02 a
C17:0	0.04 ± 0.00 a	0.04 ± 0.00 a	0.04 ± 0.00 a	0.04 ± 0.00 a	0.04 ± 0.00 a	0.04 ± 0.01 a	0.04 ± 0.00 a	0.04 ± 0.01 a
C17:1	0.07 ± 0.01 a	0.07 ± 0.00 a	0.08 ± 0.01 a	0.06 ± 0.02 a	0.08 ± 0.00 a	0.08 ± 0.00 a	0.08 ± 0.00 a	0.07 ± 0.01 a
C18:0	3.22 ± 0.02 a	3.21 ± 0.01 a	3.24 ± 0.02 a	3.21 ± 0.02 a	3.25 ± 0.01 a	3.25 ± 0.01 a	3.24 ± 0.03 a	3.22 ± 0.03 a
C18:1	80.35 ± 0.08 a	80.32 ± 0.24 a	80.32 ± 0.07 a	80.25 ± 0.02 a	80.36 ± 0.10 a	80.37 ± 0.06 a	80.35 ± 0.15 a	80.27 ± 0.07 a
C18:2	3.47 ± 0.02 ab	3.50 ± 0.03 a	3.44 ± 0.01 ab	3.48 ± 0.03 a	3.46 ± 0.03 ab	3.47 ± 0.03 ab	3.42 ± 0.01 b	3.48 ± 0.01 ab
C18:3	0.58 ± 0.02 a	0.59 ± 0.01 a	0.57 ± 0.01 a	0.58 ± 0.01 a	0.58 ± 0.00 a	0.59 ± 0.01 a	0.57 ± 0.01 a	0.58 ± 0.01 a
C20:0	0.35 ± 0.00 a	0.33 ± 0.01 a	0.34 ± 0.01 a	0.33 ± 0.02 a	0.34 ± 0.01 a	0.34 ± 0.01 a	0.35 ± 0.02 a	0.34 ± 0.02 a
C20:1	0.19 ± 0.00 a	0.18 ± 0.01 a	0.18 ± 0.01 a	0.18 ± 0.02 a	0.18 ± 0.02 a	0.17 ± 0.01 a	0.19 ± 0.01 a	0.18 ± 0.01 a
C22:0	0.06 ± 0.01 a	0.06 ± 0.01 a	0.07 ± 0.01 a	0.06 ± 0.01 a	0.07 ± 0.01 a	0.05 ± 0.01 a	0.07 ± 0.01 a	0.07 ± 0.02 a
SFA	14.49±0.07a	14.48±0.18a	14.56±0.06a	14.55±0.09a	14.49±0.06a	14.47±0.07a	14.53±0.15a	14.56±0.06a
UFA	84.92±0.07a	84.91±0.2a	84.86±0.06a	84.81±0.04a	84.91±0.06a	84.93±0.07a	84.88±0.14a	84.84±0.06a
MUFA	81.45±0.07a	81.41±0.22a	81.41±0.06a	81.33±0.04a	81.45±0.1a	81.46±0.05a	81.46±0.15a	81.37±0.06a
PUFA	4.05±0.03ab	4.08±0.04a	4.02±0.02ab	4.07±0.03ab	4.04±0.03ab	4.06±0.03ab	3.99±0.01b	4.05±0.01ab
Trial 2								
C16:0	10.17±0.09 c	10.89±0.32 a	10.29±0.04 bc	10.80±0.08 a	10.25±0.05 bc	10.93±0.08 a	10.50±0.26 abc	10.68±0.06 ab
C16:1	0.80±0.02 c	0.86±0.03 a	0.83±0.01 abc	0.83±0.02 abc	0.82±0.01 bc	0.87±0.01 a	0.84±0.02 ab	0.82±0.01 bc
C17:0	0.04±0.00 a	0.04±0.00 a	0.05±0.01 a	0.03±0.00 b	0.04±0.01 a	0.03±0.00 b	0.04±0.00 a	0.03±0.00 b
C17:1	0.07±0.00 a	0.07±0.00 a	0.07±0.01 a	0.07±0.01 a	0.07±0.01 a	0.07±0.01 a	0.07±0.00 a	0.07±0.01 a
C18:0	3.75±0.02 a	3.69±0.02 a	3.74±0.01 a	3.71±0.05 a	3.74±0.01 a	3.69±0.01 a	3.71±0.03 a	3.69±0.02 a
C18:1	79.80±0.10 a	79.07±0.25 d	79.63±0.05 ab	79.26±0.09 cd	79.69±0.08 ab	79.05±0.06 d	79.48±0.21 abc	79.35±0.03 bcd
C18:2	4.15±0.01 a	4.17±0.05 a	4.15±0.01 a	4.12±0.06 a	4.15±0.01 a	4.16±0.02 a	4.11±0.03 a	4.20±0.01 a
C18:3	0.62±0.01 ab	0.62±0.01 ab	0.63±0.02 a	0.59±0.01 b	0.63±0.01 a	0.63±0.02 a	0.62±0.02 ab	0.61±0.01 ab
C20:0	0.36±0.01 a	0.34±0.01 a	0.37±0.01 a	0.35±0.00 a	0.36±0.02 a	0.35±0.01 a	0.37±0.02 a	0.34±0.01 a
C20:1	0.18±0.01 a	0.17±0.02 a	0.18±0.01 a	0.17±0.02 a	0.18±0.01 a	0.17±0.01 a	0.18±0.02 a	0.16±0.01 a
C22:0	0.06±0.01 ab	0.05±0.01 b	0.07±0.01 ab	0.08±0.02 a	0.08±0.01 a	0.06±0.01 ab	0.08±0.01 a	0.06±0.00 ab
SFA	14.37±0.08c	15.02±0.28a	14.52±0.03bc	14.97±0.11a	14.47±0.04bc	15.06±0.07a	14.7±0.23abc	14.8±0.03ab
UFA	85±0.08a	84.35±0.28d	84.85±0.04abc	84.45±0.11cd	84.92±0.08ab	84.32±0.06d	84.68±0.22abcd	84.6±0.02bcd
MUFA	80.85±0.08a	80.18±0.24d	80.7±0.04ab	80.33±0.06cd	80.77±0.08a	80.16±0.05d	80.57±0.19abc	80.4±0.03bcd
PUFA	4.77±0a	4.8±0.05a	4.78±0.01a	4.71±0.07a	4.79±0.01a	4.79±0.02a	4.73±0.04a	4.8±0.01a

* Mean value ± SD (n = 3)
Different letters represent significant differences at p = 0.05.
C16:0 Palmitic acid, C16:1 Palmitoleic acid, C17:0 Margaric acid, C17:1 Heptadecenoic acid, C18:0 Stearic acid, C18:1 Oleic acid, C18:2 Linoleic acid, C18:3 Linolenic acid, C20:0 Arachidic acid, C20:1 Gadoleic acid, C22:0 Behenic acid. Fatty acid (FA) was grouped in saturated FA (SFA), unsaturated FA (UFA), monounsaturated FA (MUFA) and polyunsaturated FA (PUFA).

The oil fatty acid composition from all the treatments tested (Table 1) was similar to that described for `Picual´ VOO (Beltrán et al., 2004). Although significant differences were observed from the ANOVA analysis, they can be attributed to the variability in the `Picual´ fruit lot. Therefore, the fatty acids were not affected by the HPU application.

3.2. Phenolic compounds

VOO phenols generate great interest since the European Commission (2012) established a health claim specific for olive oil, that has to contain at least 5 mg of hydroxytyrosol and its derivatives (oleuropein complex and tyrosol) per 20 g of olive oil. Furthermore, their contribution to the VOO oxidative stability and their influence on the VOO sensorial characteristics (bitterness and pungent) increase their importance. The phenolic compounds identified in the VOOs obtained in this work for both harvesting dates (Table 2) were: phenolic alcohols (hydroxytyrosol, tyrosol), phenolic acids (vanillic acid, ρ-coumaric acid and ferulic acid) and secoiridoid derivatives (dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), dialdehydic form of elenolic acid linked to tyrosol (ρ-HPEA-EDA) and aldehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EA)). In general, all the treatment tested did not affect the VOO phenolic profile.

The total phenolics, sum of the identified phenols showed different behavior depending on the harvesting dates. For the first trials no significant differences were observed between treatments, whereas for the second date, the application of HPU followed by malaxation allowed an increase of the phenolic compounds respect to the reference without malaxation. This increase achieved the highest phenols content for those oils from the paste treated at 40 kHz and malaxed.

In general, total phenols showed higher concentration in those oils from malaxed pastes. Although a slight increase was observed when HPU frequency was higher significant differences were not obtained. When olive pastes were treated with HPU at 40 and 80 kHz, they gave oils with similar phenol content than those from conventional extraction procedure (malaxation and centrifugation).

Table 2: Phenolic composition (mg/kg VOO) of the virgin olive oils from the different treatments

	Centrifugation				Malaxation + Centrifugation			
	0KHz C	20KHz C	40KHz C	80KHz C	0KHz M/C	20KHz M/C	40KHz M/C	80KHz M/C
Trials 1								
Hydroxytyrosol	1.48 ± 0.08 b	1.65 ± 0.08 a, b	1.96 ± 0.49 a, b	1.88 ± 0.07 a, b	2.18 ± 0.24 a	2.16 ± 0.07 a	2.21 ± 0.18 a	2.24 ± 0.2 a
tyrosol	2.34 ± 0.1 c	2.41 ± 0.03 b, c	2.74 ± 0.39 a, b, c	2.79 ± 0.07 a, b, c	3.15 ± 0.04 a	3.05 ± 0.08 a	2.94 ± 0.32 a, b	3.06 ± 0.2 a
vanillic acid	0.16 ± 0.01 a, b	0.2 ± 0.06 a	0.14 ± 0.02 a, b	0.17 ± 0.03 a, b	0.14 ± 0.01 a, b	0.12 ± 0 b	0.13 ± 0.01 b	0.13 ± 0.01 b
p-coumaric acid	0.22 ± 0.01 c	0.23 ± 0.01 c	0.41 ± 0.18 a, b, c	0.6 ± 0.03 a	0.33 ± 0.03 b, c	0.38 ± 0.01 a, b, c	0.46 ± 0.12 a, b	0.51 ± 0.04 a, b
ferulic acid	0.27 ± 0.02 b	0.33 ± 0.03 a, b	0.39 ± 0.09 a, b	0.42 ± 0.03 a	0.36 ± 0.02 a, b	0.36 ± 0.02 a, b	0.43 ± 0.09 a	0.46 ± 0.04 a
3,4-DHPEA-EDA	196.77 ± 10.08 a	184.03 ± 3.65 a	199.25 ± 35.91 a	189.84 ± 6.71 a	192.03 ± 5.41 a	190.1 ± 3.35 a	198.25 ± 28 a	204.07 ± 16.38 a
ρ-HPEA-EDA	39.17 ± 1.05 b	37.62 ± 1.92 b	49.9 ± 14.16 a, b	50.92 ± 1.11 a, b	49.18 ± 1 a, b	54.16 ± 1.37 a, b	54.56 ± 11.92 a, b	60.32 ± 2.83 a
3,4-DHPEA-EA	104.99 ± 2.94 a	106.23 ± 3.86 a	119.32 ± 13.6 a	110.48 ± 4.77 a	116.02 ± 11.23 a	118.3 ± 4.48 a	117.86 ± 8.46 a	114.47 ± 8.76 a
Total	345.39±9.08a	332.7±5.07a	374.11±62.53a	357.1±6.57a	363.39±17.32a	368.64±4.71a	376.85±48.91a	385.26±27.74a
Trials 2								
Hydroxytyrosol	1.85±0.06a	1.35±0.66ab	1.09±0.04b	1.13±0.07b	1.35±0.04ab	1.35±0.06ab	1.38±0.07ab	1.45±0.13ab
tyrosol	1.23±0.04b	1.26±0.13ab	1.26±0.05ab	1.28±0.03ab	1.43±0.03ab	1.36±0.03ab	1.39±0.06ab	1.44±0.11a
vanillic acid	0.05±0.01a	0.05±0.02a	0.05±0a	0.04±0a	0.05±0a	0.05±0.01a	0.04±0a	0.06±0.01a
p-coumaric acid	0.22±0.03f	0.31±0.01e	0.45±0.02d	0.64±0.01a	0.36±0.02e	0.46±0.01cd	0.54±0.03bc	0.59±0.06ab
ferulic acid	0.34±0.06e	0.36±0.03de	0.42±0.06cde	0.59±0.02ab	0.48±0.06bcd	0.52±0.05bc	0.55±0.02abc	0.66±0.05a
3,4-DHPEA-EDA	252.16±5.68a	262.85±3.92a	255.74±2.86a	260.97±8.93a	281.18±7.92a	283.74±4.99a	288.31±4.88a	264.85±37.7a
ρ-HPEA-EDA	54.89±2.71f	57.66±2.91f	66.41±1.07e	83.25±1.87c	74.74±1.16d	82.73±1.38c	88.41±1.27b	93.86±0.39a
3,4-DHPEA-EA	121.08±2.74b	127.44±3.21b	142.01±4.76a	149.95±4.38a	146.94±1.38a	143.47±3.13a	146.73±2.51a	147.37±9.17a
Total	431.82±7.77d	451.27±10.49cd	467.43±7.78bcd	497.84±13.41abc	506.52±7.21ab	513.67±8.94ab	527.35±7.99a	510.28±47.46ab

* Mean value ± SD (n = 3)

Different letters represent significant differences at p = 0.05.

The phenolic alcohols, hydroxytyrosol and tyrosol, were found at higher contents in the oils from malaxed pastes. It could be explained because of hydrolysis processes described previously (Aparicio and Harwood, 2013). Also significant differences were observed between treatments because of the variability in the fruit used in the experiments. Therefore, HPU application with or without malaxation produced VOO with phenolic alcohols content similar to the conventional extraction.

Regards to the phenolic acids, they also showed different response against the treatments assayed. Vanillic acid did not show significant difference between the treatments assayed. ρ-coumaric acid was observed at the highest content for the VOO extracted directly from treated paste at 80 kHz in both trials dates, for the rest of treatments no significant effects were noted. In relation with the Ferulic acid content, the oil from HPU treated and malaxed pastes showed higher content than reference from untreated pastes.

The main phenolic group identified in this work was the secoiridoid derivatives. These compounds were the most abundant phenols representing more than the 90 % of the total amount. These compounds are known because of their antioxidant activity, heath benefits and a positive relation with bitterness and pungency of the VOO (Aparicio and Harwood, 2013). They are formed through hydrolysis of phenolic glycosides present in olives (oleuropein, ligstroside and demethyloleuropein) in the steps of crushing and olive paste malaxation involving some endogenous enzymes such as β-glucosidase, polyphenol oxidase and peroxidase (García-Rodríguez et al., 2011; Romero-Segura et al., 2012).

3,4-DHPEA-EDA as observed for total amount, when the olive paste preparation included the malaxation its concentration was slightly higher although without significant differences. In general, this phenol was not affected by HPU application for both harvesting dates.

Dialdehydic form of elenolic acid linked to tyrosol (ρ-HPEA-EDA) (Table 2), concentration showed a lineal response to the frequency increase of HPU for both with and without malaxation, achieving the higher content for 80 kHz treatments. In general p-HPEDA was observed at higher levels when the olive paste was malaxed.

Other secoiridoid derivative is 3,4-DHPEA-EA, this compound was not affected by either HPU application and malaxation.

In conclusion the HPU application did not produced alteration on total phenolic content, meanwhile the malaxation of olive pastes allowed to obtain higher concentration than the unmalaxed olive pastes. The only phenolic compound affected by the HPU treatment was the ρ-HPEA-EDA showing higher content as HPU frequency was increased.

3.3. Volatile compounds

The volatile compounds, related to the sensorial characteristics of the virgin olive oil, are biosynthesized during the olive paste preparation (Sanchez-Ortiz et al., 2012; Sánchez-Ortiz et al., 2013) and deeply affected by the processing conditions (Angerosa et al., 2001; Clodoveo, 2012; Di Giovacchino et al., 2002).

The volatiles compounds were grouped in C6 LOX and C5 LOX compounds (derived from LOX pathway) and other compounds related to negatives attributes. The total C6 LOX compounds (Table 3) were formed by C6 aldehydes (Hexanal, E-3-hexenal, Z-3-hexenal, Z-2-hexenal and E-2-hexenal), C6 alcohols (Hexanol, E-3-hexenol, Z-3-hexenol and E-2-hexenol) and C6 esters (Hexyl acetate, Z-3-hexenyl acetate and E-2-hexenyl acetate). The group of the C5 LOX (Table 4) was composed of C5 aldehydes ((Z)-pent-2-enal, (E)-pent-2-enal and pentanal), C5 alcohols (pent-1-en-3-ol, (E)-pent-2-en-1-ol, (Z)-pent-2-en-1-ol and pentan-1-ol), C5 Ketones (pent-1-en-3-one and 2+3-pentanone) and Pentene Dimers.

The total C6 LOX compounds showed higher concentration for the first trials than for the second one, reaching concentration decrease by a half. This decrease is due to fruit ripening (Gómez-Rico et al., 2006). The main components of the C6 LOX were the C6 aldehydes and especially, E-2-hexenal. In the second trials, the concentration of the E-2-hexenal showed a decrease around 70 %. Also the Hexanal content showed a decrease between first and second harvesting dates. The malaxation produced VOO with higher C6 LOX. The main compounds influenced by the malaxation were the C6 aldehydes and especially the E-2-hexenal. Its concentration was significatively higher for the oils from malaxed pastes; this trend was more accentuated for the trial 1. Malaxation did not show any effect on the oil content of C6 alcohols and C6 esters content.

The HPU treatment and malaxation of olive paste gave olive oils with higher content of LOX C6 volatiles than those obtained from untreated and unmalaxed pastes (Ref (C)).

The HPU treatment only affects the LOX C6 volatiles when it did not followed by malaxation, and for all malaxed olive pastes from all treatment testes no significant differences were observed. In the first trial, the concentration of this group of compounds increased by HPU frequency increase without posterior malaxation comparing with Ref (C). This increase was significant only for the treatment at T80 (C) reaching values as observed for malaxed olive pates. In the second trials, the total LOX C6 content tends to increase with ultrasound treatment and malaxation but not significant differences were observed from Ref (C).

Table 3: C6 volatile compounds (µg/kg VOO) derived from LOX pathway obtained in the VOO for the different treatments and different trial date.

	Centrifugation				Malaxation + Centrifugation			
	Ref (C)	T20 (C)	T40 (C)	T80 (C)	Ref (M/C)	T20 (M/C)	T40 (M/C)	T80 (M/C)
Trial1								
hexanal	972±28a	751±104a	694±116a	855±93a	977±111a	863±92a	866±44a	746±141a
E-3-hexenal	25±2a	41±4a	31±8a	36±7a	44±10a	44±1a	43±5a	36±3a
Z-3-hexenal	208±22c	477±68a	262±59bc	288±55bc	407±61ab	339±43abc	388±72ab	271±39bc
Z-2-hexenal	190±13b	256±45ab	242±41ab	283±20a	299±29a	279±23ab	294±17a	252±28ab
E-2-hexenal	5027±255c	6125±272c	7219±1127bc	9604±306a	8492±655ab	8636±448ab	8491±648ab	8574±936ab
Σ C6 aldehydes	5926±383c	7650±493bc	8448±1350bc	11067±451a	10219±840ab	10162±595ab	10082±556ab	9878±1147ab
Hexanol	374±7b	420±9ab	402±59ab	483±4a	465±23ab	443±21ab	461±10ab	420±53ab
E-3-hexenol	12±1b	12±0b	12±2ab	15±1a	14±1ab	13±1ab	13±1ab	14±1ab
Z-3-hexenol	442±50a	454±3a	452±65a	554±7a	526±24a	502±18a	516±26a	497±59a
E-2-hexenol	42±4a	43±1a	38±2a	40±3a	46±4a	44±5a	44±1a	41±1a
Σ C6 alcohols	840±0b	929±13ab	904±127ab	1092±14a	1052±51ab	1002±43ab	1034±35ab	972±114ab
hexyl acetate	792±72a	806±54a	786±27a	797±3a	795±5a	766±43a	779±16a	755±22a
Z-3-hexenyl acetate	2238±208a	2297±132a	2239±80a	2293±18a	2321±2a	2237±106a	2264±12a	2182±78a
E-2-hexenyl acetate	92±16a	69±2abc	87±3ab	62±6bcd	56±5cd	38±2d	62±11bcd	37±7d
Σ C6 esters	3122±288a	3172±187a	3083±54a	3132±28a	3172±2a	3041±147a	3105±29a	2975±98a
Total LOX C6	9722±331c	11751±693bc	12435±1516bc	15291±453a	14442±887ab	14204±696ab	14221±574ab	13824±1357ab
Trial2								
hexanal	494±17b	561±29ab	583±65ab	534±56b	549±41ab	462±17b	685±47a	540±50ab
E-3-hexenal	19±4b	25±1ab	25±2ab	30±2ab	35±6a	30±5ab	33±4a	35±7a
Z-3-hexenal	228±39a	275±7a	263±33a	240±19a	342±67a	261±47a	257±37a	266±47a
Z-2-hexenal	156±28a	191±7a	188±22a	175±11a	209±39a	180±28a	199±27a	166±1a
E-2-hexenal	1557±299b	1835±45ab	2010±162ab	2280±90a	2218±311a	2063±275ab	2400±241a	2459±278a
Σ C6 aldehydes	2214±487b	2887±74ab	2875±554ab	3260±177ab	3169±141ab	2842±98ab	3345±703a	3131±42ab
Hexanol	293±54a	335±6a	323±38a	286±21a	324±59a	290±51a	359±51a	365±23a
E-3-hexenol	10±1a	11±0a	12±1a	12±1a	11±1a	12±2a	13±1a	13±2a
Z-3-hexenol	253±41b	291±6ab	291±30ab	285±19ab	308±52ab	283±45ab	352±44ab	369±17a
E-2-hexenol	26±1c	32±1abc	31±2abc	29±3bc	34±2a	32±1abc	29±2bc	34±2ab
Σ C6 alcohols	573±98a	669±12a	658±71a	612±42a	677±114a	617±99a	753±97a	758±74a
hexyl acetate	872±68a	927±39a	897±64a	853±24a	922±35a	867±46a	898±36a	854±25a
Z-3-hexenyl acetate	1923±162a	2033±66a	1967±142a	1869±54a	2040±107a	1903±118a	2012±98a	1932±70a
E-2-hexenyl acetate	86±12a	67±11abc	80±2ab	58±9bcd	43±10d	45±3cd	39±7d	41±5d
Σ C6 esters	2881±240a	3027±96a	3063±28a	2781±75a	3006±150a	2815±166a	2949±140a	2827±97a
Total LOX C6	5669±690a	6583±85a	6947±170a	6653±290a	6851±393a	6274±359a	7048±931a	6734±172a

* Mean value ± SD (n = 3)
Different letters represent significant differences at p = 0.05.

The concentration of E-2-hexenal increased significantly with the malaxation comparing with the Ref (C) in the trials 1. All the oils obtained from malaxed olive pastes did not showed significant difference on the E-2-hexenal content. Whereas, for the oils from unmalaxed pastes, the E-hexenal showed different behavior, depending on the frequency applied. For the 20 and 40 kHz the content of this compound increased but without significant differences, meanwhile the oils from the pastes treated at 80 kHz showed a significant higher content of E-hexenal for all the oils obtained in the trails 1 then the Ref (C), treated T20 (C) and T40 (C). In the second trials the E-hexenal compounds variation showed the same trends as for the trials 1 but without significant differences at $p < 0.05$.

The group of the sum C6 esters (Table 3) did not show variation on its content with the different treatment and trials dates. Meanwhile the E-2-hexenyl acetate ester showed a decrease of it content when olive paste was submitted to HPU treatment and malaxation for both harvesting dates, this behavior was more significant for malaxed olive pastes.

For the LOX C6 alcohols (Table 3) the olive paste ultrasound treatments showed an increase of the concentration of the sum of these compounds without significant differences with the conventional malaxation. In the first harvesting date the Hexanol and E-3-hexenol were the compounds significantly affected by the HPU and malaxation treatments comparing with reference without malaxation, this increase was only significant for T80 (C) treatment. Whereas, in the second harvesting date the compound affected by the treatment were the Z-3-hexenol and E-2-hexenol.

On the other hand the LOX C5 (Table 4) compounds derived from additional reactions of the LOX pathway (Angerosa et al., 2004) was determined. The most abundant LOX C5 compounds were the pentene dimers representing more than 80 % of this group with a concentration around 3 mg/kg VOO. Other groups, C5 Ketones, C5 alcohols and C5 aldehydes were present in lower concentration. The concentration of the LOX C5 compounds did not change between the trials 1 to trials 2. With respect to the effects of the treatments on the LOX C5 compounds, the results did not showed variation or significant effect of malaxation or ultrasound treatments for both trials.

Other no LOX compounds were also identified in the oils obtained from the different treatment (Table 5). The No LOX alcohols showed an increase when olive pastes were malaxed. In general, those oils from HPU application without malaxation showed

higher alcohols content. The HPU frequencies had only effect when the HPU treated pastes were not malaxed, showing significant differences for the first trials. Attention should be paid in ethanol since is a precursor of the ethyl esters (Beltrán et al., 2015; Gómez-Coca et al., 2016). In general ethanol was observed at higher concentration for oil obtained from malaxed pastes.

Table 4. C5 volatile compounds (µg/kg VOO) derived from LOX pathway obtained in the VOO for the different treatments and different trial date.

	Centrifugation				Malaxation + Centrifugation			
	Ref (C)	T20 (C)	T40 (C)	T80 (C)	Ref (M/C)	T20 (M/C)	T40 (M/C)	T80 (M/C)
Trial 1								
(Z)-pent-2-enal	128±9a	130±0a	116±5ab	120±5ab	121±4ab	112±1ab	121±6ab	102±13b
(E)-pent-2-enal	16±1b	20±0ab	21±1a	24±1a	23±1a	23±1a	22±2a	22±2a
pentanal	43±7a	42±1abc	29±2cd	42±8ab	31±2abcd	28±2d	31±3bcd	34±3abcd
Σ C5 aldehydes	181±3abc	192±1a	165±8abc	187±12ab	175±4abc	163±1bc	174±5abc	158±18c
pent-1-en-3-ol	165±6b	186±8ab	174±31b	205±14ab	249±22a	213±15ab	233±28ab	176±27b
(E)-pent-2-en-1-ol	12±1a	13±0a	13±2a	14±0a	14±0a	14±0a	14±1a	14±1a
(Z)-pent-2-en-1-ol	73±3a	115±13a	107±10a	114±17a	102±12a	98±21a	105±11a	100±4a
pentan-1-ol	13±1a	14±0a	13±2a	15±4a	17±1a	15±3a	16±1a	14±3a
Σ C5 alcohols	183±87b	328±4a	306±40a	348±23a	382±19a	340±12a	368±22a	303±31a
pent-1-en-3-one	190±15ab	202±5a	182±13ab	205±5a	199±4a	189±1ab	197±2ab	165±21b
2+3-pentanone	4±1b	6±1ab	6±1ab	11±2ab	11±2ab	11±2a	11±3a	11±1ab
Σ C5 Ketones	193±12ab	208±4ab	185±16ab	216±3a	210±6a	200±4ab	208±2ab	176±22b
PD- 1	314±23b	407±15ab	365±49ab	444±30a	403±20ab	399±49ab	435±24a	365±73ab
PD- 2	141±1a	134±0a	122±19a	147±8a	137±7a	132±10a	142±3a	120±19a
PD- 3	1432±213ab	1498±6ab	1331±254ab	1773±85a	1555±90ab	1480±96ab	1610±32ab	1329±237b
PD- 4	208±13a	218±5a	228±14a	197±18a	225±11a	ND	244±31a	ND
PD- 5	370±43a	387±22a	365±16a	397±2a	388±6a	372±24a	381±4a	355±20a
PD- 6	685±270a	546±21a	500±5a	583±51a	551±26a	524±51a	602±45a	492±41a
Σ Pentene Dimers	3150±353ab	3189±27ab	2870±340ab	3540±144a	3258±85ab	2907±124ab	3414±33a	2661±389b
Total LOX C5	3708±365ab	3916±17ab	3521±403ab	4290±152a	4025±102ab	3610±117ab	4164±28a	3298±456b
Trial 2								
(Z)-pent-2-enal	105±11ab	112±5a	101±11abc	79±6bc	107±13ab	86±12abc	96±9abc	71±14c
(E)-pent-2-enal	16±2b	16±1b	16±2b	20±0ab	20±1ab	19±2ab	21±2a	19±2ab
pentanal	26±6c	30±1c	35±2bc	35±4bc	30±2c	23±0c	46±4ab	50±5a
Σ C5 aldehydes	147±18a	158±6a	157±11a	134±9a	157±14a	120±6a	170±2a	141±17a
pent-1-en-3-ol	159±38a	186±2a	173±21a	152±11a	197±38a	156±26a	175±27a	170±15a
(E)-pent-2-en-1-ol	12±1a	12±0a	12±1a	11±1a	13±2a	12±2a	13±1a	12±1a
(Z)-pent-2-en-1-ol	143±17a	125±8ab	112±10ab	120±6ab	92±6b	89±12b	108±14b	113±17ab
pentan-1-ol	14±1bc	16±0abc	15±1bc	14±1c	16±1abc	16±2abc	18±1a	17±0ab
Σ C5 alcohols	328±56a	339±6a	312±26a	296±9a	334±36a	278±27a	314±31a	217±92a
pent-1-en-3-one	139±22a	145±2a	133±17a	118±7a	135±22a	114±18a	129±14a	114±15a
2+3-pentanone	8±1ab	8±1ab	8±1ab	6±0b	7±1ab	9±1a	7±1ab	9±0a
Σ C5 Ketones	158±16a	153±3a	141±18a	123±7a	142±22a	123±19a	135±15a	130±9a
PD- 1	372±66a	397±31a	380±59a	347±20a	379±56a	336±66a	396±53a	439±26a
PD- 2	145±29a	114±4a	109±17a	100±7a	114±19a	103±21a	117±16a	128±1a
PD- 3	1047±251a	1189±29a	1124±172a	987±86a	1116±238a	957±198a	1205±197a	1126±238a
PD- 4	ND	192±21a	175±25a	195±14a	207±44a	203±22a	195±15a	247±46a
PD- 5	329±41a	365±11a	350±29a	331±18a	344±32a	317±29a	349±22a	329±29a
PD- 6	445±55a	478±26a	510±5a	427±27a	441±52a	422±55a	520±55a	443±54a
Σ Pentene Dimers	2497±391a	2734±27a	2804±182a	2386±167a	2601±439a	2338±391a	2781±355a	2904±23a
Total LOX C5	3169±393a	3384±32a	3437±207a	2939±188a	3122±639a	2766±511a	3344±493a	3345±28a

* Mean value ± SD (n = 3)
Different letters represent significant differences at p = 0.05.

