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Effect of botanical and physicochemical composition of Argentinean honeys on the inhibitory action against food pathogens

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ABSTRACT

Honey is a natural food with functional properties such as antioxidant and antimicrobial activities. These properties vary greatly depending on floral source, climate, and environmental and processing conditions. In this work, we characterized honeys on the basis of their botanical composition and clustered them according to their physicochemical parameters in order to find similarities, and assess their antibacterial action against microorganisms isolated from contaminated food. All honeys studied complied with international quality standards. The data showed differences between multifloral and unifloral honeys in their physicochemical parameters, as well as a direct correlation between colour, phenolic compounds, and antioxidant activity. -Antimicrobial activity resulted from hydrogen peroxide effect. Multifloral honeys with similar phenolic compounds and a botanical composition of eucalyptus and blueweed had greater inhibitory power against *E. coli, P. aeruginosa, Salmonella* spp., *S. aureus* and *B. cereus*.

1. Introduction

Honey has been used for both medical and nutritional purposes. In terms of the first, it has been used for its therapeutic action, which includes antimicrobial and anti-inflammatory properties (Libonatti, Varela, & Basualdo, 2014; Pascoal, Feás, Dias, Dias, & Estevinho, 2014, pp. 221–234). In terms of the second, it is a nutritive food widely used in the food industry, with antimicrobial and antioxidant properties that make honey a natural food preservative.

Honey is a supersaturated solution of sugars, mainly composed of fructose and glucose, and a wide range of minor components such as minerals, proteins, free amino acids, vitamins, enzymes -glucose-oxidase, and catalase-, phenolic acids, and flavonoids (Alvarez-Suarez, Tulipani, Romandini, Bertoli, & Battino, 2010; Saxena, Gautam, & Sharma, 2010). Its composition is variable and its properties vary greatly depending on floral source, climate, and environmental and processing conditions (Libonatti et al., 2014; Liu, Ye, Lin, Wang, & Peng, 2013).

The literature has widely reported on the antibacterial properties of honey (Allen, Molan, & Reid, 1991; Molan, 1992; Fangio, Iurlina, & Fritz, 2010; AL-Waili et al., 2013) which may stem from variations in plant source (Liu et al., 2013; Mundo, Padilla-Zakour, & Worobo, 2004). On the other hand, antimicrobial activity of honey is given by peroxide and non-peroxide factors. As to the first ones, some researchers have concluded that the major one is hydrogen peroxide, formed out of the oxidation of glucose by glucose oxidase during the ripening of honey (White et al., 1963). As to the non-peroxide antimicrobial factors, physicochemical characteristics -high osmolarity, acidity, peptides, lysozyme, phenolic acids, and flavonoids-are included (Feás, Iglesias, Rodrigues, & Estevinho, 2013; Kwakman et al., 2010; Molan, 1992). Some authors have shown a relationship between colour -given by carotenoids and flavonoids-phenolic compounds, and antioxidant and antibacterial activity of honey and their relation to floral source (Bueno-Costa et al., 2016; Isla et al., 2011; Liu et al., 2013).

Antibacterial activity of unifloral and mutlifloral honey against

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foodborne pathogens has been reported by various studies (Isla et al., 2011; Mundo et al., 2004). Multiflora honeys do not have any type of predominant pollen and could be mainly composed by nectar of two or more species in certain proportions. This mixture of botanical species could give honey particular physicochemical and antibacterial characteristics. However, researchers have not focused on this aspect. Thus, we hypothesized that there could be a relationship between honey inhibitory power and botanical composition in both qualitative and quantitative terms.

As a natural complex food, many variables are needed to characterize honey. The chemo metrics techniques, as principal component analysis, are the most commonly used ones to identify the natural clustering pattern and groups of variables on the basis of similarities between samples (Silvano, Varela, Palacio, Ruffinengo, & Yamul, 2014). In this work, we characterized Argentinean honey samples out of their botanical composition, and clustered them according to physicochemical parameters in order to find similarities, and assess their antibacterial action against microorganisms isolated from contaminated food. We focused on the relation between inhibition power of honey and the proportion of botanical species present in honey.

2. Materials and methods

2.1. Honey samples

Twenty four honey samples were harvested in 2014 from apiaries from three regions of Argentina (Northwestern region, Cuyo region and Pampean region) and classified according to their botanical origin. The samples were provided by beekeepers who obtained the honey by cold extraction, kept in plastic containers, and stored in a fresh, dry place until their analysis. Honey samples were clustered according to their botanical origin and classified into 10 different types of honey: 7 multifloral and 3 unifloral. The results are expressed in relation to these samples.