Jiménez et al. (2007) described the ratio of Hexanal/(E)-2-Hexenal as indicator of olive oil oxidation because (E)-2-Hexenal was formed through the LOX pathway and the Hexanal can be also formed from autoxidation process, and then give the 'rancid' off-flavor (Kalua et al., 2006). The VOO from HPU treatments showed a lower value of Hexanal/(E)-2-Hexenal ratio (Table 5) compared with Ref (C) VOOs, thus oxidation was not induced by sonication treatment.

Table 5. Other volatile compounds (µg/kg VOO) not derived from LOX pathway obtained in the VOO for the different treatments and different trial date.

	Centrifugation				Malaxation + Centrifugation			
	Ref (C)	T20 (C)	T40 (C)	T80 (C)	Ref (M/C)	T20 (M/C)	T40 (M/C)	T80 (M/C)
Trial1								
Methanol	6942±226c	7532±288bc	9034±1292ab	9058±626ab	9179±148ab	9306±609ab	10679±556a	10025±811a
Ethanol	40694±6161b	47221±433ab	46895±8563ab	55425±1130ab	58054±2367ab	54289±2798ab	61328±1379a	50105±11984ab
Σ Alcohols	47636±5936b	54753±721ab	55929±9470ab	64482±1756ab	67234±2509a	63595±3317ab	72007±1935a	60131±12793ab
Hexanal/E-2-hexenal	0.19±0a	0.12±0.01b	0.1±0cd	0.09±0.01d	0.11±0.01bc	0.1±0.01bcd	0.1±0.01bcd	0.09±0.01d
Trial2								
Methanol	8844±988c	9286±471c	9867±347bc	11466±582ab	10918±502abc	10456±311abc	11932±468ab	12346±1584a
Ethanol	37738±9105a	39111±1997a	35824±6491a	35780±3377a	43427±9927a	33627±6519a	44582±6515a	41479±10355a
Σ Alcohols	46086±8415a	48397±1710a	45692±6296a	47246±3514a	54344±10326a	44083±6208a	56514±6761a	53824±11934a
Hexanal/E-2-hexenal	0.35±0.05a	0.31±0.02ab	0.28abc0.02bc	0.23±0.02c	0.27±0.01bc	0.24±0.01bc	0.27±0.01bc	0.23±0.01bc

* Mean value ± SD (n = 3)
Different letters represent significant differences at p = 0.05.

3.4. Sensory analysis

The sensory profile of olive oil was deeply influenced by its phenolic and the volatiles composition. The results for the first experiment were presented in Figure 2. All the oils were classified into the virgin extra category according to European Commission (2013). The positive notes of fruity, bitterness and pungency were perceived for intensities higher than 3. Off flavors related with oxidation reaction `rancid´, of autoxidation phenomenon, were not detected for both reference and HPU treatment. In addition, ultrasound treatments, as techniques for rapid heating, did not induced ``burnt´´ off flavors in the VOOs.

HPU application did not affect negatively to the positive attribute of the oils such as Fruity, Bitter, pungency. HPU treatments at 40 kHz and 80 kHz without malaxation, produced an increase of the green notes of VOO until 3.5 and 3.4 respectively.

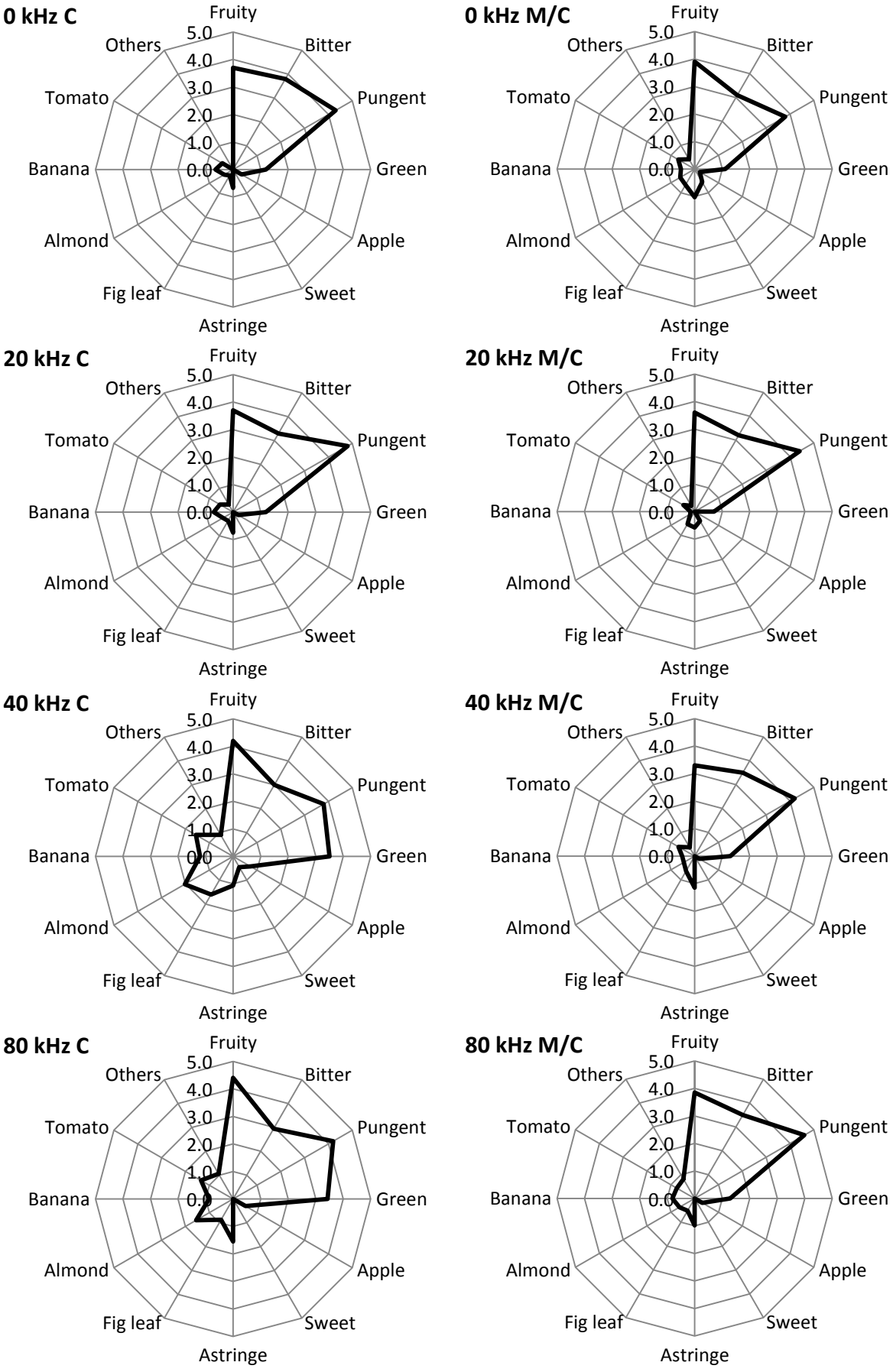

Figure 2. Sensory profile obtained for the different treatments.

The increase of the VOO green notes was in agreement with the results observed by Jiménez et al. (2007). Other changes in the oils sensory profiles were the increase of the notes of fig leaf, almond, banana and tomato attributes when the olive pastes were treated by HPU at 40 and 80 kHz. These changes observed in the VOO sensorial profile were confirmed for the HPU treatment 80 kHz volatiles compounds showing the higher concentration for the green and tomato flavor what are Hexanal, E-3-hexenal, Z-3-hexenal, Z-2-hexenal, E-2-hexenal, E-3-hexenol, Z-3-hexenol, E-2-hexenol and hexyl acetate, and for the banana flavor such as E-2-hexenal, Hexanol and Z-3-hexenyl acetate (Angerosa et al., 2004; Kalua et al., 2007; Morales et al., 2013). Also, for the oils obtained from those treatments without malaxation, the panel test perceived olfactory notes of mint.

4. Conclusions

Therefore high power ultrasound treatment of olive paste did not have any negative effect on those compounds of VOO related to the nutritional and sensorial characteristics.

HPU treatments did not affect the fatty acid composition, whereas for the phenolic compounds the ultrasound treatment allowed to obtain levels equivalent to those obtained by traditional malaxation. In general, a positive response was observed in the secoiridoid compounds when HPU frequency was increased.

VOO volatiles were affected by HPU application. The concentration of the main C6 LOX aldehyde detected, the (E)-2-Hexenal, changed depending on HPU frequency applied. For the unmalaxed pastes, let to achieve similar values than those from traditional conditions. As HPU frequency was higher, (E)-2-Hexenal concentration tends to rose, however, when further malaxation was carried out its concentration did not change.

Regarding to the VOO olive oil sensorial characteristics, all the oils obtained were classified as Extra Virgin Olive Oil. The HPU application without malaxation gave oil with more equilibrated profiles. Positive attributes were improved for oil obtained from unmalaxed pastes and treated at 40 and 80 kHz. Furthermore, ultrasound treatments did not induced off flavor as burnt or rancid was not observed.

Therefore, the application of HPU as alternative to the conventional malaxation let to obtain Extra Virgin Olive Oil without any alteration in their bioactive proprieties showing a more equilibrated sensory profile.

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Chapter 8. High power ultrasound as alternative technology to malaxation for virgin olive oil extraction.

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Contents

High Power Ultrasound as alternative Technology to Malaxation for Virgin Olive Oil extraction.

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Abstract

In this work was studied the high power ultrasound (HPU) technology as alternative to olive paste malaxation for virgin olive oil (VOO) extraction. For this purpose a prototype was designed for continuous conditioning of olive paste, where it flows in rectangular tube through which HPU were applied at three different frequencies (20 kHz, 40 kHz and 80 kHz) by `Langevin´ ultrasonic transducers this technique was compared to malaxation conditioning in conventional malaxer, the experiments were carried out at pilot plant scale for three harvesting dates. Heating capacity, oil yield, VOO quality and energy consumption were evaluated. The main results observed was the rapid heating of olive paste for all frequencies treatments tested, the best performance was for 40 kHz comparing with the 20 kHz and 80 kHz. The treatment at 40 kHz also displayed the higher oil yield similar to those obtained by conventional malaxation. The efficiency of HPU conditioning was affected by the moisture decrease of the olives. The VOO quality, nutritional and sensorial characteristics were not altered by HPU conditioning. The other aspect observed is gain of energy by the HPU conditioning comparing with the conventional malaxation.

Key words: High Power Ultrasound Frequency, Virgin Olive Oil, Malaxation, Oil Yield, Oil Characteristics.

Abbreviations: Virgin Olive Oil (VOO), High Power Ultrasound (HPU), Fatty Acid Methyl Esters (FAMEs), High Performance Liquid Chromatography (HPLC), Gas Chromatograph (GC).

1. Introduction

One of the most important steps of virgin olive oil (VOO) extraction process is the olive paste preparation that includes two unit operations: crushing and kneading or malaxation [1,2]. The olive paste kneading is a crucial operation with a major influence on the VOO extraction yield, quality and composition [2,3]. This operation is performed in machines called kneaders or malaxers, they consist in a semi-cylindrical or cylindrical tank with a jacket for warm water circulation, equipped with blades mounted on vertical or horizontal axes [1,4,5]. This operation has remained unchanged for long time and have some limitations [1,3–5]:

- Kneader needs a large space in the oil extraction room. Usually more than one malaxation container is needed to guarantee the continuous process.
- Low heating capacity for olive paste, giving longer malaxation time.
- High consumption of energy.
- Difficulties for an adequate cleaning.
- Negative influence on VOO quality, when inadequate malaxation.

Recently some design changes were carried-out to optimize the malaxer by controlling the O_2 concentration in the headspace [2,4,6,7] and some geometry change of the malaxer to improve the heat transfer [5].

In order to reduce the olive paste heating time during malaxation, recently new systems has been proposed for a rapid heating of olive paste using tubular heat exchangers [8–10]. Other technologies are the microwave treatment of olive paste before malaxation [11,12], and pulsed electric fields [13,14]. The main disadvantage of these techniques is the increase of energy consumption and the necessity of a subsequent kneading.

Most recently, high power ultrasounds (HPU) treatment of olive paste was tested for VOO extraction [15]. This technology is considered safe, environmentally friendly, reduced cost and economical competitive [16]. The HPU are low frequency sound waves going from 16 kHz to 100 kHz [17]. The main effects of propagation of HPU wave are the cavitation phenomena induced in the medium [18,19], the ‘‘sponge effect’’ generated by movement of the medium during HPU waves propagation and cell wall degradation [20].

The first works conducted by Jimenez et al. [21,15] at laboratory scale tested HPU application during malaxation using probe or horn system at 25 kHz and bath system at 24 kHz. The HPU treatment allowed a quick heating of olive paste and improved the oil extractability without alteration on VOO quality. Later, Clodoveo et al. [22,23] tested the HPU treatment at 35 kHz frequency in bath system, on olives before crushing and olive paste after crushing, this treatment allowed reduction of the malaxation time without VOO quality alteration and higher minor compounds contents when the olives were submerged in the water bath.

All these HPU studies were carried in batch conditions. Then, Bejaoui et al. [24,25] carried out the HPU treatment at 40 kHz in continuous conditions, through a device designed for laboratory scale experiments before malaxation, obtaining a quick paste heating and an increase of paste extractability without oil quality alteration.

Because the previous work were carried out as HPU pretreatment of olive paste before malaxation, using different HPU frequencies, the objective of this work was to evaluate the application of HPU at three frequencies (20 kHz, 40 kHz and 80 kHz) as alternative to malaxation measuring the effect on the process yield, energy consumption, oil quality and composition.

2. Materials and methods

2.1. Plant material

Olives from `Picual´ cultivar, *Olea europaea* L. produced during the crop year 2015/2016, were used in this work. The experiments were carried at different olive ripeness stages harvested at three different harvesting dates November-12-15, December-04-15 and December-15-15. For each experiment a lot of 8000 kg was picked from trees using a mechanical shaker, transported, then cleaned from leaves and branches and finally, homogenized and stored for less than 12 hours in stainless steel hopper. The olives were characterized by their maturity index [26] , moisture and fat content as follows.

- Olive moisture: The olive fruit was crushed and the milled paste was desiccated at 105 ºC after weighted, these two later operations were repeated until to obtain a constant weight. The results were expressed in weight percentage.

- Olive fat content: Oil content was measured, for the dried olive paste obtained from the olive moisture determination, using a Nuclear Magnetic Resonance (NMR) fat analyzer Minispec mq 20 (Bruker Analytik Gmbh). The NMR was previously calibrated and validated with Soxhlet extractor. The results were expressed on weight percent (on a fresh matter basis and dry matter basis).

2.2. Experimental procedure

The VOO extraction was performed in a pilot plant “Il Molinetto” (Pieralisi, España) installed in the olive oil experimental mill of the IFAPA Center “Venta del Llano” with a production capacity going from 150 to 300 kg/h.

The extraction plant was equipped by a mechanical hammer crusher with a 5 mm sieve and fitted with an olives loading hopper. Two systems for olive paste preparation were used: the first was composed by a kneader (capacity: 300 kg) with warm water circulation jacket (heated by an electrical resistance) and a progressive cavity pump to feed the horizontal centrifuge by olive paste. The horizontal centrifuge, series Baby 50 (Pieralisi, Spain), works at 5000 rpm. The second system tested was formed by an olive paste stainless steel hopper, a progressive cavity pump for olive paste feeding the High Power Ultrasound device and then the horizontal centrifuge for oil separation. The HPU prototype was designed for `in line´ continuous olive paste treatment by the IFAPA Center “Venta del Llano” research team and assembled by ATU Ultrasound (Valencia, Spain). The device consisted in a rectangular pipe made in food quality stainless steel, the internal volume of the pipe was 0.016 m^3. On the fourth faces of the pipe were bounded 54 ultrasound transducers applying three different resonance frequencies: 20 kHz, 40 kHz and 80 kHz (18 transducers per frequency). The transducers were mounted throughout the rectangular pipe on the 4 external faces. The piezoelectric transducers, type `Langevin´, were powered by 3 ultrasound generators with a power of 900 W. Each one generated a single frequency (20 kHz, 40 kHz or 80 kHz) and was connected to the corresponding transducers. The nominal volumetric energy density applied by the device was 56.25 W/L

The mass flow rate of olive paste was fixed at 200 kg/h for all the experiments; it allowed an adequate olive paste heating by HPU and good performance of the decanter centrifuge. After extraction, the VOO was collected in a tank for its weight.

The experiments were carried out to compare the traditional malaxation with the HPU technology, a reference without olive paste conditioning was tested too (Figure 1). The treatments applied were:

- Reference: olive paste passed through the device without HPU treatment or kneading.
- T20: HPU treatment of olive paste at 20 kHz.
- T40: HPU treatment of olive paste at 40 kHz.
- T80: HPU treatment of olive paste at 80 kHz.
- Malaxation: the olive paste was kneaded at 28 ºC for 45 minutes.

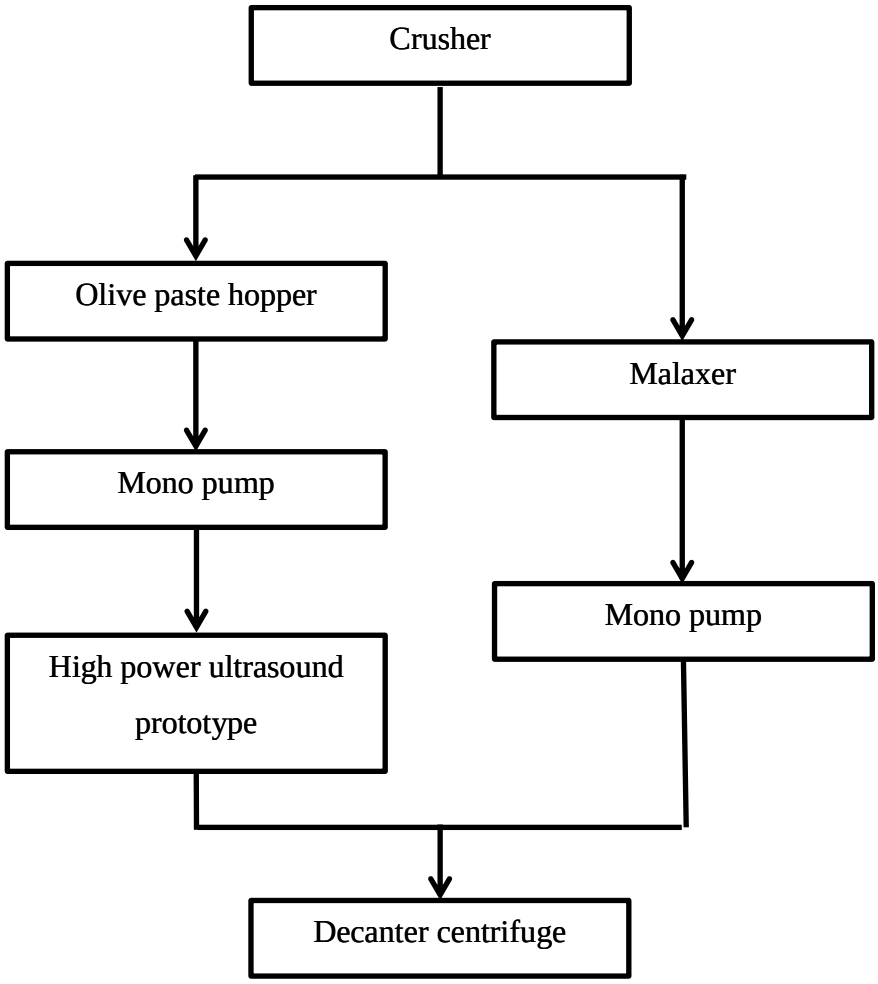

Figure 1: Virgin Olive Oil extraction procedure

The pomace samples were taken at the exit of the decanter centrifuge by triplicate for each experiment; these samples were taken after 15 minutes from the beginning of feeding of the decanter centrifuge, the replicates were taken at 15 minutes of intervals. In the same way, oil samples were taken by triplicate from the exit of the decanter centrifuge. Oil samples were filtered and stored until analysis at -24 ºC.

2.3. Process efficiency

In order to determine the HPU prototype efficiency some parameters were considered as the heating capacity, the oil industrial yield and energy consumption.

The olive paste Temperature was monitored during the experiments, for this purpose four measure points were fixed: in the crusher hopper for olives, olive paste just crushed, olive paste after malaxation and olive paste at HPU device exit. For the first harvesting date the olive paste temperature was taken each 5 minutes during the malaxation time and during HPU treatment (Figure 2).

The oil yield (OY) was determined from the oil weight obtained from each treatment, and was expressed as the percentage of the oil weight (W_{oil}) extracted from the olives weight (W_{olives}) processed [Eq 1].

$$OY = \frac{W_{oil}}{W_{olives}} \times 100 \qquad [Eq\ 1]$$

Also the extraction yield was determined by measuring the pomace oil content. The pomace moisture and oil content were determined as described for the olives. The results of pomace oil content were expressed as weight percent (on a fresh matter basis and dry matter basis).

To evaluate the energy consumption of the different olive paste conditioning methods the electric consume of the machines involved in the VOO extraction are shown in Table 3. To determine the electrical consume of the conditioning methods the unit operation of olive paste malaxation and HPU conditioning were considered since the other steps were fixed.

2.4. Olive oil quality parameters

Free acidity, peroxide values, ethyl esters and ultra-violet absorption at 232 and 270 nm (K_{232} and K_{270}) were measured as described in European Regulations EEC 2568/91 [27]. Free acidity was expressed as percent of oleic acid, peroxide values as milli-equivalents of active oxygen per kilogram of oil (mEq O_2/kg), K_{232} and K_{270} coefficients were calculated from absorption at 232 and 270 nm respectively using spectrophotometer Cary 50 UV-Vis (Varian, Spain) and ethyl esters were expressed as mg/kg [28].

Sensory Analysis was performed by the panel of the Citoliva Foundation laboratory during a series of oil-tasting sessions, in accordance with the European Regulation EEC 2568/91 [27] and International Olive Council [29]. The tasters had to rate the intensity of the different descriptors on a continuous 0 - 10 cm scale and the results were expressed as the median of the intensity perceived.

2.5. Olive oil composition

2.5.1. Fatty acid composition

The Fatty Acid Methyl Esters (FAMEs) were prepared as described by the European Regulation EEC 2568/91 [27]. FAMEs were analyzed by chromatographic separation using a Perkin-Elmer autosystem gas chromatograph (Perkin-Elmer, Spain) the operational conditions were as described by Beltrán et al. (2004). The results were expressed as relative area percentages of the total area.

2.5.2. Total phenol content

Phenolic compounds were extracted from an oil-in-hexane solution with methanol:water and their concentration was measured using Folin-Ciocalteau reagent and colorimetric measurement at 725 nm [31] using spectrophotometer Cary 50 UV-Vis (Varian, Spain). Results were expressed as mg/kg of caffeic acid.

2.5.3. Bitterness index

The bitterness index (K_{225}) was determined applying the method described by Gutiérrez Rosales et al. [32]. The bitter compounds were eluted through the solid phase extraction column with methanol:water (50:50). The absorbance of the methanolic extract recovered was measured at 225 nm using spectrophotometer Cary 50 UV-Vis (Varian, Spain).

2.5.4. Tocopherol content

Tocopherol composition was determined applying the IUPAC method 2432 [33]. Oil sample was dissolved in mobile phase (Isopropanol 0.5 % in n-hexane) and analyzed by HPLC Agilent 1200 (Agilent Technologies, Spain) system equipped with a diode array detector. Isocratic mode was used to the compounds separation in column Lichrosphere

Si60 (250 × 4.6 mm i.d., 5 µm) (Merck, Spain). Compounds were identified using standards (Sigma-Aldrich, St. Louis, Missouri, USA). Results are expressed as mg/kg of VOO.

2.5.5. Pigment content

Carotenoid and chlorophyllic pigments were determined measuring the absorbance of olive oil weighed and dissolved in cyclohexane at 470 and 670 nm using spectrophotometer Cary 50 UV-Vis (Varian, Spain), as described by Minguez-Mosquera et al. [34]. The results were expressed as mg/kg.

2.5.6. Phenolic compounds

The extraction of the phenolic compounds from the olive oil was carried as described by Beltrán et al. [35] in methanol:water solvent. Phenolic extract obtained was analyzed by RP-HPLC method as described by Beltrán et al. [35]. HPLC analysis was performed using a Agilent 1100 (Agilent Technologies, Spain) system equipped a diode array detector. The phenolic compounds were identified as described by Mateos et al. [36]. Phenolic compounds were quantified at 280 nm using syringic acid as internal standard and the response factors determined by Mateos et al. [36]. The results were expressed as mg/kg.

2.6. Data analysis

The results were expressed as mean ± standard deviation (n = 3). Analysis of variance was applied; significant differences between treatments were determined applying Tukey's test $p < 0.05$ (Statistix 9.0 software, Analytical Software, USA).

3. Results and discussion

The characteristics of the olives used in the experiments are shown in Table 1, fruit maturity index varied from 2.40 to 3.28. The olives moisture showed a wide range from 45.07 % to 56.59 %, decreasing during the olive maturity. The olive oil content was higher than 19 % on fresh mater basis for all harvesting dates.

Table 1. Olives fruit characteristics used in the three harvesting dates

	Maturity index	Moisture (%)	Oil Content (fresh matter) (%)	Oil Content (dried matter) (%)	Temperature (°C)
Olives 12-11-15	2.40	56.59±0.16a	19.03±0.73a	43.83±1.58a	13.6
Olives 04-12-15	2.78	51.44±0.53b	19.18±0.22a	39.51±0.55b	11.2
Olives 15-12-15	3.28	45.07±0.86c	19.52±0.43a	35.55±1.34c	12.1

* Mean value ± SD (n = 3)
Different letters represent significant differences at p = 0.05.

3.1. HPU process efficiency

3.1.1. HPU heating capacity

The olive paste temperature was monitored during all the experiments, data are presented in Table 2. After crushing, the olive paste temperature increased, the temperature raise varied from 2.8 ºC to 4.7 ºC. This temperature increase during crushing is due to the friction phenomenon induced during this operation [22,24,25]. The lower temperature increase was observed for the second harvesting date because the lower air temperature registered.

The HPU induced the instantaneous heating of the olive paste whereas it passed through the HPU prototype for all frequencies used. Their heating capacity depended on the olives characteristics and ultrasound frequency, obtaining temperature increases from 2.5 ºC to 9 ºC. This temperature increase can improves the oil yield since allows the oil viscosity decrease [37], helping the coalescence phenomenon of oil and fluid movement.

HPU at 40 kHz showed a higher heating efficiency than those treatments at 20 kHz and 80 kHz, since achieved olive paste temperature similar to those measured in conventional malaxation using warm water jacket. For 40 kHz HPU heating capacity was deeply dependent on the olive paste moisture as reported by Bejaoui et al. [24,25], since moisture favored the cavitation phenomenon and then the temperature raise.

Table 2. Olive paste temperature monitoring, oil yield and pomace characteristics for the different conditioning methods.

Treatments	ΔT_c (ºC)	ΔT_d (ºC)	Oil Yield (%)	Pomace moisture (%)	Pomace oil content (%) (fresh matter)	Pomace oil content (%) (dried matter)
Harvesting date November-12						
Ref	4	0	11.18	64.91±2.1b*	5.3±0.6a	15.07±1.3a
20 kHz	4	3.6	15.64	65.33±0.68ab	4.2±0.22bc	12.12±0.42bc
40 kHz	4.2	8.2	16.59	66.14±0.81ab	3.74±0.36cd	11.02±0.8cd
80 kHz	4.7	2.7	13.83	65.36±0.59ab	4.91±0.39ab	14.11±0.92ab
Malaxation	4.5	9.5	16.56	68.12±0.77a	3.14±0.08d	9.83±0.16d
Harvesting date December-04						
Ref	2.8	0	9.26	66.12±3.83a	8.17±0.93a	24.27±5.3a
20 kHz	2.8	3	11.05	68.54±0.63a	7.41±1.78a	23.49±5.2a
40 kHz	2.8	9	13.28	68.17±3.95a	6.14±1.15a	19.21±1.22a
80 kHz	2.8	4	10.26	67.94±1.34a	6.14±1.86a	18.06±4.66a
Malaxation	2.8	12	14.55	71.24±3.95a	4.8±3.41a	15.12±8.81a
Harvesting date December-15						
Ref	4.3	0	14.12	71±1.22a	4.4±0.18a	15.21±1.02a
20 kHz	4.3	3.2	15.79	69.42±2.21a	3.77±0.39ab	12.4±1.67ab
40 kHz	4.5	3.5	17.66	69.55±2.16a	3.72±0.53ab	12.19±1b
80 kHz	4.5	2.5	17.16	69.08±1.66a	3.83±0.49ab	12.35±0.99ab
Malaxation	4.5	7	18.5	72.09±2.2a	2.85±0.17b	10.22±0.2b

ΔT_c Temperature difference between olive fruit and olive paste after crushing
ΔT_d Temperature difference between crushed olive paste and olive paste after treatments
* Mean value ± SD (n = 3)
Different letters represent significant differences at p = 0.05.

Figure 2 showed the variation of temperature for both malaxation and HPU treatments for the first harvesting date. The olive paste temperature for conventional malaxation needed more than 25 minutes to achieve the same temperature reached immediately by HPU at 40 kHz. The HPU treatments at 20 kHz and 40 kHz achieved temperatures lower than for 40 kHz and conventional malaxation and thus, were more efficient.

Therefore, the heating capacity of HPU at 40 kHz was similar to the conventional malaxation although the temperature target was achieved immediately and then, was more efficient

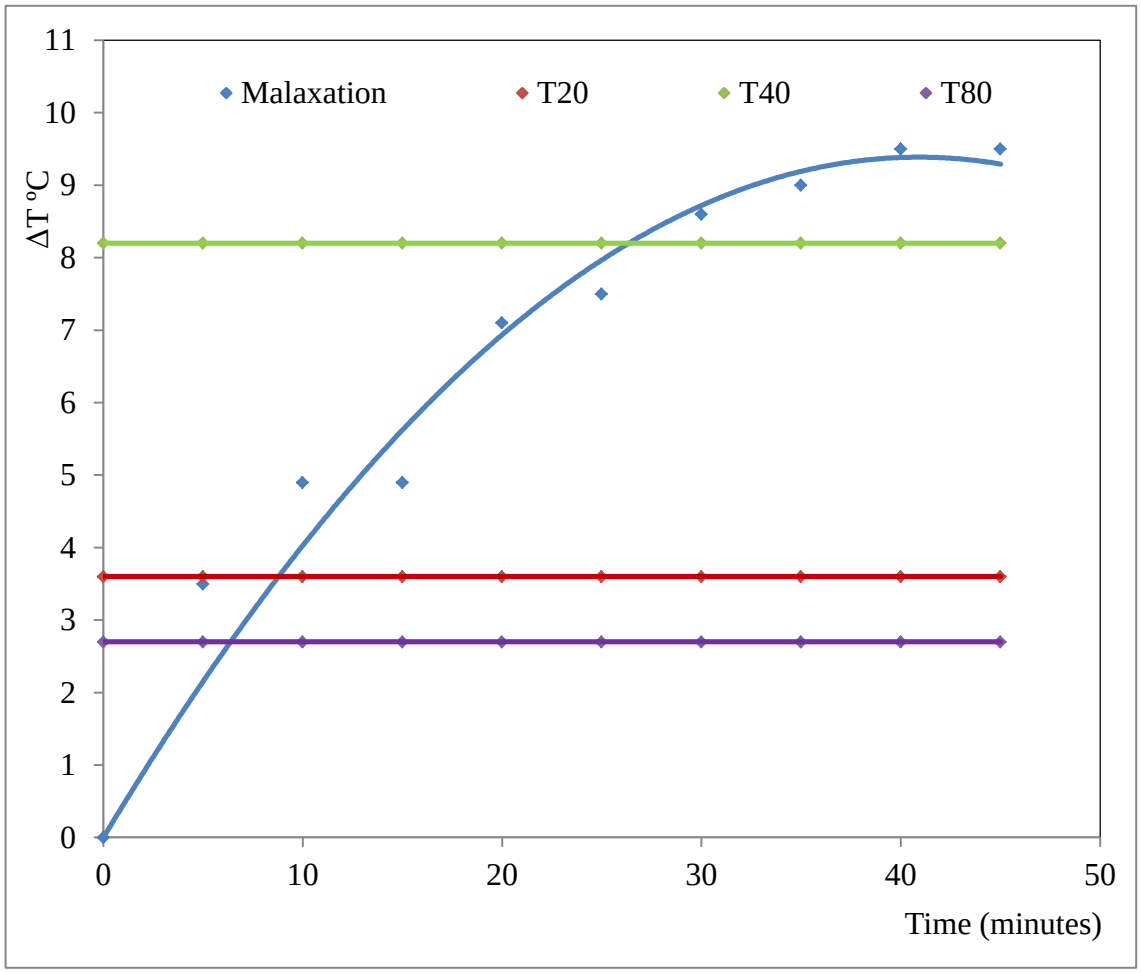

Figure 2. Olive Paste temperature increase monitoring by the different conditioning methods (Malaxation, HPU at 20 kHz, 40 kHz and 80 kHz).

3.1.2. HPU energy consumption

The HPU system was composed by two machines, the HPU prototype and the pump. HPU electrical consume (Table 3) was defined by the ultrasound generators that consume the same electric energy for all frequency used 900 W·h. The progressive cavity pump, for olive paste injection to the decanter centrifuge, consumed 37 W·h. The total consume of olive paste conditioning by HPU was 1.27 kW·h. Considering the mass flow rate of olive paste, the HPU consume per kg of olive paste processed was 6.35 W/kg.

The conventional olive paste kneading is carried out in a kneader consuming 550 W·h for blade rotation, 70 W·h to pump the warm water in the kneader jacket and 3 kW·h for an electrical resistance for water heating. This method also used a cavity pump for olive paste injection to the horizontal centrifuge consuming 37 W·h. The total consume

(Table 3) of conventional malaxation was 3.99 kW·h, this operation was divided in two step the first only by kneading consuming 3.62 kW·h and the second using total machines of this unit operation consuming 3.99 kW·h. Therefore it consumes 33.53 W/kg of processed olive paste.

Table 3. Electric consumption of the different operation for the VOO extraction.

Unit operation	Machines involved	Unit	Electric consume (kWh)
Olives Crushing	Hummer Crusher	1	3
	Olives screw conveyor	1	0.25
		1	3.25
Olive Paste Malaxation	kneader	1	3.62
	Motor for blade rotation	1	0.55
	Pump for warm water circulation	1	0.07
	Electrical resistance for water heating	1	3
	Progressive cavity pump for olive paste	1	0.37
	Total	1	3.99
Olive paste HPU conditioning	HPU prototype	1	0.9
	Progressive cavity pump for olive paste	1	0.37
	Total	1	1.27
Solid-Liquid separation	Decanter solid bowl	1	5.5
	Reducer	1	0.18
	Progressive cavity pump for pomace	1	0.37
	Total	1	6.05

Furthermore, the HPU provide continuity to the process meanwhile the conventional malaxation was carried in batch and to achieve this continuity, more than one kneader are needed. In addition ultrasound technology allows reduction of the space required for the oil extraction since the prototype used in this work has a total volume of 0.16 m^3 meanwhile the kneader of the pilot plant ¨Il molinetto¨ (Pieralisi, Spain) has a volume of 0.65 m^3.

3.1.3. HPU effect on oil yield

The performance of the olive paste conditioning methods was determined by two ways: the oil yield, as the percentage of oil extracted from the olive weight processed and as the residual oil content of the pomace. The results are shown in Table 2.

The extraction yield showed important variations between the different harvesting dates studied; the second harvesting date showed the lower oil yield meanwhile the higher yield was found for the last harvesting date. Although for the reference treatment the olive paste was not conditioned, oil yields between 9.26 % and 14.12 % were obtained. These oil yields can be attributed to the cumulative effect of the extraction steps: crushing, olive paste movement through the pipes and the oil separation in the decanter centrifuge. In general, all the conditioning treatments of olive paste improved the oil yield comparing with the reference.

For HPU treatments, oil yields showed different behavior depending on the HPU frequency and the olive characteristics. For the first harvesting date the oil yield from HPU treatments showed an increase with respect to references. The highest oil yield was observed for the HPU treatments at 40 kHz, these values were equivalent to the oil yield obtained by conventional malaxation achieving levels of 16.59 % and 16.56 % respectively. The application of HPU at 20 kHz and 80 kHz showed lower oil yields of 15.64 % and 13.83 % respectively.

Concerning the second harvesting date the HPU conditioning of olive paste increased the oil yield showing higher oil yields for malaxation and then HPU treatment at 40 kHz. HPU at 20 kHz and 80 kHz showed a small increase of oil yield comparing with reference of 1.79 % and 1 %, respectively.

For the last experiment date, the conventional malaxation also showed the higher yield as for the second harvesting date. Regarding to the HPU conditioning, the treatment at 40 kHz showed higher oil yield whereas the HPU treatment at 80 kHz and at 20 kHz achieved the lowest values.

In general, the HPU treatment at 40 kHz, for all harvesting dates, allowed to obtain oil yield equivalent or close to those obtained by conventional malaxation, whereas the treatment at 20 kHz and 80 kHz gave lower oil yields.

The results of pomace oil content obtained from the different olive paste conditioning methods and harvesting dates were presented in Table 2. In general, pomace oil content was lower for the last harvesting date whereas the highest values were obtained for the second harvesting date as observed for oil yield. Oil yield measured as pomace oil content was improved when olive paste was conditioned showing lower oil content than reference.

In general, conventional malaxation showed the lowest pomace oil content thus the best oil yield. In the first harvesting date, the pomace obtained by malaxation showed significant lower oil content than those from HPU treatment at 20 kHz and 80 kHz. However, HPU application at 40 kHz did not show significant differences with malaxation, although it showed slightly higher oil content. For the other harvesting dates the pomace oil content from malaxation treatment was lower than those observed in HPU treatments, although significant differences were not observed between treatments.

As for olive paste heating, the olive oil extractability from HPU treatments at 20 kHz and 40 kHz were positively affected by the increase of olive moisture, whereas the HPU treatment at 80 kHz was more efficient when the moisture decreased. The treatment at 40 kHz frequency was more efficient for oil extractability than the treatments at 20 kHz and 80 kHz showing values close to those obtained by conventional malaxation.

These results of oil yields observed for HPU treatment without malaxation can be attributed to some effects of the ultrasounds wave propagation through the olive paste as the sponge effect, fluid microchannels, turbulences, heat transfer and cell wall degradation [18–20,39,40].

3.2. Effect on virgin olive oil quality

For the pool of quality parameters analyzed (Table 4), all the oils were classified into the “extra virgin” category according to the European Regulation EEC 2568/91 [27].

The value of free acidity, peroxide value, ultraviolet absorption at 232 nm and 270 nm and the fatty acid ethyl esters showed level far from the limits established by the regulation [27]. No alterations were observed when HPU were applied comparing with conventional malaxation. The fatty acid ethyl esters did not exceed 3 mg ethyl esters/Kg

for the pool of oil analyzed. These results were in accordance with previous works involving HPU technology in the olive paste preparation step for extraction of VOO [15,22,24,25].

Table 4. Quality parameters of the olive oil obtained from experiments

Treatments	Free acidity (% Oleic acid)	Peroxide value (mEq O_2/kg)	Ultraviolet absorption		FAEE (mg/ kg)
			K_{232}	K_{270}	
Harvesting date November-12					
Ref	0.27 ± 0.02 a	2.47 ± 0.16 a	1.92 ± 0.16 a	0.23 ± 0.01 a	< 3
20 kHz	0.26 ± 0.03 a	3.12 ± 0.28 a	1.57 ± 0.48 a	0.2 ± 0.05 ab	< 3
40 kHz	0.23 ± 0.03 a	2.96 ± 0.97 a	0.97 ± 0.52 a	0.14 ± 0.05 ab	< 3
80 kHz	0.18 ± 0.02 a	2.06 ± 0.18 a	1.05 ± 0.38 a	0.12 ± 0.03 b	< 3
Malaxation	0.27 ± 0.02 a	3.29 ± 0.31 a	1.85 ± 0.14 a	0.22 ± 0.02 ab	< 3
Harvesting date December-04					
Ref	0.16 ± 0.02 a	3.28 ± 0.34 a	1.32 ± 0.11 a	0.14 ± 0.01 ab	< 3
20 kHz	0.19 ± 0.03 a	2.84 ± 0.06 a	0.92 ± 0.39 a	0.11 ± 0.01 b	< 3
40 kHz	0.17 ± 0 a	2.92 ± 1.02 a	0.87 ± 0.29 a	0.1 ± 0.03 b	< 3
80 kHz	0.17 ± 0 a	2.86 ± 0.19 a	1.39 ± 0.13 a	0.16 ± 0.02 a	< 3
Malaxation	0.23 ± 0.1 a	4.23 ± 0.43 a	1.33 ± 0.3 a	0.14 ± 0.02 ab	< 3
Harvesting date December-15					
Ref	0.17 ± 0 a	5.64 ± 0.08 d	1.46 ± 0.15 a	0.16 ± 0.01 b	< 3
20 kHz	0.17 ± 0 a	6.26 ± 0.05 a	1.4 ± 0.03 a	0.18 ± 0.02 ab	< 3
40 kHz	0.17 ± 0 a	6.25 ± 0.02 a	1.32 ± 0.14 a	0.16 ± 0.01 b	< 3
80 kHz	0.17 ± 0 a	5.95 ± 0.02 c	1.34 ± 0.16 a	0.15 ± 0.01 b	< 3
Malaxation	0.18 ± 0.02 a	6.13 ± 0.03 b	1.68 ± 0.17 a	0.2 ± 0.01 a	< 3

* Mean value ± SD (n = 3)
Different letters represent significant differences at p = 0.05.

The results of sensory analysis (Table 5) showed that all oils obtained by the different conditioning methods and harvesting dates were classified into ‘extra virgin’ category according to regulation of European Regulation EEC 2568/91 [27], since were not detected sensorial defects. Oils from conventional malaxation showed higher intensities

for fruity, bitter and pungent attributes. HPU technology did not affect negatively the oil fruity and pungent attributes. Regarding to bitterness, oils from HPU treatments showed lower intensity than those oil obtained by malaxation. These effects of HPU application were in agreement with those observed in previous works at laboratory scale [15,23].

Table 5. Sensory analysis of the oil obtained from all conditioning methods and all harvesting dates.

Treatments	Fruity	Bitter	Pungent
Harvesting date November-12			
Ref	5.2	4.8	5.2
20 kHz	5.55	4.65	5.7
40 kHz	5.5	4.15	5.75
80 kHz	5.65	4.3	5.9
Malaxation	6	5.55	6
Harvesting date December-04			
Ref	5	1.85	5.4
20 kHz	5.1	2.5	5.1
40 kHz	5	2	4.4
80 kHz	5.5	1.8	4.5
Malaxation	6.2	3	4.5
Harvesting date December-15			
Ref	5	2.9	5.4
20 kHz	5	3.1	5.4
40 kHz	5.75	3.5	5.4
80 kHz	5	2	3.8
Malaxation	5.95	5.15	5.95

3.3. Effect on virgin olive oil composition

The fatty acid composition was determined to evaluate if there were any effect of HPU application, the results are shown in Table 6. The fatty acids profile was similar to those described for ¨Picual¨ variety oils [30]. When ANOVA analysis was performed some significant differences were found, although they can be attributed to the variability of the fruit processed.

Table 6. Fatty acids composition of olive oils (expressed en percentage of total peak area %) obtained from all conditioning methods and harvesting dates

Treatments	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	SFA	UFA	MUFA	PUFA
Harvesting date 12-11-15															
Ref	12.36 ± 0.09 a	0.95 ± 0.04 a	0.06 ± 0 a	0.06 ± 0.01 a	3.5 ± 0.02 a	77.9 ± 0.01 c	3.67 ± 0.02 a	0.88 ± 0.02 a	0.4 ± 0.01 a	0.15 ± 0.03 a	0.07 ± 0.01 a	16.39 ± 0.06 ab	83.61 ± 0.06 bc	79.06 ± 0.06 c	5.71 ± 0.07 a
20kHz	12.13 ± 0.12 a	0.96 ± 0.13 a	0.06 ± 0.01 a	0.07 ± 0.02 a	3.41 ± 0.1 a	78.89 ± 0.15 ab	3.22 ± 0.14 b	0.76 ± 0.08 a	0.3 ± 0.06 a	0.15 ± 0.02 a	0.06 ± 0 a	15.94 ± 0.06 abc	84.06 ± 0.06 abc	80.08 ± 0.21 a	5.16 ± 0.1 b
40kHz	12.09 ± 0.2 a	0.94 ± 0.04 a	0.06 ± 0.01 a	0.05 ± 0.01 a	3.17 ± 0.08 a	79.11 ± 0.29 ab	3.28 ± 0.06 ab	0.69 ± 0.19 a	0.41 ± 0.09 a	0.14 ± 0.02 a	0.05 ± 0.01 a	15.78 ± 0.19 c	84.22 ± 0.19 a	80.24 ± 0.27 a	5.11 ± 0.19 b
80kHz	11.88 ± 0.25 a	0.88 ± 0.07 a	0.06 ± 0.01 a	0.06 ± 0.01 a	3.59 ± 0.22 a	78.62 ± 0.27 ab	3.6 ± 0.1 ab	0.73 ± 0.09 a	0.37 ± 0.09 a	0.15 ± 0.03 a	0.06 ± 0.01 a	15.97 ± 0.17 bc	84.03 ± 0.17 ab	79.71 ± 0.2 ab	5.42 ± 0.1 b
Malaxation	12.43 ± 0.25 a	0.94 ± 0.04 a	0.08 ± 0.02 a	0.07 ± 0.01 a	3.39 ± 0.08 a	78.1 ± 0.39 bc	3.55 ± 0.06 ab	0.79 ± 0.01 a	0.43 ± 0.02 a	0.15 ± 0.02 a	0.07 ± 0.01 a	16.4 ± 0.3 a	83.6 ± 0.3 c	79.26 ± 0.35 bc	5.51 ± 0.09 ab
Harvesting date 04-12-15															
Ref	11.75 ± 0.1 a	0.92 ± 0.03 a	0.07 ± 0 ab	0.08 ± 0 a	3.31 ± 0.03 a	79.12 ± 0.34 a	3.41 ± 0.12 a	0.83 ± 0.04 a	0.29 ± 0.07 a	0.16 ± 0.03 a	0.06 ± 0 a	15.48 ± 0.18 a	84.52 ± 0.18 a	80.28 ± 0.33 a	5.4 ± 0.16 a
20kHz	11.59 ± 0.24 a	0.9 ± 0.01 a	0.07 ± 0 ab	0.09 ± 0.02 a	3.67 ± 0.44 a	78.64 ± 0.52 a	3.72 ± 0.35 a	0.78 ± 0.07 a	0.29 ± 0.04 a	0.17 ± 0.03 a	0.07 ± 0 a	15.69 ± 0.2 a	84.31 ± 0.2 a	79.8 ± 0.53 a	5.67 ± 0.32 a
40kHz	11.55 ± 0.09 a	0.93 ± 0.02 a	0.07 ± 0.01 a	0.08 ± 0.01 a	3.49 ± 0.23 a	78.92 ± 0.19 a	3.57 ± 0.14 a	0.86 ± 0.04 a	0.26 ± 0.02 a	0.2 ± 0.05 a	0.07 ± 0.01 a	15.45 ± 0.15 a	84.55 ± 0.15 a	80.12 ± 0.26 a	5.63 ± 0.05 a
80kHz	11.5 ± 0.07 a	0.93 ± 0.07 a	0.06 ± 0.01 b	0.06 ± 0 b	3.34 ± 0.11 a	79.1 ± 0.36 a	3.52 ± 0.02 a	0.91 ± 0.09 a	0.3 ± 0.04 a	0.22 ± 0.02 a	0.07 ± 0.02 a	15.25 ± 0.19 a	84.75 ± 0.19 a	80.31 ± 0.29 a	5.65 ± 0.18 a
Malaxation	11.59 ± 0.13 a	0.9 ± 0.04 a	0.07 ± 0.01 ab	0.07 ± 0 a	3.24 ± 0.05 a	79.3 ± 0.2 a	3.45 ± 0.03 a	0.78 ± 0.03 a	0.39 ± 0.07 a	0.15 ± 0.02 a	0.07 ± 0 a	15.36 ± 0.13 a	84.64 ± 0.13 a	80.41 ± 0.18 a	5.35 ± 0.07 a
Harvesting date 15-12-15															
Ref	11.34 ± 0.1 a	0.91 ± 0.03 a	0.05 ± 0.01 a	0.05 ± 0.01 a	3.46 ± 0.02 a	79.7 ± 0.15 a	3.14 ± 0.03 b	0.84 ± 0.02 a	0.25 ± 0.04 a	0.2 ± 0.01 a	0.07 ± 0 ab	15.17 ± 0.08 a	84.83 ± 0.08 a	80.85 ± 0.1 a	5.14 ± 0.08 a
20kHz	11.42 ± 0.03 a	0.72 ± 0.36 a	0.06 ± 0.01 a	0.05 ± 0.01 a	3.44 ± 0.02 a	79.68 ± 0.15 a	3.15 ± 0.02 ab	0.86 ± 0.03 a	0.17 ± 0.03 bc	0.18 ± 0.06 a	0.06 ± 0.01 ab	15.16 ± 0.07 a	84.64 ± 0.41 a	80.63 ± 0.41 a	4.96 ± 0.33 a
40kHz	11.34 ± 0.04 a	0.87 ± 0.02 a	0.05 ± 0.01 a	0.05 ± 0.01 a	3.46 ± 0.02 a	79.9 ± 0.01 a	3.17 ± 0.01 ab	0.77 ± 0.03 b	0.14 ± 0.02 bc	0.16 ± 0.04 a	0.09 ± 0.01 a	15.08 ± 0.04 a	84.92 ± 0.04 a	80.98 ± 0.02 a	5.03 ± 0.04 a
80kHz	11.34 ± 0.08 a	0.87 ± 0.01 a	0.05 ± 0 a	0.06 ± 0.01 a	3.48 ± 0.02 a	79.82 ± 0.13 a	3.17 ± 0.02 ab	0.82 ± 0.02 ab	0.12 ± 0.01 b	0.2 ± 0.01 a	0.08 ± 0 a	15.07 ± 0.09 a	84.93 ± 0.09 a	80.95 ± 0.12 a	5.11 ± 0.03 a
Malaxation	11.48 ± 0.1 a	0.9 ± 0.01 a	0.06 ± 0.02 a	0.06 ± 0.02 a	3.44 ± 0.01 a	79.61 ± 0.09 a	3.2 ± 0.02 a	0.83 ± 0 ab	0.2 ± 0.03 c	0.17 ± 0.02 a	0.05 ± 0.01 b	15.23 ± 0.08 a	84.77 ± 0.08 a	80.74 ± 0.1 a	5.16 ± 0.03 a

* Mean value ± SD (n = 3)

Different letters represent significant differences at p = 0.05.

C16:0 Palmitic acid, C16:1 Palmitoleic acid, C17:0 Margaric acid, C17:1 Heptadecenoic acid, C18:0 Stearic acid, C18:1 Oleic acid, C18:2 Linoleic acid, C18:3 Linolenic acid, C20:0 Arachidic acid, C20:1 Gadoleic acid, C22:0 Behenic acid. Fatty acid (FA) was grouped in saturated FA (SFA), unsaturated FA (UFA), monounsaturated FA (MUFA) and polyunsaturated FA (PUFA).

The pigment content (Carotenoids and Chlorophylls) of the olive oils was shown in Table 7, these compounds play an important role in the oxidative activity olive oil, because of their antioxidant nature in the dark and pro-oxidant activity in the light [41]. In general, the conventional malaxation gave higher VOO pigment contents, as observed by Criado et al. [42], comparing with the reference. HPU treatments did not affect their content comparing with reference, even in the first harvesting dates HPU treatment at 20 kHz and 40 kHz showed a significant lower content comparing with reference. The conventional malaxtion gave oil with significant higher pigments content than those from HPU treatments.