2.2. Botanical origin

To determine botanical composition, ten grams of honey were diluted in 20 mL of distilled water and centrifuged at 3000 rpm for 5 min. The supernatant was poured off and the sediment was acetolyzed according to Louveaux, Maurizio, and Vorwhol (1978). The sediment was removed with a stylet, embedded in glycerin jelly, deposited on a microscopic slide, and then sealed with paraffin wax. The slides were examined using an optical microscope (40X). At least 200 pollen grains in each honey sample were counted. Pollen grains were identified using the reference collection of the Apicultural Laboratory, Veterinary Sciences Faculty, National University of Buenos Aires Province Centre. Morphological pollen types were determined with the greatest possible taxonomic approximation, achieving genus or species level when possible. Other than that, botanical families or group were determined. For multifloral honeys (MH), occurrence frequencies of pollen types were determined according to Basualdo, Pereda, and Bedascarrasbure (2006), who considered as dominant pollen (D: frequency $\geq$45% of total counted pollen grains), secondary (S: 16 < frequency< 45%) and minor importance (M: 3 < frequency< 15%). Unifloral honeys (UH) were classified following Argentinean standards (SAGPYA, 1994) according to which honeys are considered as unifloral *Eucalyptus* if the relative frequency of occurrence (RF) of *Eucalyptus* pollen reaches a minimum value of 70%, while for unifloral *Lotus* sp. the RF should be 20%, and for unifloral clovers the RF should be 45% mixture of *Trifolium* sp., *Medicago* sp. *Melilotus* sp., and *Lotus* sp. pollens (SAGPYA, 1994). Thus, a total of 10 honey types were classified according to botanical associations considering the RF.

2.3. Physicochemical analysis

Moisture was determined with an Abbé refractometer (American optical corporation), reading at 20 °C and the corresponding moisture value was obtained from the Chataway Table (1932) as cited by Wedmore (1955).

The acidity of honey was determined according to A.O.A.C. (1990). For pH determination, strips indicators (DF®) were used.

Colour measurements were performed using HI 96785 HANNA colorimeter (IRAM 15941-2, 1997). Crystallized honey was melted at 56 °C in thermostatic bath until complete dissolution of the crystals and elimination of dissolved air. The liquid honeys without air bubbles thus obtained were placed in plastic buckets and the colour was read, results being expressed in mm Pfund scale (Fell, 1978). Honey colour grades on Pfund readings are: average scale reading $\leq$8 mm: water-white; 8 < reading $\leq$16: extra white; 16 < reading $\leq$34: white; 34 < reading $\leq$50: extra light-amber; 50 < reading $\leq$85: light-amber; 85 < reading $\leq$114: amber; reading >114: dark.

2.4. Sugar profile analysis

Glucose, fructose, and sucrose were determined with high performance liquid chromatograph (Waters 1525), equipped with a differential refractive index detector (Waters 2414), (Bogdanov & Baumann, 1988). Five grams of honey were prepared with 500 μL of solution of Carrez I ($K_4Fe(CN)_6 \cdot 3H_2O$) and II ($Zn(AcO)_2.2H_2O$), in a volumetric flask of 25 mL. distilled water was added to complete the volume. The dissolution was then filtered through 0.45 μm cellulose filter prior to HPLC analysis. The injection volumes of the samples were 20 μL with a flow rate of 1.3 mL/min. The separation was performed by using a Polyamine II (4.6 × 250 mm, YMC HPLC Column) column. A mobile phase of acetonitrile/water HPLC grade; 8:2 (v/v) was used. The system was maintained at 35 °C. Identification of individual compounds was made by comparing the retention times of the honey compounds identified with commercial standards (Fluka, Switzerland). For quantification, calibration curves were developed for each compound.

2.5. Antioxidant activity and total phenolic content

The antioxidant activity of honey was evaluated by the method of inhibiting the radical $ABTS^+$ (2,2′-azinobis- [3-ethylbenzothiazol-6-sulfonic acid]). The ABTS test was performed according to Re et al. (1999). The cation radical ABTS+ was synthesised by the reaction of a 7 mM ABTS solution with a 2.45 mM potassium persulfate solution. The mixture was kept at 23 °C in the dark for 16 h. Afterwards, the ABTS + solution was diluted with ethanol until an absorbance (A) of 0.7 at 734 nm was achieved in a UV–Vis spectrophotometer. First the sample was conveniently diluted, then Aliquots of 2.7 mL from the ABTS + solution were immediately added to the sample. After 6 min, the percentage inhibition of absorbance at 734 nm was calculated for each concentration relative to the blank absorbance (ethanol).