Table 7. Olive oil nutritional parameter total content

Treatments	Bitterness K_{225}	Total phenols (mg/kg)	Pigments (mg/kg VOO)		Tocopherols (mg/kg VOO)			
			Carotenoids	Chlorophyll	α	β	γ	Total
Harvesting date 12-11-15								
Ref	0.5 ± 0.01 a	792 ± 42 b	8.34 ± 0.34 b	11.26 ± 0.27 b	321 ± 10 a	5 ± 0 a	26 ± 0 a	352 ± 11 a
20 kHz	0.46 ± 0.01 b	822 ± 75 ab	9.29 ± 0.76 ab	13.17 ± 1.56 ab	327 ± 3 a	5 ± 1 a	26 ± 1 a	358 ± 3 a
40 kHz	0.41 ± 0.01 c	927 ± 23 ab	5.33 ± 1.27 c	6.38 ± 1.98 c	325 ± 9 a	6 ± 0 a	28 ± 1 a	358 ± 9 a
80 kHz	0.41 ± 0.01 c	909 ± 36 a	5.39 ± 1.04 c	6.46 ± 1.42 c	323 ± 7 a	6 ± 0 a	26 ± 1 a	355 ± 7 a
Malaxation	0.48 ± 0.02 ab	827 ± 44 ab	10.77 ± 0.21 a	16.15 ± 0.5 a	337 ± 4 a	6 ± 0 a	26 ± 0 a	368 ± 4 a
Harvesting date 04-12-15								
Ref	0.17 ± 0.05 a	337 ± 32 a	5.11 ± 1.07 b	5.11 ± 1.54 b	323 ± 2 b	5 ± 1 a	26 ± 1 a	355 ± 4 b
20 kHz	0.16 ± 0.03 a	390 ± 15 a	5.15 ± 0.71 b	5.26 ± 0.83 b	331 ± 7 ab	5 ± 1 a	24 ± 1 a	361 ± 7 ab
40 kHz	0.16 ± 0.07 a	383 ± 42 a	5.89 ± 0.32 b	5.95 ± 0.5 b	323 ± 5 b	6 ± 0 a	25 ± 1 a	354 ± 6 b
80 kHz	0.16 ± 0.04 a	354 ± 14 a	5.51 ± 0.3 b	5.52 ± 0.43 b	325 ± 6 b	5 ± 0 a	25 ± 1 a	355 ± 6 b
Malaxation	0.21 ± 0.08 a	392 ± 18 a	9.05 ± 1.07 a	10.84 ± 1.6 a	348 ± 12 a	6 ± 0 a	25 ± 1 a	379 ± 12 a
Harvesting date 15-12-15								
Ref	0.32 ± 0.02 a	494 ± 82 a	6.37 ± 0.29 b	5.41 ± 0.24 b	291 ± 3 b	4 ± 0 a	25 ± 1 a	320 ± 4 b
20 kHz	0.29 ± 0.01 ab	461 ± 49 a	5.37 ± 1.52 b	4.3 ± 1.38 b	292 ± 4 b	4 ± 0 a	24 ± 0 a	319 ± 3 b
40 kHz	0.28 ± 0.03 ab	552 ± 76 a	6.88 ± 0.95 b	6.18 ± 1.63 b	286 ± 1 b	4 ± 0 a	24 ± 0 a	315 ± 1 b
80 kHz	0.25 ± 0.03 b	505 ± 38 a	6.15 ± 0.14 b	5.04 ± 0.09 b	293 ± 1 b	4 ± 0 a	24 ± 1 a	321 ± 1 b
Malaxation	0.35 ± 0.03 a	604 ± 52 a	10.61 ± 0.42 a	12.41 ± 0.37 a	311 ± 3 a	4 ± 0 a	24 ± 0 a	339 ± 3 a

* Mean value ± SD (n = 3)
Different letters represent significant differences at p = 0.05.

Others compounds related to the antioxidant proprieties and also for healthy properties of the VOO are the tocopherols [43,44]. The tocopherol identified are α, β and γ-tocopherol (Table 4) being α-tocopherol the major tocopherol identified as described by Beltrán et al. [45]. Tocopherols were not affected by the HPU treatment or malaxation of the olive paste.

Concerning the bitterness index (K_{225}), Table 7, olive paste malaxation gave oils with higher bitterness index achieving similar values than reference. However HPU treatment reduced the oil bitterness respect to malaxation.

In general, conventional malaxation gave oils with higher phenol content than those from HPU treatments although differences were not significant (Table 7). Results of the individual phenols identified in the oil hydroxytyrosol, tyrosol, dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), dialdehydic form of elenolic acid linked to tyrosol (ρ-HPEA-EDA), and aldehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EA) are shown in Table 8. Hydroxytyrosol was not affected by the olive paste conditioning methods assayed. For Tyrosol significant lower content was observed for HPU treatments at 40 kHz and 80 kHz frequencies. The ρ-HPEA-EDA was not affected by the olive paste conditioning methods.

Table 8. Phenolic profile of the VOO obtained from experiments expressed as mg of phenolic compound/kg of olive oil.

Treatments	Hydroxytyrosol	Tyrosol	3,4-DHPEA-EDA	ρ-HPEA-EDA	3,4-DHPEA-EA
Harvesting date November-12					
Ref	1.79 ± 0.51 a	3.57 ± 0.37 a	382.97 ± 9.62 a	312.95 ± 27.4 a	159.96 ± 10.25 b
20 kHz	1.87 ± 0.19 a	3.06 ± 0.06 ab	412.78 ± 17.05 a	337.16 ± 17.04 a	160.84 ± 13.9 b
40 kHz	1.39 ± 0.35 a	2.32 ± 0.73 b	235.22 ± 17.95 b	336.53 ± 36.09 a	142.33 ± 12.61 b
80 kHz	1.46 ± 0.29 a	1.97 ± 0.29 b	246.67 ± 21.12 b	356.49 ± 18.74 a	136.41 ± 4.59 b
Malaxation	1.88 ± 0.17 a	2.98 ± 0.37 ab	412.74 ± 26.12 a	322.64 ± 30.25 a	259.24 ± 12.13 a
Harvesting date December-04					
Ref	1.12 ± 0.07 a	1.6 ± 0.11 a	44.57 ± 15.69 b	140.17 ± 7.59 a	50.89 ± 12.84 b
20 kHz	1.67 ± 0.26 a	2.64 ± 0.24 a	80.01 ± 15.61 ab	145.74 ± 6.74 a	55.59 ± 3.6 ab
40 kHz	1.64 ± 0.54 a	1.89 ± 0.8 a	94.91 ± 18.18 a	137.93 ± 4.45 a	60.08 ± 5.99 ab
80 kHz	1.59 ± 0.15 a	2.3 ± 0.24 a	77.58 ± 22.08 ab	147.13 ± 17.87 a	54.51 ± 10.12 b
Malaxation	1.31 ± 0.13 a	2.08 ± 0.06 a	77.79 ± 20.87 ab	130.45 ± 0.17 a	79.65 ± 10.73 a
Harvesting date December-15					
Ref	1.33 ± 0.1 a	1.62 ± 0.3 a	87.24 ± 16.67 b	230.52 ± 6.25 a	66.17 ± 5.52 b
20 kHz	1.32 ± 0.25 a	1.41 ± 0.22 a	75.43 ± 10.29 b	226.06 ± 10.67 a	61.97 ± 5.88 b
40 kHz	2.16 ± 0.75 a	2.41 ± 0.86 a	100.35 ± 56.32 b	231.37 ± 6.62 a	83.37 ± 41.94 b
80 kHz	1.44 ± 0.09 a	1.4 ± 0.29 a	81 ± 24.84 b	246.99 ± 19.64 a	65.59 ± 10.24 b
Malaxation	1.89 ± 0.11 a	1.77 ± 0.14 a	201.45 ± 17.77 a	228.69 ± 18.71 a	168.84 ± 10.55 a

* Mean value ± SD (n = 3)
Different letters represent significant differences at p = 0.05.

The main phenolic compounds affected by the different conditioning methods were the 3,4-DHPEA-EDA and 3,4-DHPEA-EA. The first, in general, was found at higher content in those oils from malaxed pastes, showing lower values for HPU treatments. 3,4-DHPEA-EA content increased significantly only by malaxation for all the harvesting dates.

The reduction of phenols when HPU treatment was performed was in agreement with previous works [15,22,24].

4. Conclusions

The HPU prototype developed for VOO extraction at pilot plant was used as alternative to malaxation. The results showed that the first effect is a fast heating of the olive paste for all frequencies assayed, being 40 kHz the most efficient. For olives with lower moisture HPU heating capacity was lesser. The extraction yield was better for HPU application at 40 kHz frequency. HPU showed a slight decrease of extraction yield when the olive moisture decreased. HPU treatment at 40 kHz showed oil yield equivalent to that from conventional malaxation. The VOO quality, nutritional and sensorial characteristics were not affected negatively by HPU technology.

Furthermore, HPU technology let to reduce energy consumption respect to conventional malaxation, independently from the energy source for olive paste heating, electrical or thermal. In addition, HPU needs lowest volume and space. All these results confirmed the viability of HPU technology as alternative to malaxer in the VOO extraction.

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Conclusiones

Conclusiones

Este trabajo trata del tratamiento continuo por los ultrasonidos de potencia de las pastas de aceitunas (variedad 'Picual') para la mejora de la etapa de batido en el proceso de extracción de los aceites de oliva vírgenes. De este estudio experimental se destacan las siguientes conclusiones:

1. Los ultrasonidos de potencia permiten el calentamiento rápido de las pastas de aceitunas. La eficiencia de este calentamiento depende principalmente de las características del fruto utilizado (en particular el contenido en humedad), el tiempo de residencia o caudales de pastas usados, la frecuencia aplicada de ultrasonidos y la potencia del tratamiento

2. El pretratamiento de las pastas por ultrasonidos de potencia, previo al batido, mejora la extractabilidad de los aceites de oliva vírgenes.

3. La aplicación de ultrasonidos a 40 kHz y con caudales másicos de pastas de aceitunas de 200 kg/h, sin batido posterior, ha dado lugar a rendimientos equivalentes a un batido convencional realizado a 28 ºC. Para estas condiciones de operación en el equipo de ultrasonidos las temperaturas determinadas en las pastas de salida fueron siempre inferior a 28 ºC, con valores en el rango de 20-26 ºC.

4. En general, no se observaron alteraciones en la calidad de los aceites de oliva vírgenes extra con el tratamiento por ultrasonidos de potencia. Este tratamiento no favorece los fenómenos de oxidación de los lípidos.

5. Este tratamiento permitió obtener aceites de oliva vírgenes con mayor contenido en tocoferoles y pigmentos, mientras se observó un ligero descenso en la concentración de los compuestos fenólicos y en el amargor de los aceites de oliva vírgenes extra. En la fracción de tocoferoles se determinaron incrementos en α-tocoferol mientras que los contenidos en β y γ-tocoferol permanecieron constantes.

6. Los aceites obtenidos mediante la aplicación de ultrasonidos a 40 y 80 kHz, sin batido posterior, mostraron un incremento del atributo frutado y fundamentalmente, la aparición de otros relacionados con la percepción sensorial del verde, plátano y almendrado.

7. La tecnología de ultrasonidos de potencia puede constituir una alternativa tecnológica viable al batido de las pastas de aceitunas permitiendo un ahorro energético y de espacio en la almazara. En relación al coste energético, el sistema de extracción del aceite de oliva virgen mediante ultrasonidos de potencia permitio un ahorro del orden del 80 % con respecto a la condiciones de batido utilizadas.

En definitiva, los resultados obtenidos muestran la viabilidad de la técnica de los ultrasonidos de potencia en el proceso de extracción de los aceites de oliva vírgenes tanto a escala de laboratorio como a escala de mini planta. Esta técnica puede permitir la mejora y optimización de los sistemas de batido convencional implementados en almazaras industriales, o la sustitución del batido por el tratamiento con ultrasonidos de potencia. La eficiencia de esta técnica depende profundamente de las características de las aceitunas utilizadas (variedad, índice de madurez, contenido en humedad y materia volátil…). Se ha demostrado con estos resultados la fácil adaptación de esta técnica para las diferentes características del fruto, permitiendo su integración en el proceso de elaboración con sistemas de control y automatización.

Conclusions

In this work, continuous High Power Ultrasounds treatments of olive pastes (variety 'Picual') were used to improve the malaxation step of the Virgin Olive Oil elaboration process. From this work can be highlighted the following conclusions:

1. The High Power Ultrasounds allowed the rapid heating of olive pastes. The efficiency of olive paste heating mainly depends on the olive fruit characteristics (specifically moisture content), the olive paste flow rate or its residence time, the frequency applied and treatment intensity.

2. The High Power Ultrasound pretreatments of olive pastes, before kneading, enhanced the olive oil extractability.

3. High Power Ultrasounds application allowed to obtain, in the case of 40 kHz treatment at a flow of 200 kg/h and without kneading, oil yields equivalent to those achieved for the conventional malaxation at 28 ºC. For these operation conditions of High Power Ultrasound device, the temperature measured at the exit did not exceed 28 ºC, ranging from 20 ºC to 26 ºC.

4. In general, High Power Ultrasound treatments did not affect to Virgin Olive Oil quality comparing with the conventional malaxation. Also this treatment did not induced lipid oxidation.

5. The virgin olive oil obtained from High Power Ultrasounds treatments showed higher tocopherols and pigments content whereas the phenolic compounds and bitterness showed a reduction. The main tocopherols compound raised by High Power Ultrasound was the α-tocopherol meanwhile the β-tocopherol and γ-tocopherol remain constant.

6. The High Power Ultrasounds treatments at 40 kHz and 80 kHz without malaxation gave virgin olive oils with higher fruity sensorial attributes especially those related to green, banana and almond perception.

7. Ultrasounds technology can be used as alternative to malaxation allowing energy saving and space gain in oil mill. Regarding to energetic consumption, the HPU device allowed an energy gain of 80 % compared to the malaxation conditions assayed in this work.

Finally, the results obtained for the High Power Ultrasounds treatments showed the viability of this technique for the Virgin Olive Oil extraction process. This technique can be used as an aid to malaxation, or as alternative to the malaxer. The efficiency of this technique depends deeply on the olives characteristics (variety, maturity index, moisture…). The results obtained demonstrated the easy adaptation of this technique to the different fruit characteristics, allowing its implementation in the extraction process with control and automation systems.

Anexos: Otras contribuciones a congresos y revistas internacionales

Application of High Power Ultrasounds in the Virgin Olive Oil Extraction Previous to Olive Paste Malaxation.

Poster en Congreso. Año: 2013. Antalya, Turquía.

11th Euro Fed Lipid Congress and 30th ISF Lecture Series: Oils, Fats and Lipids new strategies for a high quality future.

Application of high power ultrasounds in the virgin olive oil extraction previous to olive paste malaxation

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IFAPA

INTRODUCTION

Virgin olive oils (VOO) are extracted from healthy *Olea europaea* L. fruit by exclusively using physical procedures. This procedures include fruit crushing, olive paste kneading, Solid-Liquid separation and finally a clarification by centrifugation and/or settling.
Kneading is a basic steps in the VOO extraction procedures, it especially important to reach high and satisfactory yield extraction. Also has several effects on the VOO quality parameters, nutritional and sensorial characteristics.
Hence a current trend is to enhance this procedure using some technics as fruit pitting before crushing, kneading in inert atmosphere and cold kneading. One of the technics recently used is the pretreatment of olive paste by high power ultrasound (HPU) (Jimenez *et al.*, 2006; 2007).

OBJECTIVES

The present work analyses the continuous application of HPU on olive paste previous to kneading and it's effects on process yield and VOO quality parameters, nutritional and sensorial characteristics compared with the VOO extraction without HPU application.

MATERIALS AND METHODS

Olive fruits from `Picual´ cultivar were collected at early véraison ripening stage, moisture and oil content were analyzed.
Two VOO elaboration procedure were realized one using 100% of the power of the HPU and the olive paste flow was 20 kg/hour to achieve 30ºC of paste temperature (VOO HPU) and the second in the same paste flow but without HPU application where the paste temperature was 20ºC (VOO Test) (Figure 1).
For the VOO samples obtained were determined the quality index (free acidity value, peroxide value, K232, K270), bitterness, phenolic content (Total and biophenols), tocopherol content and pigments.

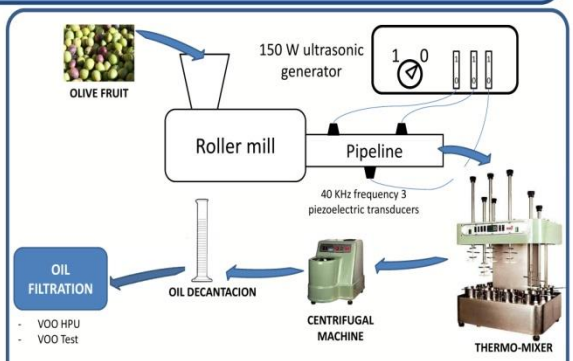

Figure 1. Schematic disposition of the experimental VOO elaboration pilot scale

RESULTS

- ✓ The application of ultrasound induced a quick heating of the paste from 20ºC to 30ºC in 3 minutes whereas it took 20 to 30 minutes without HPU application.
- ✓ This sonication treatment improves about 1 % the industrial oil yield and gave higher extractability 46.83 % without HPU application to 52.57 % using HPU (Figure 2).

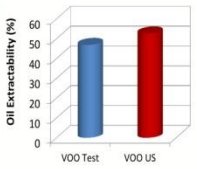

Figure 2. Effect of ultrasound treatment on oil extractability

- ✓ Oil obtained with HPU application showed a higher content of tocopherol specifically the α-tocopherol (Figure 3).
- ✓ A similar behaviour was observed for chlorophylls and carotenoids content.
- ✓ With regard to the bitterness and phenolic content (total phenols and biophenols), mainly for biophenols, decreased with HPU treatment.

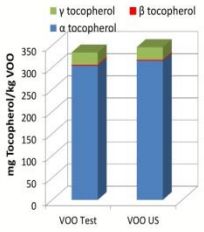

Figure 3. Effect of ultrasound treatment on oil Tocopheol content

Fatty acids	VOO Test	VOO HPU
C_{16}	13.78±0.06	13.88±0.01
C'_{16}	1.33±0.01	1.34±0.01
C_{17}	0.04±0.00	0.04±0.00
C'_{17}	0.08±0.00	0.09±0.00
C_{18}	2.67±0.01	2.78±0.01
C'_{18}	77.80±0.10	77.35±0.01
C''_{18}	3.17±0.04	3.30±0.01
C'''_{18}	0.62±0.03	0.66±0.00
C_{20}	0.28±0.05	0.33±0.01
C'_{20}	0.20±0.01	0.20±0.02
C_{22}	0.05±0.04	0.06±0.01

Quality parameters	VOO Test	VOO HPU
Free Acidity (% oleic fatty acid)	0.12±0.01	0.12±0.01
Peroxides (meq O2/kg)	3.30±0.07	3.98±0.08
K232	0.1077±0.0107	0.1016±0.0044
K270	1.5588±0.0958	1.4020±0.0193
Nutritional parameters	**VOO Test**	**VOO HPU**
Bitterness	0.2705±0.0070	0.2667±0.0077
Phenols content (ppm)	374±10	362±11
Pigments Carotenoid (ppm)	1.96±0.06	2.73±0.04
Pigments Chlorophyll (ppm)	1.83±0.09	3.40±0.04

VOO did not show changes in the quality parameters (free acidity value, peroxide value, K232, K270), and the fatty acid composition

Phenolic Compounds	VOO Test (mg/kg olive fruit)	VOO HPU (mg/kg olive fruit)
Hydroxytyrosol	0.312±0.016	0.134±0.001
Tyrosol	0.422±0.004	0.240±0.015
Vainillic Acid	0.068±0.014	0.033±0.009
Coumaric acid	0.099±0.006	0.085±0.001
Ferulic Acid	0.137±0.061	0.069±0.009
34dhpeaeda	17.827±0.387	10.902±0.137
phpeaeda	15.419±0.624	10.249±0.048
Pinoresinol	0.301±0.018	0.221±0.017
Cinammic Acid	0.069±0.004	0.050±0.002
Acetoxy pinoresinol	0.402±0.025	0.275±0.029
34dhpeaea	7.174±0.299	3.958±0.138
Luteolin	1.300±0.046	0.619±0.043
phpeaea	0.729±0.091	0.275±0.068
Apigenin	1.537±0.037	1.116±0.023
Total	45.796±1.408	28.228±0.376

CONCLUSIONS

Continuous high power ultrasound application on olive paste has shown some effect on malaxation and virgin olive oil:

- It provides a quick-heating of olive paste
- Improve process extractability
- No oxidation or hydrolysis effects were observed in oils when ultrasounds were applied
- Ultrasound treatments gave oils with significant higher contents in tocopherol, carotenoids and chlorophylls
- Lower bitterness level and phenols content in VOO

References : Jiménez, A., Beltrán, G., Uceda, M. and Aguilera, M.P. (2006). Empleo de ultrasonidos de potencia en el proceso de elaboración del aceite de oliva virgen. Resultados a nivel de planta de laboratorio. Grasas y Aceites. Vol 57(3), 253-259.
Jiménez, A., Beltrán, G. and Uceda, M. (2007). High-power ultrasound in olive paste treatment. Effect on process yield and virgin olive oil characteristics. Ultrasonics Sonochemistry 14 (6), 725–731.

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CONSEJERÍA DE AGRICULTURA, PESCA Y DESARROLLO RURAL

ACKNOWLEDGMENTS: This work has been supported by the project of excellence P10-AGR-6099: 'Estudio de la inertización y control de atmósferas durante el batido de la pasta. Influencia en las características bioactivas y sensoriales del aceite'.

Unión Europea

High power ultrasound treatment at pilot plant scale for virgin olive oil extraction.

Poster en Congreso. Año: 2014. Montpellier, Francia.

12th Euro Fed Lipid Congress "Oils, Fats and Lipids: From Lipidomics to Industrial Innovation".

High power ultrasound treatment at pilot plant scale for virgin olive oil extraction

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Introduction

High Power Ultrasound (HPU) pretreatment of olive paste before malaxation at laboratory scale during the Virgin Olive Oil (VOO) elaboration process was recently investigated to improve the efficiency and reduce the duration of this step. The main effects of the HPU treatment on olive paste are a rapid heating of olive paste and better extractability.

Objectives

The study of the effects on the process yield and quality of a pilot plant scale device designed to the olive paste HPU continuous treatment..

Experiments

'Picual' olives was crushed in an industrial hammer mill and after pumped through the HPU device with a flow rate of 200 kg/h. Different HPU treatments were assayed as described below.

Milled olive Paste

Olive paste reception tank

High Power ultrasound Pilot Device

- Ref = Reference, untreated olive paste
- T20 = First treatment, 20 khz , 900 W
- T40 = Second treatment, 40 khz , 900 W
- T80 = Third treatment, 80 khz , 900 W

M/C

Malaxation

Centrifugation

C

To determine the effect of HPU the olive paste was extracted in an `ABENCOR´ system. For each treatment two extraction conditions were carried: malaxation (at 28ºC for 30 minutes) and centrifugation (M/C) against direct olive paste centrifugation (C).

Direct olive paste centrifugation

The liquid phases were recovered in a laboratory test-tube and after settling the volume of the oily phase was taken to determine the industrial yield.
After filtration oil quality indices and composition were analyzed:

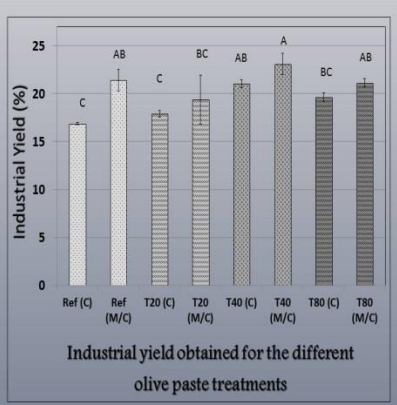
- Free Acidity
- Peroxides
- Ultraviolet (K232 and K270)
- Tocopherols content
- Total Polyphenols
- Bitterness
- Pigments

Results

The principal effect of the HPU treatment on the olive paste compared with the untreated paste was:

- the instantaneous of olive paste
- Higher process yield

Therefore, only with 40 khz HPU treatment the process yield obtained was similar to that obtained for olive paste malaxed at 28ºC during 30 minutes.



Industrial yield obtained for the different olive paste treatments

Quality indices	Free Acidity (% oleic fatty acid)	Peroxides (meq O2/kg)	Ultraviolet	
			K232	K270
Ref (M/C)	0,17±0,00	6,17±0,15	1,59±0,02	0,13±0,01
T20 (M/C)	0,17±0,00	6,40±0,53	1,62±0,02	0,15±0,02
T40 (M/C)	0,17±0,00	5,87±0,42	1,61±0,01	0,13±0,01
T80 (M/C)	0,17±0,00	6,16±0,07	1,68±0,03	0,17±0,01
Ref (C)	0,17±0,00	5,97±0,18	1,63±0,04	0,16±0,03
T20 (C)	0,17±0,00	6,06±0,21	1,60±0,02	0,15±0,01
T40 (C)	0,17±0,00	6,27±0,20	1,64±0,03	0,17±0,02
T80 (C)	0,17±0,00	5,33±0,32	1,57±0,05	0,14±0,02

There are no difference observed between virgin olive oil obtained using HPU treatment and reference.

Regards to the oil composition, there are not alteration for the nutritional parameters (Tocopherols and Polyphenols contents)

Oil Composition	Tocopherols content (ppm)				Total Polyphenols (mg/kg)	Bitterness	Pigments (ppm)	
	α	β	γ	Total			Carotenoid	Chlorophyll
Ref (M/C)	350±3	1±0	24±0	362±0	577±21	0,37±0,01	7,33±0,42	4,57±0,81
T20 (M/C)	351±0	1±0	24±0	360±2	546±17	0,37±0,01	7,20±0,10	4,30±0,00
T40 (M/C)	343±10	1±0	24±1	365±7	553±62	0,38±0,02	6,67±0,84	3,97±0,75
T80 (M/C)	353±2	1±0	24±0	364±2	598±11	0,35±0,01	8,30±0,20	6,30±0,36
Ref (C)	337±0	1±0	24±0	375±3	571±52	0,37±0,02	6,93±0,21	4,77±0,25
T20 (C)	336±2	1±0	23±1	376±0	521±17	0,37±0,03	7,07±0,21	5,10±0,50
T40 (C)	341±6	1±0	23±1	368±10	575±57	0,39±0,02	7,77±0,65	5,77±0,85
T80 (C)	339±2	1±0	24±0	378±2	541±2	0,39±0,01	6,90±0,10	4,87±0,21

CONCLUSIONS

The study of treatment of the olive paste with the HPU device designed for pilot plant, showed that the treatment improve the olive oil yield and in the case of the overripe olive fruit used during the experiment it can be considerate as an alternative to malaxation. Regards to the olive oil characteristics, the HPU treatment did not cause alteration on VOO quality indices and composition.

ACKNOWLEDGMENTS: This work has been supported by the project of excellence P10-AGR-6099: 'Estudio de la inertización y control de atmósferas durante el batido de la pasta. Influencia en las características bioactivas y sensoriales del aceite'.

How high power ultrasound treatment on olive paste affects the virgin olive oil process yield.

Comunicación oral. Año: 2014. Barcelona, España.

PLENARY LECTURE. Chemical Engineering and sustainable development: Shaping our future. Nº 31_001_O

13th Mediterranean Congress of Chemical Engineering.

HOW HIGH POWER ULTRASOUND TREATMENT ON OLIVE PASTE AFFECTS THE VIRGIN OLIVE OIL PROCESS YIELD.

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Scientific Topic: Applied Chemical Engineering: Food Engineering

Ultrasound, depending on the frequency, is used in various processes in the food industry and where a reproducible food processes can be performed with high reproducibility. The use of this technic is recently investigated for the pretreatment of olive paste step, which has an important impact on the virgin olive oil process yield. As reported by Jimenez *et al.* (2006; 2007), the use High Power Ultrasound (HPU) during olive paste malaxing has two major effects which are a rapid heating of olive paste and better extractability.

Previous experiment at laboratory scale showed that the continuous application of HPU gave a higher process efficiency and lower cost, especially on the olive paste heating. A pilot scale device for continuous HPU application was performed for its use at experimental plant level. The device was composed of a rectangular pipeline where the ultrasound piezoelectric transducers were placed on the four surfaces of the pipeline, and applied three frequencies 20, 40 and 80 khz supplied by a generator of 900 watt. The experiments were carried out using olive paste, obtained after olive fruit crushing without kneading and then treated through the HPU device with an olive paste flow rate of 200 kg/h. Four treatment were assayed the reference without sonication and the remaining applying the three frequencies. After HPU treatment the olive paste was collected and the oil was extracted at `ABENCOR´ laboratory scale virgin olive oil extraction system. Two extraction conditions were compared, malaxation at 28 ºC for 30 minutes and centrifugation against direct paste centrifugation. The liquid phases were recovered in a laboratory test-tube and after settling the volume of the oily phase was taken in order to determine the virgin olive oil process yield and extractability. The experiment was performed using olive fruits from `Picual´ cultivar collected at two different harvesting date, their moisture and oil content were analyzed.

The principal effect of the HPU application on the olive paste was the instantaneous heating when it flows through the device for all the frequencies used when compared with the untreated paste.

Regarding to the process efficiency, in general the application of HPU improve the process yield with respect to the untreated paste. Therefore, the single use of the HPU treatment was similar to that olive paste kneading at 30 ºC. HPU treatment at 40 khz frequency followed by kneading produced higher olive oil extractability. These results may lead to think about the viability of this technique as a system of improvement of kneading or even as an alternative process.

- Jiménez A., Beltrán G., Uceda M., Aguilera M.P. (2006). Empleo de ultrasonidos de potencia en el proceso de elaboración del aceite de oliva virgen. Resultados a nivel de planta de laboratorio. Grasas y Aceites, **57**(3), 253-259.
- Jiménez A., Beltrán G., Uceda M. (2007). High-power ultrasound in olive paste treatment. Effect on process yield and virgin olive oil characteristics. Ultrasonics Sonochemistry, **14**(6):725-731.

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Letter

pubs.acs.org/JAFC

Ethanol in Olive Fruit. Changes during Ripening

ABSTRACT: Ethanol is one of the precursors of ethyl esters, the virgin olive oil quality parameter for the "extra" category recently adopted by the European Union and International Olive Oil Council. Although ethyl ester content has great importance for virgin olive oil classification, the origin of ethanol is not clear. A possible source of ethanol may be the olive fruit itself while it remains on the tree. Variation of fruit ethanol content during ripening was studied for three different olive cultivars: 'Picual', 'Hojiblanca', and 'Arbequina'. Ethanol was measured in fruit homogenates by HS-SPME-GC-FID. The ethanol content varied between 0.56 and 58 mg/kg. 'Hojiblanca' fruits showed the highest ethanol concentration. For all of the cultivars, ethanol content of fruit increased during the ripening process, although a clear cultivar-dependent effect was observed because 'Hojiblanca' fruits showed the most significant raise. Therefore, results indicated that ethanol can be accumulated during fruit maturation on the olive tree.

KEYWORDS: *Olea europaea L., olive fruit, ethanol, ripening, cultivar*

INTRODUCTION

Olive (*Olea europaea* L.) growing has great importance in the Mediterranean basin because is the base of two important food industries, table olives and virgin olive oil production. Although it has great importance, information about the raw material characteristics is scarce, mainly in olives for oil mill use.

In this way, ethanol in olive fruit is a metabolite that has achieved great importance for the past two years because it is one of the precursors of ethyl esters. The level of ethyl esters is a virgin olive oil quality parameter adopted recently by both the European Commission and the International Olive Oil Council[1,2] to discriminate "extra virgin olive oil" produced from healthy and high-quality olive fruits. In fact, for oil classification into "extra virgin" category ethyl ester content must be <40 mg/kg. This limit will be reduced in sequence for the next crop years: 35 mg/kg in 2014/2015 and, finally, 30 mg/kg for the crop year 2015/2016.

Although a close relationship between virgin olive oil quality and ethyl esters was described,[3−5] their biosynthesis and the source of ethanol as ethyl ester precursor are not clear and have to be analyzed.

Conte et al.[6] proposed that ethanol can be solely produced by fermentation during virgin olive oil extraction and storage process. However, a possible source of ethanol may be the synthesis in the olive fruit itself. The production of ethanol in olives may originat on the tree, during fruit collection or transport, and/or in the postharvest treatments until processing. However, to our best of knowledge there are no data for ethanol content in olive fruit and its possible biosynthesis from the olive tree to the oil mill.

Ethanol in fruits is formed by the enzyme alcohol dehydrogenase (ADH; EC 1.1.1.1); it has been described for oranges and pears to increase during maturation.[7,8] Fruits have been described as the source of ethanol, as during ripening on the tree it activates reactions to produce aroma and other components requiring the synthesis of anaerobic metabolites, among them ethanol.[9]

Furthermore, ethanol is accumulated in different fruits that remain for long periods on the tree such as oranges and grapefruits. In these cases the concentration of ethanol showed a fast increase while the fruits were on the tree.[10] Litchi fruits increased ethanol production during maturation on tree.[11] Peaches and nectarines showed increases in aroma volatiles and ethanol during maturation.[12]

Ethanol can be accumulated in over-ripe and senescent fruits than remain on the tree for a long period. In apple cultivars, although a small amount of ethanol can be detected, it can be synthesized at higher concentrations in over-ripe and senescent fruits.[13]

Olive is a nonclimateric fruit that usually remains for long periods on the tree until harvest. This period can oscillate between 5 and 9 months, depending of olive cultivar, including fruit development, ripening, and overmaturation. The mean ripening period described for World Olive Germplasm Bank cultivars varied between 53 and 69 days for the earliest and latest olive cultivars, respectively.[14] However, a considerable part of the olive production can remain on the tree until overmaturation (2−3 months) because of adverse climatic conditions during the harvesting period or high crop yields. Thus, it is of great importance to determine how ethanol content varies in fruit during the ripening process in order to evaluate if ethanol is synthesized on the tree; if so, harvest date may have effect the levels in fruit, olive paste, and finally virgin olive oil of ethanol and ethyl esters.

The objective of this work was to study the ethanol content in olive fruit and its variation during ripeness. To achieve this aim three olive cultivars were selected according to their importance in world olive oil production: 'Picual', 'Hojiblanca', and 'Arbequina'. Acetaldehyde content, as ethanol precursor, was analyzed, too.

MATERIALS AND METHODS

Plant Material. The study was carried out for the crop year 2012/2013. Olive trees of three olive cultivars, 'Picual', 'Hojiblanca', and 'Arbequina', were selected by their crop load homogeneity (3−4). Two trees were selected for each cultivar. The 25-year-old trees were grown in the Wold Olive Germplasm Collection of IFAPA Center Venta del Llano in Mengibar, Jaen, Spain. The trees were spaced 7 × 7 m and grown under irrigation and traditional techniques.

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Journal of Agricultural and Food Chemistry Letter

Table 1. Sampling Dates of Olive Fruits from 'Picual', 'Hojiblanca', and 'Arbequina' Olive Cultivars

olive cultivar	Sept 11 (14 WAF[a])	Oct 16[b] (21 WAF)	Oct 24 (22 WAF)	Nov 13[b] (26 WAF)	Dec 20[b] (30 WAF)
'Picual'		0		1.8	2.4
'Hojiblanca'		0		1.5	2.2
'Arbequina'	0	0.3	1.0	2.0	2.4

[a]WAF, weeks after flowering. [b]Harvest dates common for the three olive cultivars.

Olive Samples. Two kilograms of olives was collected from each tree and cultivar at three harvest dates for 'Picual', 'Hojiblanca', and 'Arbequina'. In addition, for 'Arbequina' more harvest dates were included because of the stepped maturation of its fruits. Fruit ripening index was measured according to the method proposed by the Estacion de Olivicultura y Elaiotecnia.[15] The fruit sampling dates and ripening index are shown in Table 1. After harvesting, the fruits were washed using Milli-Q water, dried with filter paper, and processed immediately (15 min) to avoid any alteration.

Fruit Homogenates. To measure the ethanol content, a fruit homogenate approach was followed.[16] For this purpose, 4 g of olive fruit mesocarp, from at least 20 olive fruits, was homogenized with 8 mL of distilled water by means of a homogenizer Ultra-Turrax T-25 at the highest speed (24000 rpm) for 2 min. After an equilibrium period of 5 min at 25 °C, homogenate aliquots of 2 mL were taken into 10 mL vials containing 2 mL of a saturated $CaCl_2$ solution to deactivate the enzyme systems, which were sealed and stored at −18 °C until analysis. Two homogenates were prepared in duplicate for each olive sample.

Analysis of Ethanol and Acetaldehyde. Solid-phase microextraction (SPME) followed by GC-FID was used to analyze the ethanol and acetaldehyde in the samples studied according to the method described by Sanchez-Ortiz et al.[16] Briefly, homogenate samples were conditioned to room temperature and then placed in a 10 mL vial fitted with a silicone septum heater at 40 °C. After 10 min of equilibrium time, ethanol from headspace was adsorbed by exposing the SPME fiber DVB/Carboxen/PDMS 50/30 μm 1 cm (Supelco Co., Bellefonte, PA, USA) for 50 min at 40 °C in the headspace of the sample and then retracting it into the needle, followed by immediate transfer and desorption for 5 min into the injection port of a gas chromatograph equipped with an FID.

Ethanol and acetaldehyde were analyzed using a Varian CP 3800 GC equipped with a Supelcowax 10 capillary column (30 m × 0.25 mm, 0,25 μm, Sigma-Aldrich Co. LLC). Operating conditions were as follows: He was the carrier gas; injector and detector at 250 °C; and column held for 5 min at 40 °C and then programmed at 4 °C min^{-1} to 200 °C. Compound identification was carried out on an ISQ single-quadrupole MS (Thermo Fisher Scientific, Austin, TX, USA) operating in EI mode (70 eV) under identical conditions for GC-FID, matching against the Wiley/NBS Library, and by GC retention time against standard. Quantification was performed using individual calibration curves in the matrix (olive mesocarp homogenate). Results were expressed as milligrams per kilogram of fruit.

Data Analysis. Data for harvest dates are shown as mean value and standard deviation, whereas for cultivar content as mean value and standard error. ANOVA was performed to establish the effect of cultivar and ripening stage considering the three common harvest dates for the three olive cultivars. Tukey's test was applied to establish differences between means, $p = 0.05$. Statistical analyses were performed with Statistix 8.0 software.

RESULTS AND DISCUSSION

ADH activity catalyzes the reversible reduction of aldehydes to alcohols using reduced pyridine nucleotides as cofactors. In previous works ADH activity was measured only in crude extracts prepared from acetone powders of olive mesocarp and seed tissues,[17] whereas it was not detected in crude extracts from fresh tissues.[16] To determine the effect of homogenization process in ethanol synthesis, a previous experiment was carried out. Fruit frozen tissue was ground in a cold blender containing $CaCl_2$ at 0.33 g g^{-1} tissue to inhibit enzyme activities during homogenization compared with the method used in this work. Both methods showed similar contents of ethanol (data not shown).

Ethanol content in fruit varied depending on olive cultivar. For 'Hojiblanca' fruits ethanol concentration varied between 6 and 58 mg/kg, whereas 'Picual' cultivar oscillated from 0.56 to 2.90 mg/kg. 'Arbequina' olives showed an ethanol content that ranged between 1.5 and 11.5 mg/kg.

When ANOVA was performed, the olive cultivar was observed to be mainly responsible for the variability of ethanol concentration, although the harvest date showed similar values (Table 2). Among the olive cultivars analyzed, 'Hojiblanca' showed the highest ethanol content in fruit and 'Picual' achieved the lowest concentration (Table 3). Significant differences between olive cultivars were obtained ($p = 0.05$). These differences in ethanol content between cultivars were described for apples and nectarines previously.[16] There are no previous works describing differences in olive ethanol content between cultivars. The differences observed in this work may be explained by the ADH content/activity in different olive cultivars as described for 'Coratina' and 'Carolea' olive cultivars for C6 volatile alcohols.[18] In our case, the levels of ethanol in fruits of different cultivars corresponded to similar differences for its precursor, acetaldehyde (data not shown).

Harvest date showed a highly significant effect on ethanol content in olive fruits (Table 2). During olive fruit ripening,

Table 2. Partial Mean Squares from Analysis of Variance for the Effect of Cultivar and Harvest Date on Fruit Ethanol Content from the Olive Cultivars 'Picual', 'Hojiblanca', and 'Arbequina'

source	DF[a]	MS[b]	SSTo[c]	p
cultivar	2	942.828	36.36	0.0000
harvest date (HD)	2	938.539	36.20	0.0000
cultivar × HD	4	353.892	27.30	0.0000
error	9	0.805	0.14	
total	17			

[a]DF, degrees of freedom. [b]MS, mean squares. [c]SSTO, partial mean squares for the effect, expressed as percentage, of the total corrected sum of the squares.

Table 3. Mean Ethanol Content of Olive Fruits from 'Picual', 'Hojiblanca', and 'Arbequina' Olive Cultivars

olive cultivar	ethanol (mg/kg)
'Picual'	3.4 ± 1.7 c[a]
'Hojiblanca'	26.1 ± 10.2 a
'Arbequina'	5.7 ± 1.9 b

[a]Different letters indicate significant differences between means for p value of 0.05.

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ethanol concentration increased slightly at the beginning of maturation, and then a fast increase was detected (Figure 1).

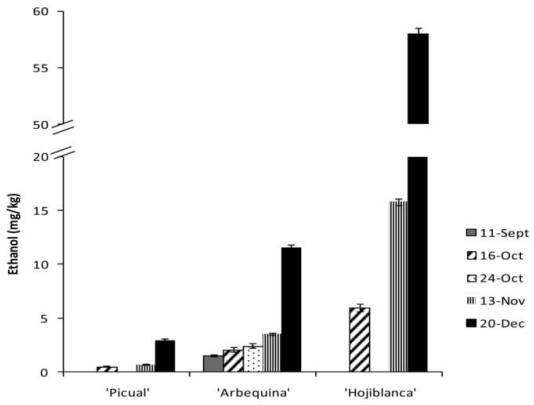

Figure 1. Changes during the ripening process in ethanol concentration of fruits from the olive cultivars 'Picual', 'Hojiblanca', and 'Arbequina'.

Although 'Arbequina' has a fruit stepped maturation and was studied for more harvest dates, the behavior was similar to that of the other cultivars. In the first raise, ethanol showed an increase between 23% for 'Picual' cultivar and 62% for 'Hojiblanca'. For the last harvest date the fastest increases were detected; the increases obtained varied around 70% for the three olive cultivars. Between the first sample collection and the final one, ethanol content rose around 80% for 'Picual' and 'Arbequina', whereas 'Hojiblanca' fruits achieved an increase of 89%. Therefore, 'Hojiblanca' fruits achieved the highest ethanol content during fruit maturation. These differences between cultivars in the biosynthesis of ethanol may be explained by the high significance obtained for the interaction between olive cultivar and harvest date in the ANOVA (Table 2). Ethanol is formed from acetaldehyde by the enzyme ADH. Salas et al.[17] described an increase of ADH activity from 13 weeks after flowering up to 25 weeks after flowering when fruits are still green. After this point ADH activity decreased during the ripening process. Therefore, the increase of fruit ethanol content observed for the three cultivars may not be explained by a higher ADH activity because the period analyzed corresponded to a reduction of ADH activity.

Both acetaldehyde and ethanol have been shown to accumulate in various fruit (both climacteric and non-climacteric) that remain on the tree for long periods.[9] The increase in anaerobic respiration in over-ripen fruit may be explained by a reduction of mithocondrial activity in its tissues. In our case both of them are accumulated in the fruit during maturation, although ethanol showed a faster increase, giving a reduction of the ratio acetaldehyde/ethanol (Figure 2). This ratio can be used as an indicator of fruit anaerobic respiration.

The increase of ethanol content observed for the three cultivars was similar to those described for both climacteric and nonclimateric fruits during the ripening and their residence on the tree for long periods,[10,19] although in our case over-ripening was not achieved.

The results confirm that ethanol is produced naturally in olive fruits while they remain on the tree because for this work their processing was immediate. Ethanol content in olives had a very important genetic component because significant differences were observed between cultivars. 'Hojiblanca' fruits showed the highest ethanol concentration. During fruit ripening ethanol concentration rose, showing a slight increase at the beginning and a fast rise for the last harvest date. Ethanol production during fruit ripening was affected by the cultivar, too. Therefore, ethanol was accumulated in the olive fruit during its residence on the tree as a result of anaerobic respiration. Further works should be focused on the formation of ethanol from olive tree to virgin olive oil and how ethyl esters are synthesized.

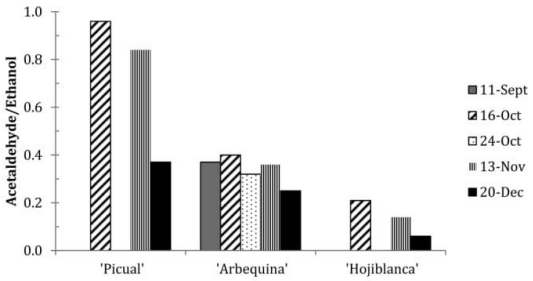

Figure 2. Changes during the ripening process in the ratio of acetaldehyde to ethanol of fruits from the olive cultivars 'Picual', 'Hojiblanca', and 'Arbequina'.

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Notes
The authors declare no competing financial interest.

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Modeling the settling behavior in virgin olive oil from a horizontal screw solid bowl

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ABSTRACT

Nowadays, in the oil mill, settling tanks are used as an alternative clarification technology to the vertical centrifuges used to clarify the virgin olive oil from the decanter. Despite the fact that these settling tanks are being implemented, there is limited knowledge on the settling process. In this preliminary work, the effect of room temperature (15, 20 and 30 °C) in the static settling of virgin olive oil ('Picual' variety) in a settling column has been studied. First, the particle-size distribution in oil was analyzed resulting in a d50 of around 165 μm. As expected, a temperature of 30 °C showed higher values of settling efficiency compared to lower temperatures (15 and 20 °C). Finally, a simulation study of this static settling case was carried out using computational fluid dynamics (CFD) in which good agreement was found compared to experimentally determined process behavior.

1. Introduction

Virgin olive oil (VOO) is obtained exclusively from the fruit of the olive tree (*Olea europaea L.*) using mechanical and physical processes, such as washing, decantation, centrifugation and filtration. The latter does not lead to alterations in the oil, exclusion of oils obtained using solvents or re-esterification processes and of any mixture with other oils (IOOC, 2006).

Currently, the so-called two phase continuous extraction system is the most widespread used in the VOO extraction process (Jiménez et al., 1995; Piacqadio et al., 1998). In this system, the horizontal screw solid bowl, so-called 'decanter', is used for a primary separation of the oil fraction from the water and solid pomace (Altieri, 2010; Altieri et al., 2013). However, the oil resulting from the 'decanter' still contains moisture and suspended solid particles, which both have to be removed (Uceda et al., 2006, 2008). If not, this can lead to the alteration of VOO since it can induce anaerobic fermentation, hydrolysis and oxidation reactions during storage, which reduces the oil quality and sensory characteristics causing the emergence of undesired off-flavors such as fusty or muddy sediment (Ranalli, 1989; Ambrosone et al., 2002; Tsimidou et al., 2005; Gómez-Alonso et al., 2007; Jiménez and Carpio, 2008; Di Giovacchino et al., 2002; Morelló et al., 2004; Baiano et al., 2014).

Traditionally, after liquids are sieved, the separation of liquid phases with different density is performed by vertical centrifuges. These centrifuges are characterized by a high water consumption, high wastewater production and a considerable energy consumption (Masella et al., 2009, 2012). For these reasons, a growing trend appeared in the olive oil industry to substitute the centrifuges by conical bottom settling tanks in the clarification step. These tanks have a cone angle between 45° and 60° and a capacity between 400 and 10,000 L. They are equipped with a purge system (manual or automatic) and can be used both for batch and continuous operation (Humanes and Humanes, 2011; Altieri et al., 2014). Despite the fact that settling tanks are being implemented, little knowledge on the settling process of VOO from HSSB is available. The main factors affecting this separation processes are the density difference between liquid and solid particles, the particle size, the liquid viscosity, among others (Davis, 2010). Some of these parameters, such as oil density and viscosity, have already been studied for several vegetable oils, including olive oil (Esteban et al., 2012; Bonnet et al., 2011; Fasina et al., 2006). These physical

Abbreviations: VOO, virgin olive oil; HSSB, horizontal screw solid bowl; CFD, computational fluid dynamics; SPC, solid particles content; MSPC, moisture and solid particle content.

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properties depend on the VOO fatty acid composition and are strongly affected by temperature (Gila et al., 2015). However, for other essential parameters, such as solids density and solid particle size distribution, no data was found in the literature.

Direct measurement of these separation processes are the best way to understand the behavior of liquid and solid particles flows, however they cannot be ascertained until the tank is available. Computational fluid dynamics (CFD) offers an alternative way to predict the behavior of this separation process. This tool permits to save costs and time, because it is less expensive than experiments, physical modifications are not necessary, and it can also predict which design changes are most crucial to improve performance. CFD techniques have already been applied in other separation processes (Romaní and Nirschl, 2013; Vieira and Barrozo, 2014; Narasimha et al., 2005) including wastewater treatment that uses settling tanks to separate suspended solid sludge particles from the purified water (Stamou, 2008; Goula et al., 2008a, 2008b; Patziger et al., 2012). For these reasons, introduction of CFD technology in the study of oil clarification step can be very useful to obtain extra information and to improve some aspects of the extraction process in olive oil mill.

The aim of this work was the analysis of the clarification process of VOO from HSSB in settling columns at three different temperatures (15, 20 and 30 °C). In addition, a CFD model was developed for the settling column that represents and simulates this separation step.

2. Material and methods

2.1. Origin of the oil

For this work, around 9 L of VOO were taken directly from the HSSB after the vibratory sieve (with 1 mm of diameter). The oil was obtained from the 'Picual' variety olive fruits in the experimental oil mill of the research center IFAPA 'Venta del Llano' in Mengíbar, Jaén (Spain). The experimental plant pilot was equipped with a metallic hammer mill with a 6 mm sieve, a thermobeater formed by three containers (Pieralisi, Spain) of 600 kg each, a two–three phases horizontal centrifuge Pieralisi SC-90 (working at two phases way) with a theoretical processing capacity of 45,000 kg/day. The olive paste was loaded at 750 kg/h in the horizontal centrifuge, being controlled by the automation system. The operation work conditions were: crusher velocity of 2250 rpm; kneading temperature of 28 ± 2 °C and a kneading time of 45 min.

2.2. Experimental assays

Laboratory graduated cylinders (with a volume capacity of 1000 mL) were used as settling column, with an internal diameter and height of 6 cm and 35.37 cm respectively. An aliquot of 1000 mL of VOO sample was poured into of each settling column. Prior to starting the assay, the column was shaken, in order to obtain a homogeneous suspension. The assays were carried out simultaneously at three different room temperatures (15, 20 and 30 °C) in different temperature controlled chambers. Assays were performed in triplicate. Oil samples were taken from each column with a pipette (5 mL), at 10 cm of depth from the oil upper level (Fig. 1) at different times: 0, 5, 10, 20, 40, 80, 160, 320, 640 and 1280 min.

2.3. Experimental analyses

2.3.1. Moisture and solid particle content

To determine the moisture content, approximately 5 mL of olive oil was weighed in a ceramic capsule with filter paper.

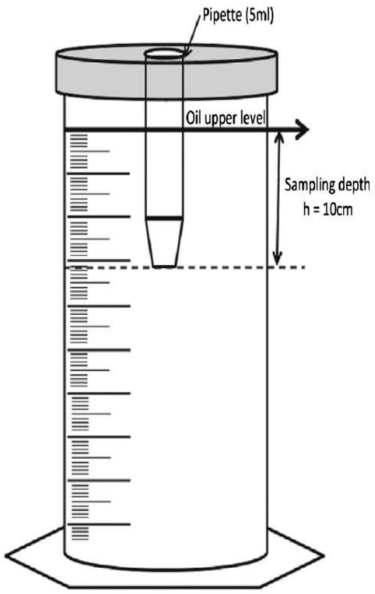

Fig. 1. Experimental device. Laboratory test tube with pipette coupled.

Subsequently, the oil sample was dried in an oven at 105 °C until weight stabilization. The loss of weight yields humidity (%) and volatile matter (UNE 55-020-73, 1973). After drying, impurities were retained by the paper filter and the oil was extracted with petroleum ether in a Soxhlet system. Finally, the filter paper was dried and weighed to determine impurities amount (%) (UNE 55002:1962, 1962).

2.3.2. Particle-size distribution

Particle size distribution analysis was performed using a gravitational method adopted from the ISO 13317-2:2001 (ISO, 2001; Farmer and Beckman, 1984). The latter uses a device consisting of a laboratory cylinder with pipette coupled, as described in Section 2.2 (Fig. 1). Similar to the experimental assays, an aliquot of 1000 mL of VOO sample was poured into each of the settling columns and shaken to obtain a homogeneous suspension. Subsequently, during solid particle settling at 20 °C, a volume was pipetted at a fixed depth (10 cm) and at different settling times. Next, the solid particle content of the sample was determined (Section 2.3.3), each time measuring the quantity of the still suspended particles in the suspension. Using these obtained results, combined with the known values of VOO density and viscosity according to Gila et al. (2015), the solid particle density of 1025 kg/m^3 (Alba, 2008), the sampling time and depth, the gravity value (9.81 m/s^2) and applying the equation based on the Stokes law's (Eq. (1)), the distribution curve (Fig. 2) can be calculated:

$$d_p = \sqrt{\frac{18 \cdot h \cdot \mu}{t \cdot g(\rho_s - \rho_l)}} \quad (1)$$

where d_p is the particle diameter (m), h is the depth of sampling point (m), μ is the olive oil viscosity (Pa s) at 20 °C, t is the sampling time (s) after starting the assay, g is the gravity (m/s^2), ρ_s is the solid particle density (kg/m^3) and ρ_l is the liquid density (kg/m^3) at 20 °C.

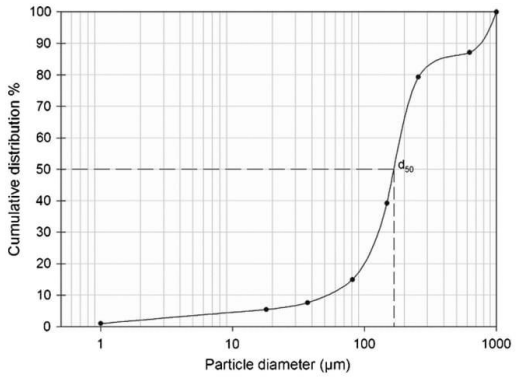

Fig. 2. Particle size distribution of a VOO sample from HSSB.

Table 1
Physical properties of virgin olive oil and solid particles used in the simulations.

	Phase				
	Primary	Secondary			
Physical properties	Olive oil	Solid 1	Solid 2	Solid 3	Solid 4
Density (kg/m^3)	908	925	1025	1125	1225
Viscosity (Pa s)	0.084	–	–	–	–

2.3.3. Settling efficiency

The settling efficiency at 80 min was estimated using following relationships (Eq. (2)):

$$n_{80} = \left[\frac{(c_i - c_{80})}{c_i}\right] \cdot 100 \quad (2)$$

where n_{80} is the settling efficiency at 80 min expressed in %, c_i is the initial moisture and solid particle content (MSPC) expressed in % and c_{80} is the MSPC at 80 min expressed in %.

2.4. CFD simulation

2.4.1. Problem description

The primary phase was the olive oil and the secondary phase was the solid particles (Table 1). The range of the suspended solids was divided into 7 distinct classes (Table 2) of particles based on the discretization of the measured size distribution obtained previously (Fig. 2). The settling column contained a uniform solids volume fraction of 0.36% at the beginning of the simulation (uniform agitation of the column). Then the particles started settling due to gravity and difference between densities.

2.4.2. Geometry, meshing and boundary cells

The design software GAMBIT 2.2.30 was used to build and to mesh the geometry of the model. The 3D settling column was 0.3537 m in height and 0.06 m in diameter, this is not a closed container, so the upper surface of the tube was modeled as 'free wall' whereas all others zones were modeled as 'wall zone'. The selected grid was comprised of 20,492 hexagonal elements. Two other grids (one finer with 11,475 elements and one coarser with 39,889 elements) were also used to determine the effect of the overall grid resolution on predictions. While the predictions obtained using the coarse grid were found to be different from those resulting from the selected one, the difference between the predictions made by the selected and fine grids were insignificant. As a result, the solutions from the grid of 20,942 hexagonal elements were considered to be grid independent.

Table 2
Classes of solid particles used to account for the total suspended solids in the settling column.

Class	Range of particle size (μm)	Mean particle size (μm)	Volume fraction (%)
A	627–1000	813.5	0.0756
B	256–627	441.5	0.1440
C	148–256	202.0	0.0864
D	81–148	114.5	0.0252
E	37–81	59.0	0.0108
F	18–37	27.5	0.0144
G	0–18	9.0	0.0036
Total volume fraction			0.3600

2.4.3. Physics of the model

The computational fluid dynamics code FLUENT 6.3.21 was used to carry out the simulations (ANSYS-Fluent, 2006). The flow was modeled as laminar, (Re < 2100). This case presents a liquid–solid interaction (olive oil and solid particles), so the Eulerian granular multiphase model was activated. The granular viscosity model chosen for this case was Syamlal–O'Brien (Syamlal and O'Brien, 1988), since this model was more appropriate for low volume fractions (less than 5% at the beginning of the simulation). No special boundary conditions have been defined for the walls.

2.5. Statistical analysis

For parameters b and m in Table 3, means and standard deviations were calculated using the statistical package Statistix, Version 9.1 (USA). The Statistix software was used to perform an analysis of variance (ANOVA) and Tukey's honest significant difference test at a 95% confidence level ($p < 0.05$) in order to identify differences among groups.

3. Results and discussion

3.1. Particle size distribution

Fig. 2 presents the measured cumulative particle size distribution of VOO from the HSSB after the vibratory sieve (1000 μm). The distribution curve shows a sigmoidal shape. Around 15% of the solid particles is smaller than 81 μm and 20% of the particles is larger than 256 μm. The remaining 65% correspond to a size range between 81 and 256 μm. This results in a d50 of 165 μm. To the best of our knowledge, no other data about the particle size distribution of VOO form HSSB have been reported, thus the results could not be compared.