The scavenging capability of the ABTS + radical (%AS) was calculated using the following equation:

$$\% AS = 100(Acontrol - Asample)/Acontrol$$

Where “A control” is the absorbance control obtained from the ABTS + radical alcoholic solution, and “A sample” is the absorbance radical in presence of the sample or the trolox standard. The results were expressed as SC50 ± sd, where SC50 represents the sample

concentration required to obtain half the ABTS + radical scavenging activity and sd is the standard deviation.

The results were expressed as SC50 (g of honey per mL), the minimum sample concentration required to obtain half the ABTS + radical scavenging activity. Therefore, lower values indicate higher antioxidant activity.

Phenolic compounds were determined according to Singleton, Orthofer, and Lamuela-Raventos (1999), using Folin-Ciocalteu reagent and saturated sodium carbonate solution (75 g/L). The tubes were left in the dark at room temperature for two hours for colour development, and then absorbance was read at 765 nm by a spectrophotometer diode array (Hewlett Packard 8452A). A calibration curve was made by using a solution of gallic acid.

2.6. Antibacterial activity and bacterial strains

Antibacterial activity of samples was tested by an agar diffusion test (Koneman, Allen, & Dowell, 2008) against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* spp. and *Escherichia coli*, all of them isolated from contaminated food -strain collection from the Department of Technology and Food Quality, UNCPBA.-; and *Bacillus cereus* -spore suspension, Difco, 0959-36-9-. The strains were incubated on nutrient agar at 35 °C for 24 h. The bacterial inoculum, prepared according to tube number 1 of McFarland scale (3×10^8 CFU/mL), was placed in Petri dishes and homogenized with Mueller-Hinton agar. Four wells of 7 mm were punched in solid agar with a cork borer. One of the four wells was used as a control well to which sterile distilled water was added.

Honey samples were tested after dilution to 1:2 and 1:4 w/v with sterile distilled water. One hundred microliters of honey dilution were placed in triplicate into each well with a sterile micropipette. The plates were incubated for 24 h at 35 °C. After incubation, bacterial inhibition zones around each well were measured by using Vernier scale. In order to inhibit the peroxide effect of honeys, the samples showing antimicrobial activity were treated with catalase solution (SIGMA, ALDRICH, 3050. Co bovine liver catalase, C9322-16, 2000–5000 units/mg protein) according to Allen et al. (1991) methodology. After being treated with catalase, the honeys lost their antimicrobial capacity. Thus, we assume that the antibacterial activity of the honeys used in this work resulted from the peroxide effect. We refer to the antimicrobial capacity as the presence or absence of inhibition zones and inhibition power as the diameter of the zone of inhibition measured in mm.

2.7. Data analysis

Analyses were performed using InfoStat software. A principal component analysis was conducted to examine the relationship between honey physicochemical parameters, phenolic compounds, antioxidant activity, and botanical origin. The physicochemical parameters grouped as MH and UF were compared by multivariate analysis of variance MANOVA using Wilks-Lamba statistics. The power of inhibition between two honey dilutions was compared by using a Kruskal-Wallis test. Data of antibacterial activity of each dilution of honey were analyzed separately. Differences in antibacterial activity of honeys against the different bacterial strains were compared by using the chi-square test. The inhibitory power of 1:2 honey dilution against the different bacterial strains was compared by using ANOVA, and the differences of inhibitory power between these honeys assorted according to botanical origin were compared by using an ANOVA and Fisher tests.

3. Results

3.1. Botanical origin

The UH were Eucalyptus, Lotus, and clover. In MH, the presence of *Eucalyptus* sp. pollen was frequent, being the dominant pollen in most honeys, representing over 45% of the pollen spectrum (Table 1). The secondary pollens found in MH were blueweed, sunflower, and clover. Honey MF7, from a different geographical origin, showed a distinctive pollen spectrum, including genus of the Fabaceae family as secondary pollen and *Cissus* sp. and *Eupatorium* sp. as a minor importance pollen.