3.2. Static settling assay

The effect of room temperature (15, 20 and 30 °C) on the static settling of VOO from HSSB in a column is shown in Fig. 3: (A) solid particles content, SPC, and (B) moisture and solid particle content,

Table 3
Coefficients for MSPC values (from 5 to 80 min) of hindered settling of the VOO from HSSB and settling efficiency for 80 min at three different temperatures (15, 20 and 30 °C).

Temperature (°C)	$m^{(*)}$	$b^{(*)}$	R^2	Settling efficiency (%)
15	-0.042 ± 0.002^a	5.341 ± 0.011^a	0.924	55
20	-0.046 ± 0.006^b	5.363 ± 0.355^a	0.929	56
30	-0.051 ± 0.000^c	4.807 ± 0.187^a	0.905	77

(*) Mean ± sd. Eq. (3): $c = b + m \cdot t$. For letters a–c, the same letters in the same column do not significantly differ ($n = 3$, $p < 0.05$).

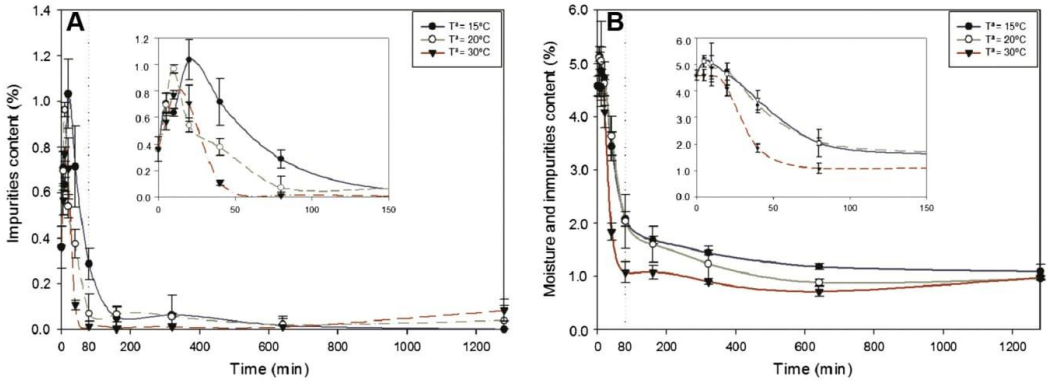

Fig. 3. Effect of temperature (15, 20 and 30 °C) on the static settling of the VOO from HSSB in a settling column: (A) solid particles content (SPC) and (B) moisture and solid particle content (MSPC).

MSPC. The MSPC of the initial VOO is 4.58%, of which 0.36% corresponds to SPC.

As can be observed, the settling curve for the three temperatures, for both SPC and MSPC, could be divided in three phases. The first phase of hindered settling corresponded to a linear model (Eq. (3));

$$c = b + m \cdot t \tag{3}$$

where c is the MSPC expressed in %, t is the time (min), b is the intercept and m is the slope. The values of m and b for the MSPC have been calculated for each temperature (Table 3). The parameter b shows values of -4.2×10^{-2}, 4.6×10^{-2} and 5.1×10^{-2}%/min for 15, 20 and 30 °C, respectively. However, a somewhat higher slope ($m = -0.051$) with a smaller intercept ($b = 4.807$) are achieved for 30 °C.

The second phase of settling, called transition phase, shows a lower rate which starts around 80 min (Kynch, 1952). As settling continues, a compressed layer of particles begins to form at the bottom of the tank. As can observed in Fig. 3, after 80 min the settling velocity decreases until that the MSPC and SPC were stabilized around 1% and 0.04%, respectively. This third phase where the rate is stabilized is called compression settling and also was described by Kynch (1952).

In addition, settling efficiency at 80 min (inflection point between linear and hindered phases) was estimated (Table 3) by Eq. (2). After 80 min of settling similar values of n_{80} were obtained for 15 °C and 20 °C, i.e. 55% and 56% respectively, while for 30 °C the value of n_{80} was significantly higher, around 77%. This increase of the settling efficiency, according Stoke's law (Eq. (1)), can be explained by the correlation between temperature and oil viscosity, where the oil viscosity tends to decrease when the temperature increases, and thus facilitates the settling of solid particles. An Arrhenius type model describes the effect of temperature on oil viscosity (Bonnet et al., 2011; Gila et al., 2015), which is expressed by Eq. (4):

$$\mu = A \exp(E_a/RT) \tag{4}$$

where μ is the oil dynamic viscosity (Pa s), A the pre-exponential factor (Pa s), E_a is the activation energy (J/mol), R is the gas constant (8.314 J/mol/K) and T the absolute temperature (K). These results about settling efficiency of the VOO from HSSB cannot be compared since there are no previous works available.

Finally, it is noteworthy that during the first minutes of the oil settling an increase of SPC is observed. This increase can be explained by the fact that VOO from HSSB is freshly prepared and contains air microbubbles which drag the solid particles to the column top at the beginning of the settling process.

3.3. CFD simulation

As far as the comparison of the CFD model with the obtained experimental values is concerned, Fig. 4 presents both the experimentally measured and the simulated values of the SPC of the VOO from HSSB during settling in a column. For this purpose, the experimental values of the SPC of the assay at 20 °C are used. Simulated values are obtained using values of physical properties of the VOO (Table 1), such as viscosity (0.084 Pa s) and density (908 kg/m^3) borrowed from a previous study (Gila et al., 2015). 28 simulations are carried out, analyzing the settling process of 7 solid particle classes (Table 2) with four different density values (Table 1).

As can be observed in Fig. 4, a linear settling of the solid particles, expressed as SPC, occurs both for experimental, apart from the microbubble phenomenon, and simulated values for particles with densities of 1025, 1125 and 1225 kg/m^3. However, for particles with density of 925 kg/m^3 the settling is slower during the entire simulation. Similarly, as observed for the experimental values of the SPC, the simulated values for these particles also initially follow a hindered settling regime (i.e. linear decrease), after which it remains more stable until the end of the simulation. The SPC values achieved, at 10 cm depth, after 1280 min of simulation were 0.052%, 0.043%, 0.039% and 0.026% for the solid particles with density of 925, 1025, 1125 and 1225 kg/m^3, respectively. A similar value of SPC (0.038%) was obtained in the experimental assay at the same time (1280 min). Therefore, the model CFD demonstrates that the solid particle density is situated in these density ranges, i.e. similar density values (1025–1200 kg/m^3) previously reported by Alba (2008). In Fig. 5 the contours of volume fraction of solid particles (Table 2) with a density of 1125 kg/m^3 are shown after 80 min of simulation. As expected, according to Stoke's law (Davis, 2010), the solid particles of larger size settled faster than the smaller. This behavior is also observed in the simulation, where the solid particles of larger size (A, B, C and D) are found at the bottom whereas the smaller particles (F and G) remain homogeneously distributed in the column without settling. However, for solid particles with intermediate size, as is particle E, although part of the solids particles had settled during the first 80 min, most of them still remain in suspension.

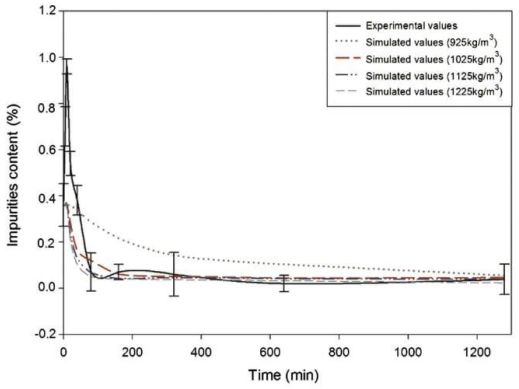

Fig. 4. Simulated (CFD) and experimental values for SPC of VOO from HSSB. Simulations of different particle densities (925, 1025, 1125 and 1225 kg/m^3) are shown.

3.4. Outlook and perspectives

Up to now, to our best of knowledge, no data is available about VOO settling from a two ways 'decanter', so that this work is a first attempt to quantify the behavior of the VOO from two-ways 'HSSB' during settling in settling column.

Normally, the room temperature in the oil mill ranges between 10 and 25 °C and the oil temperature is about 15–30 °C during the settling in conical bottom tanks. Therefore, as expected, temperature is an important factor to consider in these kinds of separation processes, since the oil viscosity is strongly influenced by temperature (Gila et al., 2015). Besides temperature other factors affect this separation process, such as the density difference between liquid and solid particles, the particle size and shape among others (Davis, 2010). Further, the simulation CFD of this settling case allowed obtaining an idea of the influence of particle density in the settling process.

Thus, further works focused in these aspects should be carried out and CFD models could be developed for current settling tanks, both in batch and continuous operation, or investigate new designs, using the results of this work as a starting point.

4. Conclusion

A first approach was presented about the effect of temperature in the static settling of the VOO from HSSB in a settling column model. The particle size distribution was obtained (particle sizes between 10 and 1000 μm), which showed a curve with a sigmoidal shape, where the major percentage (around 65%) of the solid particles ranged in sizes from 81 to 256 μm, obtaining a particle size of d50 around 165 μm. The three temperatures used in the assays described settling curves that showed two steps, a linear settling for the first minutes (around 80 min) and a hindered settling where the settling rate was decreased slowly till values of MSPC and SPC of 1% and 0.04%, respectively. The temperature of 30 °C showed higher values of settling efficiency (77%) compared to the lower temperatures (15 °C and 20 °C). Besides, in this study a first approach of the use of a CFD model in the VOO clarification step was presented. Simulations showed the same settling trend than that of the experimental measurement. According to the four solid particles density values studied in the simulations, the values that ranging between 1025 and 1225 were those that showed best

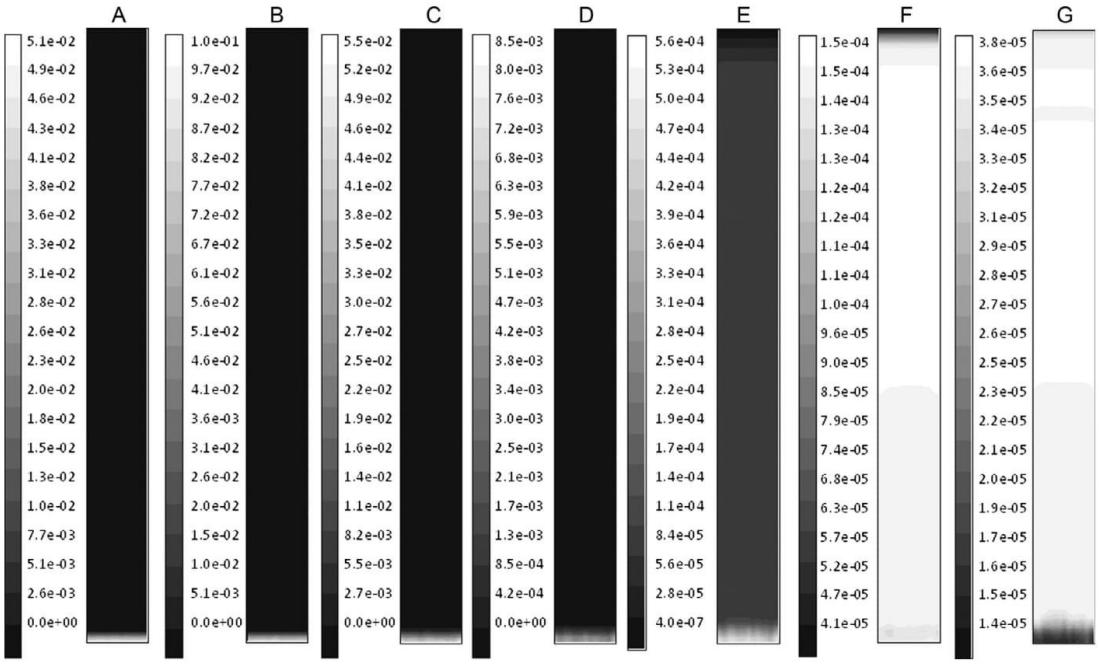

Fig. 5. Contour plots of volume fraction with solid particle with density of 1125 kg/m^3 after 80 min. Particles diameter: *A* = 813.5 μm; *B* = 441.5 μm; *C* = 202 μm; *D* = 114.5 μm; *E* = 59 μm; *F* = 27.5 μm; *G* = 9 μm.

settings with respect to the experimental values, excluding 925 kg/m^3 which showed lower settling rate. The solid particles with larger size (813.5, 441.5, 202.0 and 114.5 μm) at 80 min settled well, whereas smaller particles did not settle. Hence, CFD modeling seems to be a useful tool to design oil mill devices or improving actual designs.

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Research Article

Application of oxygen during olive fruit crushing impacts on the characteristics and sensory profile of the virgin olive oil

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A novel crushing process which involves increasing the level of oxygen during the virgin olive oil (VOO) extraction has been applied to develop new knowledge on the biosynthesis of the compounds responsible for sensory, nutritional, and technological quality of VOO. The singular composition of VOO is the result of a series of physical, chemical, and biochemical processes that occur during its extraction procedure. The extraction conditions, temperature, time, addition of water, and oxygen availability, can modulate the final composition of VOO. The last parameter, oxygen, is a known co-factor for the many endogenous enzyme activities present in the olive fruit, mainly oxide-reductases. The present study focuses on the influence of oxygen during the crushing of olive fruits on enzyme activities and the process yield. Three different cultivars, namely "Picual," "Arbequina," and "Blanqueta", were selected for the study. The increase of oxygen concentration during crushing produces a significant change in the volatile composition and sensory profile; however, no significant differences were shown on the content of fatty acid composition, total phenols, pigments, tocopherols, and qualitative parameters. This could be a useful analysis tool while designing new prototypes of mills or while working with breeding selection programs.

Practical applications: The application of oxygen during olive fruit crushing in the oil extraction process has been studied. The information provided by this study enables to increase the knowledge on metabolism pathway in olive fruit while working with breeding selection programs. The present study can be useful to clarify the optimum time, crushing, or malaxation to apply atmospheric control to the VOO extraction. Moreover, the results obtained in this work suggest new research opportunities in order to design new prototypes of mills that allow the modification of the sensory characteristics VOO.

Keywords: Atmosphere control / Crushing / Oxygen concentration / Sensory characteristics / Virgin olive oil quality

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1 Introduction

Virgin Olive Oil (VOO) is the name given to the olive fruits juice obtained by mechanical processes of extraction including: washing and crushing of olive fruit, malaxation, and centrifugation of the olive paste and finally, decantation and filtration of the oil obtained. In this process special importance is attributed to the action of the endogenous oxidoreductase enzymes present in the olive fruit, which are classified according to the nature of the oxidizing substrates such as dehydrogenases, oxygenases (monooxygenases and dioxygenases), oxidases, and peroxidases involving some type of free radical and/or oxygen species. Most of the relevant enzymatic reactions, both desirable and undesirable, occur in the presence of oxygen. In particular, the most desirable one regards the synthesis of volatile compounds thought the lipoxygenase pathway [1]. In olive fruit there are several proteins with oxidoreductase activity; specifically, lipoxygenase (LOX) and alcohol dehydrogenase (ADH), both are part of the so called LOX pathway. This pathway

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Abbreviations: ADH, alcohol dehydrogenase; **LOX,** lipoxygenase; **NMR,** nuclear magnetic resonance; **POD,** peroxidase; **PPO,** polyphenol oxidase; **VOO,** virgin olive oil

combines the actions of three enzymes: Lipase, LOX, and HPL converting lipidic substrates, such as C18:2 and C18:3 fatty acids, into short chain volatiles. These reactions, triggered by cell membrane disruptions, produce compounds known as green leaf volatiles, which are C6 or C9-aldehydes [2]. The lipoxygenases, after their release owing to cellular disruption of fruits, immediately become active and transform the unsaturated fatty acids, linolenic, and linoleic acids, into C6 and C5 compounds that contribute to green odor notes [3, 4]. In the same way, a pool of enzymes associated with phenolic metabolism is present in the olive tissue, such as β-glucosidase, peroxidase (POD), and polyphenol oxidase (PPO). The first one, β-glucosidase is involved in the hydrolysis of main phenolic glycosides found in olive fruit: oleuropein, ligstroside, and demethyloleuropein giving rise to the main phenolic components found in VOO, denominated as secoiridoids compounds. The latter enzymes, peroxidase (POD) and polyphenol oxidase (PPO), are related to phenolic compounds oxidation (i.e., secoiridoids compounds) resulting in a reduced phenolic concentration in the oil [1, 5]. Phenolic compounds present nutritional, organoleptic, and oxidative stability properties, hence their activity produces an important impact on VOO final composition [6, 7].

Currently, the demand of higher quality or special characteristics VOOs is promoting the study of all the parameters related to the quality of VOO affected by the oil processing conditions: temperature and time of malaxation paste and oxygen concentration. The last variable, oxygen concentration, probably modulates the activity of oxidoreductase enzymes within the metabolism of oil bioactive compounds during the crushing and malaxation steps [8, 9]. These observations were confirmed by Amirante et al. (2012) [10] that measured the concentration of O_2 inside the olive paste in an hermetic malaxer: the initial concentration of oxygen was equal to 18%. This value was halved after 5 min and becomes equal to 5% after 10 min. After this period, a rapid increase in concentration was observed followed by a gradual decrease in emission rate. The depletion of oxygen in the early minutes can be ascribable to enzymatic reactions that are not associated to the production of carbon dioxide [1]. After this initial period, the effects of cellular respiration became prevalent and the CO_2 saturated the head space of the malaxer, depriving the lipoxygenase pathway of oxygen, thereby inhibiting the synthesis of other volatile compounds. However, more exhaustive researches on the kinetics of the various endogenous enzymes immediately after the crushing are needed in order to better elucidate how many time are effectively required to obtain the desirable products synthesis.

On the other hand, a special interest has emerged on the development of new extraction processes able to enhance the oils with those compounds responsible for the bioactive, organoleptic properties, and oxidative stability such as polyphenols, tocopherols, pigments, and volatiles among others [11, 12].

The great majority of studies are focused on the design and development of devices in order to control the oxidation of VOO compounds during the malaxation phase. Even several reviews have analyzed all the aspects related to this stage [13–16]. In this sense, during the last years oxygen monitoring and its control during the paste malaxation have been widely studied showing interesting results to improve the VOO quality [10, 17–27].

Likewise, less attention has been paid to the oxidation produced by the above-mentioned oxidoreductase enzymes during the crushing step. It should be noted that LOX pathway is trigged through tissues disruption when the fruit is milled as has been mentioned previously. In fact, Clodoveo et al. (2014) [28] suggested that it is important to develop innovative crushing systems able to modulate atmosphere composition inside the crushing chamber to obtain a strategic control the oxygen concentration in the olive paste. An example of a plant that allows modulation of the atmosphere composition from the crusher to the malaxer has been described in a recent patent, which the atmosphere upstream of the crusher is balanced by the hydrostatic pressure of a water vat, whereas downstream of the crusher is delimited by the hermetic closure of the malaxer. The atmosphere composition can be modulated, choosing the appropriate mix of gas, both in the crusher and in the malaxer by means of a valve implemented on the malaxer [29]. The great influence of the type of milling on nutritional and sensory qualities of VOO have already been established in several studies [30–35]; even recently commercial devices has been developed as a strategy to control the atmosphere composition into the crusher such us a new small-size extraction plant named the “Apollo-Culitvar 500” that operates under vacuum in order to reduce the olive paste exposure to oxygen obtaining a product richer in polyphenols. However, this new equipment has not yet been validated by scientific research and a probable weakness of vacuum employment in oil processing could be the loss of aromas due to their low vapor pressure. Very recently, Zoani et al (2014) [36] has proposed a new method employing solid-state carbon dioxide, commonly known as “dry ice” added to the olives before crushing; the solid-state carbon dioxide after few seconds become gas and causes the inertization of the atmosphere into the crusher increasing the oil yield respect to the traditional method. Despite growing interest in using atmospheric control crushers are very restricted data available in the international literature on the influence of the atmosphere composition in contact with the olive paste during crushing, crucial for the control of atmosphere composition inside the crusher.

Therefore, the aim of this work was to investigate the influence of oxygen concentration during fruit crushing on process parameters, VOO final composition and quality parameters in three olive cultivars: “Picual,” “Arbequina,” and “Blanqueta.” The present study can be useful to clarify if an atmospheric control limited to the malaxer machine may

not be effective because of the high rate of enzymatic reactions during crushing, which makes useless any subsequent modulation attempts. Finally, the results obtained will provide the basic to know the role of the oxygen on enzymes determining the nutritional and sensory characteristic of VOO and therefore the information can be used to design new VOO extraction equipment for example while testing cultivar breeding program in order to develop strategies to increase nutritional and sensory qualities of VOO.

2 Materials and methods

2.1 Plant material

Three different olive cultivars (*Olea europaea* L.) were selected in this study: "Picual," "Arbequina," and "Blanqueta." The olive trees were grown in the experimental orchard of IFAPA Venta del Llano in Mengíbar (Jaén, Spain) using standard growing techniques. The work was carried out during crop season 2013–2014. Two adult 25-year-old olive trees spaced $7 \times 7\,m^2$ were chosen in a random manner for each variety for the study.

2.2 Solvents, reagents, and standards

All solvents and reagents, analytical or HPLC grade, were obtained from Merck (Germany). All reference compounds were supplied by Sigma (St. Louis, MO, USA).

2.3 Determination of initial characteristics of olive fruit and paste

Olive samples were hand-picked by hand and batch homogenized at random were used. In order to determine the ripening index (RI) and mean weight, 100 fruits were arbitrarily chosen. The ripening index for olives was determined according to the method of Olive Growing and Extraction Techniques Station of Spain, IFAPA Venta del Llano [37]; it is based on a scoring system for each stage of the skin and flesh coloring. Only healthy fruits without any kind of infection or physical damage were processed.

Further analyses were carried out to determine the moisture and oil content of olive fruit. The humidity expressed in percentage in weight was determined by drying olive paste in heater at 105°C until constant weight is reached. The total oil content expressed in percentage in weight (on wet and dry basis), was carried out using a nuclear magnetic resonance (NMR) Minispec mq 20 (Bruker Analytik Gmbh), the NMR was previously calibrated and validated with a Soxhlet extractor according with official method described in Regulation EEC 2568/91 of the Commission of the European Union [38].

2.4 Crushing condition and oil extraction

Oil extraction was performed using an Abencor laboratory oil mill (Abengoa, Spain) equipped with a hammer mill, a thermobeater, and a paste centrifuge. The extraction was repeated twice for each cultivar. The milling of the olive fruits was performed using a stainless steel hammer mill operating at 3000 rpm provided with a 5 mm sieve. The mill was modified to increase the atmospheric oxygen concentration from 20% (control) to 60% (tested) during the fruit crushing by a polyethylene flexible container sealed around the hammer head. The container was equipped with an inlet to allow continuous oxygen blew during the milling. Oxygen concentration was monitored using a portable electrochemical Oxygen analyzer (Witt-Gasetechnik model Oxybaby, Germany) with a 0.1% resolution. Simultaneously, the paste temperature was measured using a digital thermometer (Ebro® TTX100, Germany). Right after, the olive paste malaxation was carried out at 28°C with kneading at 50 rpm for 45 min. Centrifugation of the kneaded olive paste was performed in a basket centrifuge at 3500 rpm for 1 min. After centrifugation, the oil obtained was decanted, filtered and stored in amber glass bottles at −24°C in the dark without headspace until analysis. Finally, industrial yield (oil percentage in weight on a fresh matter basis) was calculated using the following equation:

$$IY = \left[\frac{(OV \times 0.915)}{WOP}\right] \times 100$$

where OV is the oil volume obtained from the ABENCOR system (L), the value 0.915 corresponding to the olive oil density in kg/L and WOP is the weight of olives fruit paste (kg). Extractability was calculated according to ref. [39].

2.5 Oil quality indices

Free acidity, peroxide value, and UV spectrophotometric indices (K_{232}, K_{270}) were evaluated according to the official methods described in Regulation EEC 2568/91 of the Commission of the European Union [38]. All parameters were determined in triplicate for each sample.

2.6 Oil analyses

2.6.1 Bitterness K_{225}

Bitterness K_{225} was determined by SPE of bitter compounds, using SPE C18 cartridges of 6 mL filled with 500 mg solid phase (J.T. Baker) [40]. The oil was dissolved in n-hexane, the cartridge was conditioned by eluting with methanol and n-hexane, and the oil was applied to the SPE column. The column was washed with hexane that was run through the cartridge and discarded. The bitter compounds were eluted with methanol/water (50:50). The absorbance of the

methanolic extract was measured at 225 nm. The results were expressed as the absorbance of 1 g in 100 g (K_{225}). All the absorbance measurements were performed in an HP8452A diode array spectrophotometer (Hewlett Packard, Spain).

2.6.2 Tocopherol content

Tocopherol content was analyzed by IUPAC 2432 method [41]. Briefly, 1.5 g of oil was dissolved in 0.5% isopropanol in n-hexane to 10 mL. The chromatographic separation was performed using a PerkinElmer liquid chromatograph equipped with an isocratic pump LC200 and a UV–vis detector Lc295. A normal phase column Lichrosphere Si60 (250 mm length, 4.6 mm id, and 5 μm particle size, Merck, Germany) was used with a volume injection of 20 μL and a flow rate of 1.0 mL/min. The mobile phase was 0.5% isopropanol in n-hexane. The absorbance was measured at 295 nm and the data was collected in a PerkinElmer 1020 data recorder. Tocopherols were identified by comparing the retention time with those of pure standards. Quantification was performed by external standard calibration curves using five concentrations of the corresponding standard: α-tocopherol, β-tocopherol, and δ-tocopherol. The results were expressed as mg of tocopherol per kg of oil.

2.6.3 Pigments

Pigments carotenoids and chlorophylls were determined as described by Mínguez et al. (1991) [42]. For this purpose, 7.5 g of oil were weighed, dissolved in cyclohexane and taken to a final volume of 25 mL; carotenoid and chlorophyll pigments were determined by measuring the absorbance at 470 and 670 nm, respectively. The results were expressed as mg/kg oil. All the absorbance measurements were performed in a Cary 50 Bio spectrometer (Varian Inc., USA).

2.6.4 Oxidative stability

Oxidative stability was measured using a Rancimat Model 679 (Metrohmn, Switzerland); 2.5 g of oil was weighed and heated at 98°C, and air was bubbled through the oil at a flow rate of 10–12 L/h; each oil sample was analyzed twice. The results were expressed as induction time in hours [43].

2.6.5 Fatty acid composition

The fatty acid methyl esters (FAMEs) were prepared as described by the EU official method [38]. The chromatographic separation was carried out using a Perkin–Elmer Autosystem gas chromatograph (Perkin–Elmer, Spain) equipped with an autosampler, split/splitless injector, flame ionization detector (FID), and a fused silica capillary column BPX70 of 50 m length, 0.25 mm id, and 0.25 μm of film thickness (SGE Scientific PTY Ltd., Australia). Helium was used as carrier gas and the oven temperature was maintained at 198°C. The injector and detector temperatures were 235 and 245°C, respectively. A mixture standard of FAMEs was used to the allocation of corresponding fatty acid in the chromatogram. The results were expressed as relative area percentages of the total area.

2.6.6 Volatile compounds analysis

Solid-phase microextraction (SPME) followed by GC-FID were used to analyze the volatile fraction of the virgin olive oil obtained. Olive oil samples were conditioned to room temperature and then placed in a vial heater at 40°C. After 10 min of equilibrium time, the volatile compounds from headspace were adsorbed on a SPME fiber DVB/Carboxen/PDMS 50/30 μm (Supelco Co., Bellefonte, PA). Sampling time was 50 min at 40°C. Desorption of volatile compounds trapped in the SPME fiber was directly done into the GC injector. Volatiles were analyzed in triplicate using a HP-6890 gas chromatograph equipped with a Supelcowax 10 capillary column (30 m × 0.25 mm, 0.25 μm, Sigma–Aldrich Co. LLC). Operating conditions were as follows: Helium as carrier gas; injector and detector at 250°C; the column was held for 5 min at 40°C; and then programmed at 4 °C min^{-1} to 200 °C. Quantification was performed using individual calibration curves for each identified compound by adding known amounts of different compounds to redeodorized high-oleic sunflower oil.

Detection and identification was performed using a mass spectrometer (ISQ single quadrupole MS, Thermo Fisher Scientific, Austin, Texas, USA) operating in EI mode (70 eV) under identical conditions for GC, matching against the Wiley/NBS Library, and by GC retention time against standards. Ion source and transfer line temperatures were 200 and 240°C, respectively. Mass spectra were obtained in scan mode in the 29–250 mass-to-charge ratio range at a scanning speed of 7 scan/s. Chromatograms and spectra were recorded and processed using the Thermo Xcalibur software (Thermo Fisher Scientific, San Jose, California, USA).

2.6.7 Phenolic compounds

A sample of virgin olive oil was weighed (1.5 g) and 100 μL of a standard solution (0.002 g of syringic acid/100 mL of methanol) was added [44]. The oil was dissolved in n-hexane (1 mL), the phenolic compounds were extracted with 1.25 mL of methanol/water (60:40 v/v) twice, and the extracts were washed with n-hexane rising to a final volume of 2.5 mL. HPLC analysis was performed using a HP1100 system equipped with an autosampler, quaternary pump, and diode array detector. A reversed-phase C18 Pecosphere column (83 × 4.6 mm i.d., 3 μm particle size, Brown Lee Columns) was used with an injection volume of 20 μL and a flow rate of 0.45 mL/min. The mobile phase was a mixture of water/acetic acid (98:2 v/v) (solvent A) and methanol/acetic acid (98:2 v/v) (solvent B). The total run time was 70 min, the

solvent gradient changed according to the following conditions: 90% A–10% B for 10 min, 80% A–20% B in 8 min then remained for 2 min, 60% A–40% B in 10 min, 50% A–50% B in 10 min, and 100% B in 10 min up to the end of the run. Phenolic compounds were quantified at 280 nm using syringic acid as internal standard and the response factors determined by Mateos et al (2001) [45].

2.6.8 Sensory analysis

Sensory Analysis was performed by the panel of the Citoliva Foundation laboratory during a series of oil-tasting sessions, in accordance with the European Community (EC/2568/91) [45] and subsequent amendments (EC) N° 640/2008 [46]. Based on these regulations, the tasters smelt and tasted each olive oil sample to evaluate its positive attributes according to the list of descriptors included in this method. The sensory profile of each VOO sample is expressed as the average value for each descriptor. Moreover, the tasters evaluated direct or retronasal aromatic olfactory sensations (fruity, green leaf/fresh-cut grass, apple, almond, artichoke), and other positive attributes; gustatory sensations (bitterness and sweetness); and tactile/kinesthetic sensations (pungency) according to COI/T.20/Doc. no. 22 (2005) [47]. The tasters had to rate the intensity of the different descriptors on a continuous 0–10 cm scale.

2.7 Statistical analysis

Each experiment was carried out in duplicate. Results are expressed as mean values of at least three measurements. Analysis of variance (ANOVA) was applied using Statistix, version 8.0. Tukey's test ($p < 0.05$) was used to determine significant differences between means.

3 Results and discussion

3.1 Olive fruit characteristics and parameters of extraction process

The olive fruit was characterized in order to study the cultivar differences. Harvest date, ripening index, medium weight, humidity, and oil content are shown in Table 1. In spite of carrying out the fruit harvest on the same date the ripening index was different for each variety, "Arbequina" was the most unripe and "Picual" the most ripe; however "Arbequina" shows the lowest medium weight of fruit. "Picual" contained the highest humidity percentage in opposite to "Arbequina" that contained the highest oil content in wet and dry weight.

The influence of the oxygen concentration during the fruit crushing on the oil extraction yield has been studied in reference to paste temperature, industrial yield, and extractability (Table 2). In general, a decrease in the paste temperature was observed when oxygen was increased to 60% during the fruit milling. The influence of the crushing temperature during the olive paste preparation has been studied by several authors [30, 48]. They described how higher temperatures in the crusher during olive processing lead to shorter preservation of the oils, although did not analyze the effect on process yield.

This decrease, about 4°C, was similar for the three varieties. Concurrently, the results indicated a decrease in the oil extraction yield with decreasing temperature of olive paste malaxation. However, a reduction of about 10% in the extractability percentage was only observed for the "Picual" cultivar, probably because after the malaxation step at 28°C for 45 min the paste temperature was practically the same for both treatments. This effect has been studied by Kalua et al. (2006) [49] with similar results.

3.2 Quality characteristics of VOO

The commercial category of VOO is determinated by a series of quality indices and sensory tests grouped in the Regulation CEE n° 2568/91 [38] with subsequent amendments, hence, any modification in the extraction process of the oil must be analyzed. Table 3 shows the values obtained using the official analytical methods to determine the free acidity, peroxide index, and UV absorption for each cultivar. The oils examined were defined as belonging to the commercial class of "extra virgin olive oil" according to the resulting data, since all data analyzed were within the ranges established by the current regulation.

A clear effect of oxygen increasing the free acidity values was not found (Table 3), although varietal differences could

Table 1. Initial characteristics of olive fruits from three cultivars studied: "Picual," "Blanqueta," and "Arbequina"

	Olive fruit characterization						
	Harvest date	Ripeness index	Medium weight (g)	Humidity (%)	Oil content (% wet weight)	Oil content (% dry weight)	Olive fruit temperature (°C)
Picual	22/01/2014	5.02	4.12	46.78	23.85	44.84	15
Blanqueta	22/01/2014	3.24	1.34	44.55	27.91	50.34	22
Arbequina	22/01/2014	3.6	0.96	45.49	24.32	44.63	15

Table 2. Parameters of the process of extraction of virgin olive oils obtained by Abencor system.

	Paste temperature (°C)*	Industrial yield (%)	Extractability (%)
Picual			
Control (20%)	20.00	19.71	0.83
Oxygen (60%)	14.00	16.29	0.68
Blanqueta			
Control (20%)	24.00	23.53	0.84
Oxygen (60%)	20.25	22.65	0.81
Arbequina			
Control (20%)	24.00	17.17	0.71
Oxygen (60%)	19.50	16.64	0.68

*Paste temperature was measured just after crushing in degree Celsius (°C) according to section 2.4.

be observed. "Arbequina" oils showed lowest values of free acidity. A lipase from olive fruits has been reported by Panzanaro et al. (2010) [50] which may determine the level of free fatty acids (FFA). The enzymatic activity of lipase is genetically dependent, explaining the observed difference respect to the cultivar.

The presence of higher percent of oxygen may induce oxidation processes in the olive paste and thus in the oil. A very important parameter related to oil oxidation is the peroxide value. The data does not show any notable differences with the increase of oxygen, although the peroxide index values depend on the variety analyzed, where the highest value for "Arbequina" oils was documented. The determination using specific ultraviolet wavelengths provides information on the quality of a fat, its state of preservation and changes brought about by technological processes. Both measurements at the ultraviolet wavelengths: K_{232} and K_{270}, showed similar values in all samples analyzed. The effect of oxygen application on oil preservation was analyzed measuring oxidative stability. These values using both oxygen percentages did not show significant differences, although slight variations were observed for each cultivar (Table 3).

In relation to the effect of increasing oxygen on the compounds involved in nutritional properties of VOO such as: tocopherols and pigments the data are shown in the Table 3. Tocopherols contribute to the antioxidant properties of olive oil since they not only act as a free radical trapping agent but also as a singlet oxygen quencher. Oils from the three cultivars processed under oxidizing conditions did not show changes in comparison with control. The range was between 284 and 317 mg/kg. The α-tocopherol, which was predominant in the oil tocopherols showed a similar concentration in all the studied samples. In fact, in the "Arbequina" oils it represents the 100% because only

Table 3. Qualitative characteristics of the virgin olive oils from "Picual," "Blanqueta," and "Arbequin" cultivars obtained at two different concentrations of oxygen during the crushing process

	Picual		Blanqueta		Arbequina	
	Control (20%)	Oxygen (60%)	Control (20%)	Oxygen (60%)	Control (20%)	Oxygen (60%)
Free fatty acid (% oleic acid)	0.22 ± 0.00a	0.22 ± 0.00a	0.29 ± 0.01b	0.32 ± 0.01b	0.19 ± 0.00bc	0.17 ± 0.00c
Peroxide value (meq O_2/kg)	2.51 ± 0.03b	2.38 ± 0.49b	4.96 ± 0.13a	5.3 ± 0.14a	1.31 ± 0.01c	0.99 ± 0.05c
K_{232}	1.68 ± 0.01d	1.66 ± 0.01de	2.02 ± 0.01a	1.93 ± 0.01b	1.72 ± 0.01c	1.63 ± 0.01e
K_{270}	0.16 ± 0.00ab	0.15 ± 0.01ab	0.17 ± 0.01a	0.17 ± 0.01a	0.14 ± 0.01ab	0.13 ± 0.01b
Oxidative stability (hours)	200 ± 9.77a	202 ± 6.9a	91 ± 5.76cd	84 ± 1.13d	113 ± 6.9b	102 ± 5.7bc
Total phenols (mg/kg caffeic acid)	776 ± 19b	731 ± 0.01b	959 ± 26a	826 ± 42b	619 ± 23c	533 ± 18c
K_{225}	0.49 ± 0.02ab	0.53 ± 0.02b	0.60 ± 0.01a	0.55 ± 0.04ab	0.53 ± 0.01ab	0.50 ± 0.01b
α-tocopherol (mg/kg)	291 ± 13a	289 ± 8a	283 ± 1.41a	284 ± 5.66a	296 ± 5.66a	284 ± 1.41a
β-totopherol (mg/kg)	1 ± 0.01	n.d	1 ± 0.01	1 ± 0.01	n.d	n.d
γ-tocopherol (mg/kg)	25 ± 2.83a	27.5 ± 2.12a	16 ± 0.00a	16 ± 0.00a	n.d	n.d
Total tocopherols (mg/kg)	317 ± 9.90a	317 ± 9.90a	300 ± 1.41ab	301 ± 5.66ab	296 ± 5.66ab	284 ± 1.41a
Carotenoids (mg/kg)	4.65 ± 0.35bc	4.45 ± 0.07bc	4.25 ± 0.21bc	3.7 ± 0.01c	6.3 ± 0.42a	5.15 ± 0.35b
Chlorophylls (mg/kg)	3.25 ± 0.35b	2.95 ± 0.07b	4.15 ± 0.21ab	3.1 ± 0.02b	4.9 ± 0.71a	3.6 ± 0.42ab
Total pigments (mg/kg)	7.9 ± 0.71b	7.4 ± 0.14b	8.4 ± 0.42b	6.8 ± 0.01b	11.2 ± 1.13a	8.75 ± 0.78ab

Mean values ± SD from two determinations in two different experiments followed by different letters in the same row indicated significant differences at $p < 0.05$ for all cultivars analyzed.

α-tocopherol was detected. Data about the influence of the extraction system on tocopherols are limited but the results showed are in accordance with Ranalli and Angerosa (1996) and Gimeno (2002) [51, 52], therefore the technology does not seems to affect the levels of α-tocopherol. This protective effect of the tocopherols against photooxidation is enhanced by other antioxidant compounds presents in the VOO, such as β-carotene. We observed a slight reduction in the carotenoids and chorophylls content obtained at higher oxygen concentration but the data did not show significant differences (Table 3). The mean content of total pigments for all samples was of 8.4 mg/kg oil according to fruits at advanced ripening stage. On the other hand, both type of compounds, tocopherols, and pigments are related to oxidative stability as shown in Table 3. Picual oils displayed the highest oxidative stability, followed by Arbequina and Blanqueta oils, although the increase of oxygen produced no significant effects on these parameters within each variety. Likewise oxidative stability is not directly related to any antioxidant group of compounds, but rather is the result of synergic effect of different compounds such us: fatty acid, tocopherols, pigments, and phenols.

Finally, K_{225} or bitter index is an analytical method developed by Gutiérrez-Rosales et al. 1992 [40] to evaluate the intensity of the bitter taste in virgin olive oil and the data are shown in the Table 3. The oils obtained by the increase of oxygen during fruit milling respect to the control displayed a slight increase in Picual oils and slight decrease in Arbequina and Blanqueta oils. These results are not in accord with the bitter intensity from sensory analysis by panel test in the same oils. This could be due to compounds responsible for the bitter attribute in panel test, which showed different absorbance or response at 225 nm of wavelengths.

3.3 Fatty acid composition

Most of the fatty acids of edible oils are present as triacylglycerols, in olive fruit these are accumulated by olive fruit in its development. Olive oil, like other vegetable oils, shows a high concentration of oleic acid and low concentration of saturated fatty acid in position—2 of the triacylglycerol molecules. Fatty acids present in the olive oils analyzed are shown in Table 4. The fatty acid composition was not affected by the crushing at high levels of oxygen. Gimeno et al. (2002) and Inarejos-García et al. (2010) [52, 53] did not observed any change in the fatty acid composition by crushing conditions.

Nevertheless, fatty acid composition showed significant differences relative to different cultivars. The oleic acid was the highest percent fatty acid in all oils, considering ranges from 65.10% in "Blanqueta" oils to 80.33% in "Picual" oils, followed by palmitic acid in "Picual" (11.30%) and "Blanqueta" (14.42%) and linoleic acid in "Blanqueta" (15.34%) and "Picual" (3.32%). These data are in agreement with those obtained by Uceda et al. (2008) [54] for fatty acid composition in the same varieties.

3.4 Phenolic compounds

The POX and POD enzymatic activities involved in the phenolic metabolism are oxidoreductases and use oxygen as cofactor. The phenolic composition of the olive oils obtained at different oxygen concentrations during the fruit crushing

Table 4. Fatty acid composition (%) of the virgin olive oils from "Picual," "Blanqueta," and "Arbequina" cultivars obtained at two different concentrations of oxygen during the crushing process

	Picual		Blanqueta		Arbequina	
	Control (20%)	Oxygen (60%)	Control (20%)	Oxygen (60%)	Control (20%)	Oxygen (60%)
C16:0	11.26 ± 0.14c	11.35 ± 0.34c	14.42 ± 0.00a	14.41 ± 0.18a	13.97 ± 0.13a	14.01 ± 0.04a
C16:1	1.00 ± 0.05b	1.00 ± 0.09b	1.09 ± 0.01b	1.10 ± 0.00b	1.38 ± 0.01a	1.37 ± 0.01a
C17:0	0.03 ± 0.00b	0.04 ± 0.01b	0.12 ± 0.01a	0.13 ± 0.00a	0.11 ± 0.01a	0.11 ± 0.00a
C17:1	0.08 ± 0.01c	0.07 ± 0.01c	0.26 ± 0.01a	0.26 ± 0.01a	0.23 ± 0.00ab	0.22 ± 0.00b
C18:0	2.95 ± 0.10a	2.93 ± 0.09a	2.26 ± 0.00b	2.32 ± 0.05b	1.88 ± 0.01c	1.96 ± 0.01c
C18:1	80.33 ± 0.09a	80.12 ± 0.12a	65.25 ± 0.02c	65.10 ± 0.20c	71.22 ± 0.03b	71.45 ± 0.00b
C:18:2	3.32 ± 0.12c	3.43 ± 0.23c	15.34 ± 0.04a	15.21 ± 0.36a	10.06 ± 0.14b	9.72 ± 0.04b
C18:3	0.49 ± 0.01b	0.51 ± 0.00bc	0.56 ± 0.00a	0.56 ± 0.01a	0.47 ± 0.01cd	0.45 ± 0.00d
C20:0	0.32 ± 0.04a	0.34 ± 0.00a	0.41 ± 0.01a	0.42 ± 0.01a	0.36 ± 0.04a	0.39 ± 0.01a
C20:1	0.18 ± 0.01b	0.18 ± 0.01b	0.20 ± 0.01a	0.21 ± 0.01a	0.24 ± 0.01a	0.24 ± 0.02a
C22:0	0.07 ± 0.00b	0.07 ± 0.01b	0.09 ± 0.00a	0.10 ± 0.00a	0.09 ± 0.00a	0.09 ± 0.01a
∑saturated	14.63 ± 0.00c	14.72 ± 0.25c	17.30 ± 0.02a	17.37 ± 0.11a	16.40 ± 0.18b	16.55 ± 0.04b
∑unsaturated	85.38 ± 0.00a	85.29 ± 0.25a	82.69 ± 0.01c	82.43 ± 0.19c	83.59 ± 0.18b	83.44 ± 0.03b

Mean values ± SD from two determinations in two different experiments followed by different letters in the same row indicated significant differences at $p < 0.05$ for all cultivars analyzed.

Table 5. Major phenolic compounds (mg/kg oil) in VOOs obtained from "Picual," "Blanqueta," and "Arbequina" with regards to concentration of oxygen during crushing.

	Picual		Blanqueta		Arbequina	
(mg/kg oil)*	Control	Oxygen	Control	Oxygen	Control	Oxygen
Hydroxytyrosol	1.90 ± 0.10a	2.05 ± 0.06a	1.56 ± 0.37ab	1.78 ± 0.03a	0.80 ± 0.12c	0.95 ± 0.17bc
Tyrosol	3.68 ± 0.22a	4.20 ± 0.72	0.95 ± 0.14b	0.91 ± 0.00b	0.84 ± 0.10b	0.86 ± 0.02b
p-Coumaric acid	0.32 ± 0.04c	0.32 ± 0.10	0.63 ± 0.07c	0.57 ± 0.03c	2.39 ± 0.15a	1.27 ± 0.01b
Ferulic acid	1.05 ± 0.01a	1.21 ± 0.02b	n.d	n.d	n.d	n.d
3,4-DHPEA-EDA	456.95 ± 22.99d	422.03 ± 0.72d	881.52 ± 27.84a	678.50 ± 57.71b	668.53 ± 56.89bc	525.10 ± 23.40cd
p-HPEA-EDA	59.60 ± 3.22d	59.49 ± 4.02d	207.35 ± 1.89bc	175.27 ± 17.18c	143.74 ± 11.48a	137.93 ± 4.36ab
Pinoresinol	20.51 ± 1.70a	20.71 ± 0.15	8.81 ± 0.24b	8.27 ± 0.09b	6.53 ± 0.46b	6.88 ± 0.36b
3,4-DHPEA-EA	123.15 ± 16.24a	118.76 ± 7.09a	114.44 ± 3.12a	97.01 ± 5.28a	26.99 ± 2.56b	23.65 ± 0.32b
p-HPEA-EA	9.86 ± 1.41a	10.96 ± 0.69a	n.d	n.d	n.d	n.d
Total phenolic	677.01 ± 45.23cd	639.74 ± 3.42d	1215.27 ± 26.21a	962.30 ± 80.33b	849.82 ± 71.76bc	653.52 ± 34.04cd
Secoiridoids Oleuropein deriv.**	582.00 ± 39.34c	542.84 ± 7.87c	997.52 ± 31.33a	777.28 ± 63.02b	696.32 ± 59.56bc	549.70 ± 23.90c
Secoiridoids Ligstroside deriv.***	73.14 ± 4.85d	74.65 ± 5.43d	208.30 ± 2.03a	176.18 ± 17.19ab	144.59 ± 11.58bc	138.79 ± 4.38c

Different letters in the same row indicated significant differences at $p < 0.05$ between the three cultivars analyzed.
*Mean values ± SD from three determinations in two different experiments.
**Secoiridoids oleuropein derivative: 3,4-DHPEA, 3,4-DHPEA-EDA, and 3,4-DHPEA-EA
***Secoiridoids ligstroside derivative: p-HPEA, p-HPEA-EDA, and p-HPEA-E

is shown in Table 5. Phenolic alcohols (p-HPEA and 3,4-DHPEA), phenolic acids (ferulic and *p*-coumaric acid), lignans (pinoresinol) and secoiridoids (3,4-DHPEA-EDA; p-HPEA-EDA; 3,4-DHPEA- EA; and p-HPEA-EA) were the major fractions for the three varieties, although their profile varied with the applied treatment. Secoiridoid: 3,4-DHPEA-EDA was found as the most abundant phenolic compound in all oils, followed by p-HPEA-EDA in "Blanqueta" and "Arbequina" and 3,4-DHPEA-EA in "Picual". *P*-HPEA-EA and ferulic acid were not detected in "Arbequina" and "Blanqueta" samples. Phenolic acids were less abundant in all the samples.

The qualitative and quantitative composition of VOO hydrophilic phenols is strongly affected by the agronomic parameters such as the fruit maturity and the cultivar, as reported by different studies [55–57]. These studies have shown a decrease in polyphenols content through fruit ripening. Besides, according to Uceda et al (2008) [54] "Picual" and "Blanqueta" VOO are characterized by having medium-high concentration of phenols while "Arbequina" has medium-low phenol content. In our study "Blanqueta" and "Arbequina" oils showed higher phenolic contents than "Picual" oils. These results can be explained by the higher ripening index for "Picual" fruits (5.02) in comparison with "Arbequina" (3.6) and "Blanqueta"(3.24) cultivars. The enzymatic oxidation of derivatives of secoiridoids catalyzed by PPO and POD can explain the phenolic loss during the processing. Similar results have been reported in other studies related to malaxation steps [20, 24, 58], which connect the presence of oxygen in the headspace of the malaxation with a decrease in the oil phenolic compounds.

Oxygen increase during crushing caused a decrease in the phenolic compounds with respect to the control (Table 5). Among the cultivars studied, "Blanqueta" and "Arbequina" oils showed the greatest decrease in phenolic compounds with reduction values of 20.82 and 23.10%, respectively. Regarding the phenolic profile for all the samples, oleuropein secoiridoids derivatives suffered a higher reduction than other phenols such as ferulic and *p*-coumaric acids or pinoresinol. On the contrary, phenolic alcohols, (hydroxytyrosol and tyrosol) in "Picual" oils showed an increase with respect to the control oils, which can be due to a release in secoiridoids hydrolysis.

3.5 Volatile compounds

The sensory properties of VOO, for instance the "green" characteristic odor, is highly related to its volatile compounds, which are biosynthesized by LOX pathway forming aldehydes, alcohols, and their corresponding esters of five and six carbons. The effect of oxygen treatment during fruit crushing on volatile compounds is reported in the Fig. 1. The results show from a quantitative point of view, a strong change in the profile of the volatile. In fact, a positive correlation was observed between increasing oxygen and the total volatile compounds from LOX pathway in the three cultivars studied. For each cultivar this increase was different:

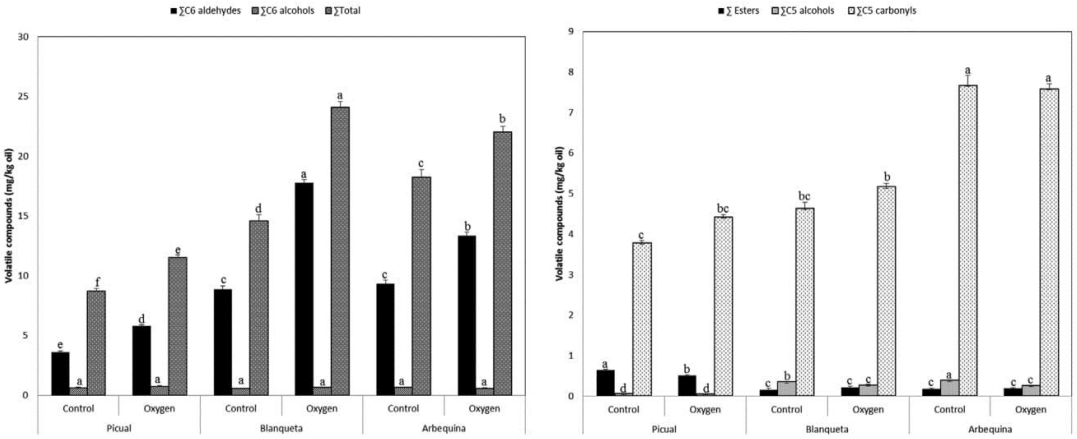

Figure 1. Volatile compounds (mg/kg oil) derived from LOX pathway in VOO from "Picual" "Arbequina," and "Blanqueta" cultivars obtained at two different concentrations of oxygen: atmospheric (control, 20% O_2) and increased (oxygen, 60% O_2) during fruit crushing. *Each bar represents a group of volatile compounds: ∑C6 aldehydes (E)-3-hexenal, (Z)-3-hexenal, (Z)-2-hexenal, (E)-2-hexenal, and Hexanal; ∑C6 alcohols: (E)-3-hexen-1-ol, (Z)-3-hexen-1-ol, (E)-2-hexen-1-ol, (Z)-2-hexen-1-ol; ∑Esters: (Z)-3-hexenyl acetate, (E)-2-hexenyl acetate, and hexyl acetate; ∑C5 alcohols: (E)-2-pentenol, (Z)-2-pentenol, 1-penten-3-ol, and 1-pentanol; ∑C5 carbonyls: (Z)-2-pentenal, (E)-2-pentenal, 1-penten-3-one, pentanal, 3-pentanone, and pentene dimers. Data show the mean value ± SD from three determinations in two different experiments. Different letters in the same group of compounds indicated significant differences at $p < 0.05$.

65% to "Blanqueta", 32% for "Picual" and 20%, for "Arbequina". Volatile compounds concentration in oils obtained by oxygen increase during crushing was strongly modified with respect to the control oils in all cultivars studied. "Arbequina" control oil displayed higher concentration of total volatile compounds than the other control oils and for "Blanqueta", the oxygen increase presented higher level of volatiles than the other oils tested. Consequently, there is a relationship between oxygen availability of oxidoreductase enzymes and biosynthesis of volatile compounds, according to Sánchez-Ortiz et al (2008) [19]. Likewise, the correlations between availability of oxygen during processing and volatile synthesis by LOX activity has been reported in several studies [19, 20, 24], although always for reduced oxygen concentrations.

The analysis of volatile compounds (listed in the Fig. 1) synthesized through LOX pathway disclosed information about the effect of oxygen increase during fruit crushing in the test completed. The group of aldehydes with six carbon atoms was the major fraction in all the oils analyzed and they presented the highest increase of volatile compounds; this increase was about 100% in the "Blanqueta" cultivar. The ratio C6/C5 compounds was between 1.3 and 1.9 except for "Blanqueta" where it was 3.9 after treatment with oxygen. On the other hand alcohols with five carbon atoms displayed a reduction about 30% in all the oils. The rest of group of volatiles showed different response depending on cultivar studied. For instance, esters suffered a decrease in "Picual" oils.

3.6 Sensory analysis

Sensory analysis is a crucial tool to evaluate the quality of extra virgin olive oils. The results of the sensory profile of the oil samples are shown in Fig. 2. None of the analyzed oils in this study presented any sensory defects. Sensory attributes according to official methods of analysis such as fruity, pungent, and bitter were detected in all samples tested. Fruity notes ranged from 3.70 to 5.25. The intensity of fruity attribute was medium according to the high rate of fruit ripening. These results are in accordance to the results obtained by other authors [59, 60].

The use of oxygen during olive fruit crushing had impact on olive oil sensory descriptors with respect to the control oils in all samples analyzed, however this effect was cultivar-dependent. Similar dependency on cultivar was found by Tura et al. (2008)[61]. "Picual," "Blanqueta," and "Arbequina" oils showed quite differences in terms of sensory profile in control oils. The fruity, bitterness, pungency, and almond, banana, and tomato descriptors were perceived in all samples of the three varieties. The increase of oxygen during the milling fruit produced an increase in the fruity attribute in the three cultivars studied. "Green and almond" notes tended to increase in "Picual" oils obtained by oxgygen increase and by contrast the data displayed a decrease in "Arbequina" oils in comparison with control. "Tomato" notes increased in all samples tested. "Fig leaf" notes were only perceived in "Picual" oils. Regarding others attributes, "mint" and "nuts" notes were detected in "Picual" and

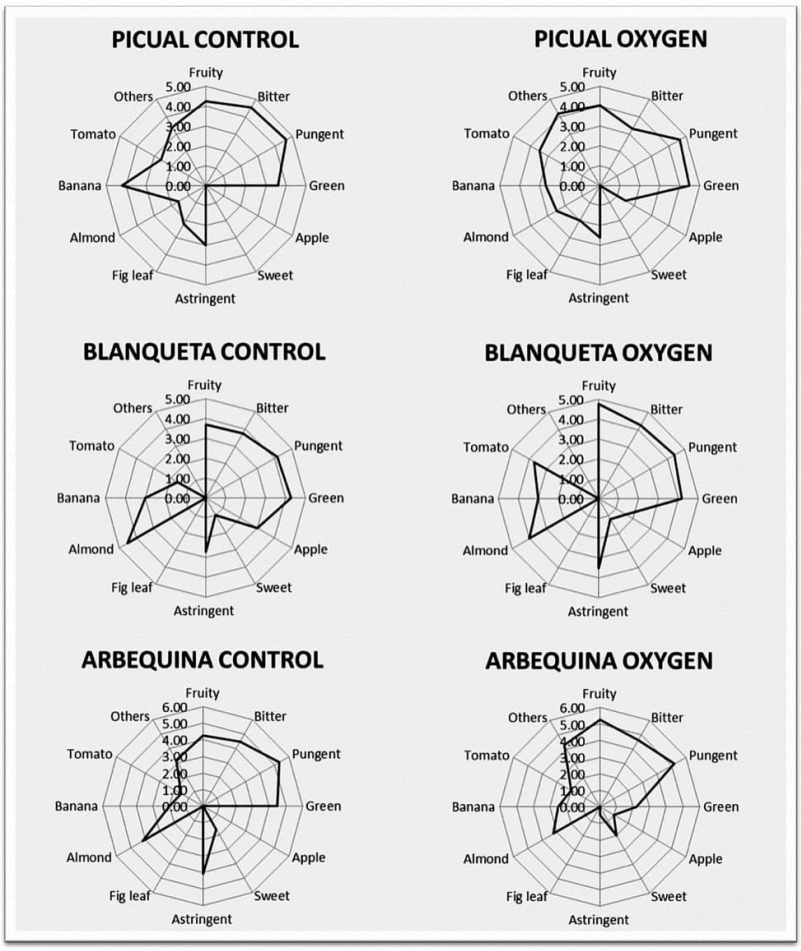

Figure 2. Sensory profile for olive oils from "Picual," "Arbequina," and "Blanqueta" cultivars obtained at atmospheric concentration (control, 20% O_2) and increased concentration of oxygen (oxygen, 60% O_2) during fruit crushing.

"Arbequina" oils, respectively; although the intensity of these attributes was higher in the oxygen treated oils than in the corresponding controls.

4 Conclusions

In conclusion, this study provides first results on the effect of increased oxygen during the crushing step. The data obtained suggest that the activity of the oxidoreductase enzymes related with the biosynthesis of the compounds responsible of the sensory characteristics of VOOs (LOX, ADH, POD, and POX) are strongly affected by the oxygen concentration during the oil extraction process.

Moreover a control of oxygen concentration in the milling process could promote the volatile compounds and sensory characteristics without compromising the oil yield, fatty acid composition, and natural antioxidants such as phenolic compounds or tocopherols. In this sense the results obtained also reveal a cultivar dependency. This might be of great importance to modulate cultivar with unbalanced profile of volatile (poor in flavor) or phenols (too bitter). On the other hand, the knowledge obtained with the oxygen increase during the milling suggests new research opportunities in order to obtain additional information about the enzymatic loading present in the fruits of the different cultivars. This could be a useful analysis tool while designing new prototypes of mills or while working with breeding selection programs.

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Research Article

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How 'ground-picked' olive fruits affect virgin olive oil ethanol content, ethyl esters and quality

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Abstract

BACKGROUND: Olives dropped on the ground naturally sometimes are not separated from those fresh and healthy collected from the tree for harvest and processing. In this work we compared the quality, ethanol content and bioactive components of virgin olive oils from ground-picked olives, tree-picked fruits and their mixture.

RESULTS: Ground-picked olives produced 'Lampante' virgin olive oils; these are of a lower quality category, because of important alterations in chemical and sensory characteristics. Ethyl esters showed the highest values, although under the regulated limit. The mixture of ground and tree-picked olives gave oils classified as 'virgin' because of sensory defects, although the quality parameters did not exceed the limits for the 'extra' category. Ethanol content showed a significant increase in the oils from ground- picked olives and their mixture with respect to those from tree-picked fruits. Furthermore, bioactive compounds showed a significant decrease as fruit quality was poorer.

CONCLUSION: Ground-picked olives must be harvested and processed separately since they produce low-quality virgin olive oils with sensory defects and lower concentrations of bioactive compounds. The higher acidity and ethanol concentration observed in oils from ground-picked fruits or their mixture may help ethyl ester synthesis during storage.

Keywords: virgin olive oil; quality; ethyl esters; ground-picked olives; ethanol; bioactive compounds

INTRODUCTION

Extra virgin olive oil (EVOO) is obtained directly from olives just harvested from the tree using only physical and mechanical methods. EVOO can be differentiated from other olive oils of lower quality by its bioactive and sensory characteristics.

Sensory attributes and minor compounds related to healthy and sensory properties are not found at the same intensities and concentration in all the EVOOs. They are affected by several factors, such as the fruit harvest date. In general, most of these components and sensory descriptors show a decrease during the harvest period as fruit ripens, although EVOO can be obtained even for ripened olive fruits.[1–3]

Associated with fruit ripening a natural olive fall occurs because of the reduction of fruit retention force and adverse weather conditions. When the olives drop to the ground, due to the impact they suffer loss of epidermal integrity, and alteration and degradation can then be observed while they remain on the ground, until harvesting. The fruit alteration can be helped by frosts and high humidity, usually registered during the autumn and winter.

When natural fruit drop occurs olive growers have to accelerate fruit harvesting in order to avoid quality losses and a generalized fruit drop. Furthermore, adverse weather conditions (wind, rain and frost) complicate olive harvesting, and olives can then remain on the ground for a longer time.

Although at this date EVOO can still be obtained from olives collected from the tree, some bad practices may be carried out by olive growers and/or oil mills. For example, olives can be shaken mechanically from the tree and harvested together with those that remained for a long time on the ground. Another non-recommended practice is when olives are harvested separately (ground and tree) and the oil mills do not discriminate between them at fruit reception and processing.

Although the negative effect of these practices may be considered obvious,[4] results concerning the effect of these bad practises on oil quality and sensory characteristics have not been sufficiently reported. Nowadays, because of the recent adoption of ethyl ester as a quality parameter[5,6] to distinguish 'extra' virgin olive oils it is essential to describe whether this parameter is affected by the bad practices described above. Ethyl esters have been related to low-quality VOOs[7] and were associated with oils from damaged and low-quality olive fruits.[8] Conte *et al.*[9] described the formation

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of ethyl esters during oil conservation. Ethyl esters can be formed by direct esterification of free fatty acids or by transesterification of fatty acids from triacylglycerides as described by Biedermann *et al.*[8] Their alcoholic precursor – ethanol – may be synthesized in the own olive fruit whereas it remains on the tree,[10] but any alteration of the fruit can result in fruit enzyme activation or attack by microorganisms because of the loss of fruit integrity during its period on the ground. Furthermore, an increase in ethanol content and free acidity, although values were always below the regulated limits, may induce the synthesis of ethyl esters in VOO. Free fatty acids can act as catalysts, improving ethyl ester synthesis.

The aim of this work was to describe the effect of no separation of ground-picked olives from those harvested directly from the tree on VOO quality, ethyl esters, ethanol as ethyl ester precursors and minor compounds.

MATERIAL AND METHODS

'Picual' olive fruits were harvested from selected olive trees grown in the experimental orchard of Centro IFAPA Venta del Llano, Mengibar, during the crop year 2013/14. The adult olive trees were grown under traditional conditions and irrigation.

A batch of homogeneous olive trees, with approx. 50% of fruits fallen naturally on the ground and the rest remaining on the tree, was selected. The olives from the ground (3000 kg) were harvested first using olive blowers, whereas those fruits from the tree (3000 kg) were picked by a mechanical shaker. Both fruits were transported, by separate means, immediately to the oil mill for processing. For the ground-picked olives the branches, stones and sand were removed by cleaning and washing. For olive washing fresh water was used. Olives harvested from the tree were cleaned by removing small branches and leaves without washing.

The two fruit types were stored separately in a divided hopper with a common exit, allowing processing of each fruit type separately or a mixture of both types in appropriate proportions.

The experiment was carried out as follows. Olives harvested from the tree were processed first; then the mixture of ground- and tree-picked olives (50:50); and finally, the ground-picked olives. Two batches of 500 kg were processed separately for each fruit type. Olive fruit storage time was less than 8 h.

Oil extraction was performed in the experimental oil mill of IFAPA Centro Venta del Llano. The oil mill was equipped with a continuous extraction system (Pieralisi, Spain) working in a two-phase way, formed of a hammer crusher, three containers, malaxer, a horizontal centrifuge SC90 (HC) and a vertical centrifuge P1500 (VC). The process was automated and monitored by the automation software Procioleo (Procisa, Spain).

The extraction conditions were: crusher grid diameter 6 mm; malaxation temperature 20 °C for 45 min; olive paste load 750 kg h^{-1}; and a ratio of 1:1 between oil and water in the VC.

Samples

Fruit samples were collected for each fruit type in triplicate. Oil samples were taken from both the HC after the vibrating sieve and VC for the last 15 min of processing each malaxer container. Oils without filtration were used for ethanol analysis and filtered oils were used for oil analysis including quality parameters, composition and ethanol.

Fruit characterization

Fruits were characterized by measuring the ripening index[11] and analysing the oil content and moisture. Moisture was determined in milled olive paste drying in an air-forced oven to constant weight. Oil content was measured in dry olive paste by a nuclear magnetic resonance fat analyser (minispeq q10, Bruker, Spain). Results are expressed as a percentage. The fruit characteristics are shown in Table 1.

Table 1. Characteristics of 'Picual' olive fruits (two batches) used for the experiments in the oil mill

Fruit	Humidity (%)	OFW (%)	ODW (%)	RI	Mean weight (g)	P/S
Tree 1	42.67	25.76	44.95	4.8	2.2	3.58
Tree 2	43.54	27.57	48.84	5.1	2.56	3.65
Ground + tree 1	43.24	26.43	46.56	–	2.06	2.89
Ground + tree 2	43.02	25.90	45.48	–	2.03	2.98
Ground 1	42.37	24.05	41.74	–	1.71	2.42
Ground 2	43.19	24.46	43.10	–	1.73	2.68

OFW, oil content on fresh weight; ODW, oil content on dry weight; RI, ripening index; P/S: pulp/stone ratio.

Oil analytical determinations

Oil quality was characterized by measuring ethyl esters, acidity, peroxide value, and UV absorbance at 232 and 270 nm according to EU Regulations.[12] Results were expressed as mg kg^{-1}, % oleic acid, meqO_2 kg^{-1} and extinction coefficient, respectively.

Analysis of ethanol

Solid-phase microextraction (SPME) followed by gas chromatography–flame ionization detection (GC-FID) were used to analyse the ethanol in the samples studied, according to the method described by Sanchez-Ortiz *et al.*[13] Briefly, olive oil samples were conditioned to room temperature and then placed in a 10 mL vial fitted with a silicone septum heater at 40 °C. After 10 min equilibrium time, ethanol from the headspace was adsorbed by exposing the SPME fibre DVB/Carboxen/PDMS 50/30 μm, 1 cm (Supelco Co., Bellefonte, PA, USA) for 50 min at 40 °C in the headspace of the sample, and then retracted into the needle and immediately transferred and desorbed for 5 min into the injection port of a gas chromatograph equipped with FID.

Ethanol was analysed using a Varian CP 3800 gas chromatograph equipped with a Supelcowax 10 capillary column (30 m × 0.25 mm, 0,25 μm, Sigma-Aldrich). Operating conditions were as follows; He was the carrier gas; injector and detector temperature 250 °C; and column held for 5 min at 40 °C and then programmed at 4 °C min^{-1} to 200 °C. Compound identification was carried out on an ISQ single-quadrupole mass spectrometer (Thermo Fisher Scientific, Austin, TX, USA) operating in EI mode (70 eV) under identical conditions for GC-FID, matched against the Wiley/NBS Library, and by GC retention time against standard. Quantification was performed using individual calibration curves for ethanol in refined olive oil. Results were expressed as mg of ethanol per kilogram of oil.

Polyphenol content was determined according to the method described by Vázquez-Roncero *et al.*[14] using Folin–Ciocalteu reagent and absorbance measurement at 725 nm. The results were expressed as milligrams per kilogram of caffeic acid. The absorbance measurements were performed in a UV–visible spectrophotometer (Cary Bio50, Varian, Spain).

Tocopherols were determined by high-performance liquid chromatography (HPLC) applying IUPAC method 2432.[15] Detection and

Table 2. Effect of olive fruit quality (ground, ground + tree and tree) on the regulated quality parameter for horizontal centrifuge (HC) and vertical centrifuge (VC) virgin olive oils

	HC			VC		
Quality parameter	Ground*	Ground + tree	Tree	Ground	Ground + tree	Tree
Acidity (%)	0.65 ± 0.03a	0.32 ± 0.02b	0.20 ± 0.04c	0.50 ± 0.12a	0.37 ± 0.03b	0.26 ± 0.05b
Peroxide value (meq O_2 kg^{-1})	32.46 ± 0.58a	14.16 ± 1.03b	7.47 ± 1.19c	23.57 ± 5.60a	16.19 ± 1.97b	11.49 ± 2.21b
K_{232}	1.74 ± 0.07a	1.5 ± 0.04b	1.49 ± 0.02b	1.62 ± 0.05a	1.54 ± 0.02b	1.58 ± 0.05ab
K_{270}	0.20 ± 0.04a	0.12 ± 0.02b	0.12 ± 0.02b	0.15 ± 0.34a	0.13 ± 0.01a	0.15 ± 0.02a

Mean values ($n = 6$). Different letters in the same row indicate significant differences ($P = 0.05$) between fruit types for HC and for VC oils.
Limits for EVOO of quality parameters: acidity ≤ 0.8%; peroxide value ≤ 20 meq O_2 kg^{-1}; $K_{232} \leq 2.50$ and $K_{270} \leq 0.22$.
Limits for VOO of quality parameters: acidity ≤ 2%; peroxide value ≤ 20 meq O_2 kg^{-1}; $K_{232} \leq 2.60$ and $K_{270} \leq 0.25$.
Limits for lampante olive oil of quality parameters: acidity > 2%; peroxide value ≤ 20 meq O_2 kg^{-1}.

quantification were carried out in an Agilent 1200 HPLC instrument equipped with a quaternary pump and UV–visible detector set a 295 nm. Results were expressed in milligrams per kilogram.

HPLC analysis of phenolic compounds

Extraction of phenolic compounds

A sample of VOO was weighed (1.5 g) and 100 μL of a standard solution (0.002 g of syringic acid 100 mL^{-1} methanol) was added. The oil was dissolved in *n*-hexane (1 mL), the phenolics were extracted twice with 1.25 mL methanol–water (60:40, v/v), and the extracts were washed with *n*-hexane, rising to a final volume of 2.5 mL.[16]

Reversed-phase HPLC determination of phenols

HPLC analysis was performed using an HP1100 system equipped with an autosampler, quaternary pump and diode array detector. A reversed-phase C18 Pecosphere column (83 × 4.6 mm i.d., 3 μm particle size; Brown Lee Columns) was used with an injection volume of 20 μL and a flow rate of 0.45 mL min^{-1}. The mobile phase was a mixture of water–acetic acid (98:2, v/v) (solvent A) and methanol–acetic acid (98:2, v/v) (solvent B). The total run time was 70 min. The solvent gradient changed according to the following conditions: 90% A–10% B for 10 min; 80% A–20% B for 8 min; then, after a further 2 min, 60% A–40% B for 10 min; 50% A–50% B for 10 min; and 100% B for 10 min until the end of the run. Phenolic compounds were quantified at 280 nm using syringic acid as internal standard and response factors as determined by Mateos *et al.*[17]

Sensory tests were performed by the VOO analytical Sensory Panel of Fundacion Citoliva, formed of eight informed and trained tasters as described by EU Regulation.[18] The results were expressed as the median of the intensity of the sensory attributes.

Statistical analysis

Analysis of variance analysis was performed using Statistix 8.0 analytical software in order to determine the effect of fruit quality on the oil parameter analysed. Tukey's test ($P = 0.05$) was applied to determine differences between means.

RESULTS AND DISCUSSION

In general, considering the set of quality parameters analysed, the VOOs obtained from tree-picked olives were classified as 'extra virgin' olive oils whereas the oils from the mix of ground and tree-picked olives were included in the 'virgin' category. Samples from ground-picked olives were classified as 'lampante'.

Results of quality parameters, acidity, peroxide value and UV absorbance analysed in the VOOs obtained in the experiments are shown in Table 2.

Acidity in VOOs shows hydrolytic alterations; the values obtained in the oils from the different fruit qualities showed values lower than 0.8%, the limit established for 'extra virgin' olive oil. The highest acidity values were found in VOOs from ground-picked olives, whereas the lowest were observed for oils from tree-harvested fruits. The mixed fruits (ground + tree) gave oils with acidity higher than those from tree-picked olives. Significant differences between the three VOO types were detected for both HC and VC oils. The higher-acidity values obtained for the oils from ground-picked olives can be explained because of fruit integrity loss and the action of microorganism with hydrolytic activity. The presence of ground-picked olives also produced a significant increase in oil acidity, as described.

Peroxide value indicates the oxidation status of a fat. The peroxide value achieved higher values (32 meq O_2 kg^{-1}) for those HC oil from ground-picked olives, being classified as 'lampante' since they exceed the limit of 20 meq O_2 kg^{-1} for 'extra' virgin olive oil. When olives harvested from the ground were not separated the oils showed a peroxide value higher than that from fruits harvested directly from the tree. Although with slightly lower values, the VC oils showed similar changes in peroxide value when fruit quality changed. As explained for acidity, the loss of fruit integrity and the action of microorganisms in the ground-picked olives gave a peroxide value raise.[19]

Ultraviolet absorbance at 232 and 270 nm (K_{232} and K_{270}) showed lower values in the oil extracted from tree-picked olives. These parameters rose as the percentage of damaged fruits from the ground was higher (Table 2). All the oils analysed showed K_{232} and K_{270} values below the limits regulated for EVOO: 2.5 and 0.22 respectively.[18]

Ethyl esters have been adopted recently as a quality parameter to distinguish EVOOs from those VOOs of lower quality.[5,6] The limit established for crop year 2014/15 was 35 mg kg^{-1}; although a reduction was planned for the current crop year to 30 mg kg^{-1}, the limit of 35 mg kg^{-1} will be maintained until June of 2016 as the result of a moratorium.

The lowest ethyl ester content was observed in VOO from olives picked directly from the tree (<3 mg kg^{-1}) (Fig. 1). When fruits from the ground were not separated from healthy and fresh fruits, an increase in ethyl ester concentration was observed, although a very low concentration was detected. VOOs obtained

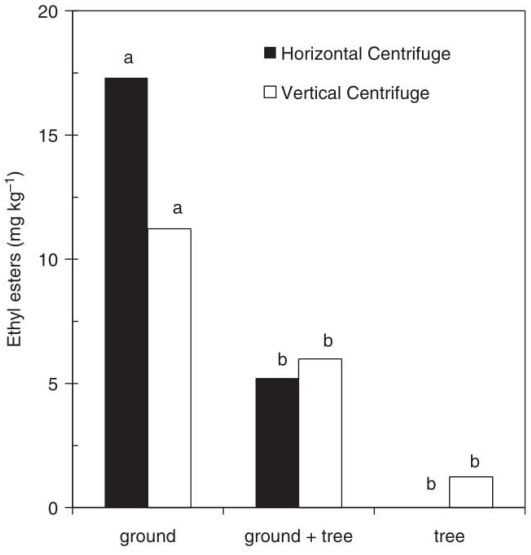

Figure 1. Effect of fruit quality (ground, ground + tree and tree) on the ethyl ester concentration of 'Picual' virgin olive oil. Different letters indicate significant differences ($P = 0.05$) between fruit types for HC and VC oils.

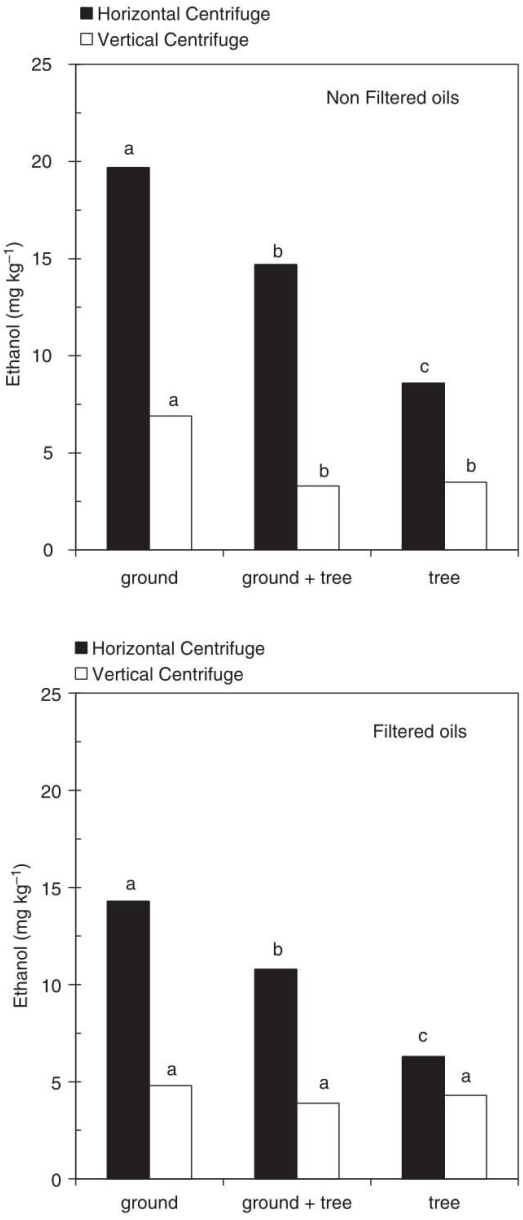

Figure 2. Effect of fruit quality (ground, ground + tree and tree) on the ethanol concentration of 'Picual' virgin olive oils: filtered and non-filtered. Different letters indicate significant differences ($P = 0.05$) between fruit types for HC and VC oils.

from ground-picked olive fruits achieved the highest ethyl ester concentration: around 17 mg kg^{-1}. Significant differences were obtained for the oils from the different fruit qualities. Attention should be paid to the oils from ground-picked olives since their ethyl ester content was below the limit established for EVOO although defective olive fruits were used for their extraction. Thus the capacity of these compounds to discriminate EVOOs from those of lower quality may be in question. The ethyl ester content of the ground-picked olives may be explained because of the short time period that olives remained on the ground, which was not long enough to synthesize ethyl esters at higher concentrations.

Although methyl esters are not included in the regulations, they were analysed too. Their content varied between 9.4 mg kg^{-1} for the oils from ground-picked olives and values lower than the detection limit for oils extracted from olives harvested directly from the tree (data not shown).

Because of the great discrimination capacity of ethyl esters for oil quality classification, there is increasing interest in their chemical precursors – mainly ethanol. The importance of ethanol is due to its capacity to synthesize ethyl esters during oil storage.[9] For this compound both filtered and unfiltered oils were analysed.

The highest ethanol content was found in the unfiltered oils. This higher content may be explained because ethanol may be dissolved in the aqueous phase that remains in these oils (Fig. 2) since, owing to its chemical characteristics, ethanol can be mixed with water.

In general, ethanol concentration was higher in those oils taken directly from the HC, since they contain higher percentages of humidity and solid particles. For the oil after VC a significant decrease in ethanol concentration was obtained. This decrease may be due to the 'washing' effect of the water added to the VC.

Ethanol content was also affected by the olive fruit quality. VOO from the ground-picked olives showed the highest concentration of ethanol, whereas the lowest were measured in the oils from tree-picked olives. No separation of those fruits collected from the ground gave an ethanol content higher than that from fresh and healthy fruits, with significant differences with respect to the oils from the other fruit qualities.

Therefore, those oils of fruits collected from the ground, showing the highest ethanol content and acidity, may give a higher ethyl

Table 3. Effect of olive fruit quality (ground, ground + tree and tree) on the main sensory attributes and category of virgin olive oils

Sensory descriptor	Ground	Ground + tree	Tree
Fruity	n.d.	2.5	3.4
Musty–humid–earthy	2.9	2.5	n.d.
Fusty/muddy sediment	4	2.2	n.d.
Winey–vinegary	2.6	n.d.	n.d.
Category	'Lampante'	'Virgin'	'Extra'

ester content during storage. However, attention should be paid to the oils from fresh olives mixed with ground-picked fruits since they showed a significant increase of acidity and ethanol content and, then, ethyl ester may be formed faster than in those oils from tree-picked olives.

The sensory characterization of oils from olives harvested from the tree were classified as 'extra virgin', whereas those extracted from the mixture for ground- and tree-picked olives belonged to the 'virgin' category (Table 3). However, the oils obtained from the fruits harvested from the ground were classified as 'lampante'. 'Fruity' attribute of VOOs from tree-picked fruits showed the highest intensity, whereas a 50% reduction of 'fruity' intensity was observed for the olives without any quality distinction. For the oil obtained from ground-picked olives the attribute 'fruity' was not detected.

However, olives harvested from the ground generated some sensory defects or negative attributes, such as 'musty–humid–earthy', 'fusty/muddy sediment' and 'winey–vinegary'. The 'musty–humid–earthy' defect, characteristic of the ground-picked olives, was detected for an intensity of 2.5 in those oils from the mixture of ground- and tree-picked fruits; an intensity of 2.9 was achieved for the oils from olives harvested on the ground (Table 3). The mixture of the olives harvested directly from the tree and those from the ground gave oils with 'fusty/muddy sediment' defect with an intensity of 2.5, whereas the oils from the ground rose to 4.

Because of their greater importance in VOO quality and related health claims,[20] phenolics and tocopherols were also analysed. The results obtained for these compounds are shown in Table 4. In general, the oils from tree-picked olives showed the highest concentration for all of them, whereas the oils from the ground-picked olives achieved the lowest values, with significant differences between them. The oils extracted from the mixture of ground- and tree-picked olives showed a significant decrease with respect to those from the tree.

For individual phenols (Table 4), a significant decrease was observed for secoiridoid derivatives as the fruit quality was poorer. However, tyrosol and hydroxytyrosol showed an increase that can be explained by the hydrolysis of secoriridoids because of fruit degradation and microorganism attack during its permanence on the ground. Therefore, the health benefits related to VOO phenolic compounds were affected negatively by fruit quality.

CONCLUSIONS

The oils from ground-picked oils showed the highest alterations of sensory and chemical characteristics. These oils showed sensory defects and peroxide values that allowed classification in the 'lampante' VOO category. When ground-picked olives were not separated from the healthy and fresh olives harvested, their oils showed an increase of those quality parameters related to hydrolytic and oxidative alterations, ethyl esters and the presence of sensory defects compared with those from olives harvested from the tree. Therefore, the oils from non-separated fruit quality were classified in the virgin category. Ethanol content was detected at the highest concentration in the oils from ground-picked olives. The mixture of bad-quality olives with fresh and healthy olives produced oils with a higher ethanol content than good-quality fruits. The minor compounds responsible for the health claims related to VOO showed a significant decrease as fruit quality was lower. Therefore, ground-picked olives must be harvested and processed separately since their presence negatively affects oil quality and bioactive components.

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Table 4. Effect of olive fruit quality (ground, ground + tree and tree) on the minor compounds and bitterness index of horizontal centrifuge (HC) and vertical centrifuge (VC) virgin olive oils

	HC			CV		
Parameter	Ground	Ground + tree	Tree	Ground	Ground + tree	Tree
Tocopherols (mg kg^{-1})	364c	374b	384a	364b	366b	378a
Total phenols (mg kg^{-1})	198c	292b	402a	221c	282b	356a
Phenolic fraction (mg kg^{-1})						
Hydroxytyrosol	12.1a	3.3b	2.9b	2.9a	3.1a	3.3a
Tyrosol	12.5a	3.9b	2.7b	2.9a	2.9a	2.7a
3,4-DHPEA-EDA	18.2c	68.6b	130a	45.4c	67.3b	89.2a
p-HPEA-EDA	31.7c	65.9b	95.7a	44.1c	59.7b	74a
3,4-DHPEA-EA	40c	60.5b	77.1a	56.3b	66b	82.2a
Total	115c	202b	308a	152c	199b	251a

Different letters in the same row indicate significant differences ($P = 0.05$) between treatments for horizontal centrifuge (HC) oils and vertical centrifuge (VC) oils.
3,4 DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; p-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, aldehydic form of elenolic acid linked to hydroxytyrosol.

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