3.2. Physicochemical parameters, phenolic compounds, and antioxidant activity

Acidity ranged from 12.5 to 20 meq/kg and pH values ranged from 3 to 4.5. Honey MF7 had higher acidity than the rest with a mean value of 36 meq/kg (Table 1). Moisture showed values between 16% and 19.5%. The glucose content was between 23% and 43%, the fructose content was between 37% and 49%, and the fructose/glucose ratio ranged from 1.08 to 1.63. Colour ranged from 48 to 150 mm in the Pfund classifier (Table 1), which corresponds to extra light-amber, light amber, amber, and dark amber.

Antioxidant activity of honeys ranged from 0.24 to 0.61 g/ml, and the content of phenolic compounds was between 0.32 and 0.44 mg GAE/g honey. Honey MF7 had phenolic compounds of 1.24 mg GAE/g above the rest, and also showed a higher antioxidant activity with a minimum concentration of honey required to scavenge the ABTS radical of 0.24 g/mL (Table 1).

3.3. Principal component analysis

The Principal Component (PC) analysis explained 78% of the total variation of physicochemical parameters, composition, and antioxidant activity of honey in its first two components (PC) (Fig. 1). PC1 explained 61.2% of the variation and was mainly defined by phenolic compounds, colour, and acidity in a positive direction together with glucose and sucrose content and SC50 value, but in the opposite direction. The greatest variation of honey characteristics according to botanical origin could be explained by these parameters. On the other hand, PC2, with 16.9% of variation, was defined by moisture and pH.

The angle between the vectors that represents the variables can be understood as the correlation between the variables. Values below or above 90° suggest a positive or negative correlation between the variables, respectively. Colour, phenolic compound, and sucrose content showed a high correlation in a positive direction each, while phenolic compounds showed a negative correlation with the glucose content and SC50 value.

MH were grouped in the negative sector of PC1, indicating higher content of glucose and fructose, fewer antioxidant activity -due to higher SC50 value- and low values of acidity, pH, colour, sucrose, and phenolic compounds, as compared with UH. Honey MF7 was located in the positive sector of PC1 and showed amber dark colour, greater acidity, acidity antioxidant, and phenolic compounds than the other MH. UH line up in the PC2 axis, differentiating by moisture and showing average values of acidity, pH, sugar contents, colour, phenolic compounds, and antioxidant activity. Eucalyptus and clover UH were located in the negative sector of PC2 and had the lowest humidity values, while the lotus UH, located in the positive sector of PC2, had the highest moisture content (Fig. 1).

Significant differences were detected between UH and MH

Table 1
Physicochemical parameters, sugars, phenolic compounds, and antioxidant activity of honeys.

Honey type[a]	Botanical composition	Acidity (meq/kg)	pH	Moisture (%)	Fructose (%)	Glucose (%)	Ratio fructose/glucose	Sucrose (%)	Colour (mm PFUND)	Total Phenolic content (mg GAE/g honey)	Antioxidant Activity SC50 (g/mL)
MF1	*Eucalyptus* sp.$^{(D)}$, *Echium plantagineum*.$^{(S)}$, clovers.$^{(M)}$ (*Trifolium* sp. *Lotus* sp., *Melilotus* sp.)	16.63 ± 2.10	3.95 ± 0.05	16.64 ± 0.65	46.87 ± 1.03	42.94 ± 1.71	1.10 ± 0.06	2.55 ± 0.31	49.25 ± 8.39	0.32 ± 0.02	0.58 ± 0.12
MF2	*Eucalyptus* sp.$^{(S)}$, *Helianthus annuus*.$^{(S)}$, clovers $^{(M)}$ (*Trifolium* sp., *Lotus* sp., *Melilotus* sp.)	12.50 ± 2.50	4.25 ± 0.25	17.71 ± 0.22	46.07 ± 0.22	43.42 ± 4.98	1.08 ± 0.12	2.78 ± 0.94	76.00 ± 21.00	0.38 ± 0.11	0.48 ± 0.20
MF3	*Eucalyptus* sp.$^{(D)}$, *Echium plantagineum*.$^{(S)}$ y *Helianthus annuus* $^{(M)}$	16.14 ± 1.26	3.94 ± 0.04	17.33 ± 0.16	45.05 ± 0.71	41.01 ± 1.09	1.10 ± 0.03	2.16 ± 0.18	48.71 ± 7.43	0.35 ± 0.05	0.56 ± 0.05
MF4	*Eucalyptus* sp.$^{(D)}$, *Cytisus scoparium*.$^{(S)}$, *Echium plantagineum*$^{(M)}$	20.00	4.20	17.77	46.16	37.54	1.23	2.23	53.00	0.36	0.47
MF5	*Eucalyptus* sp.$^{(S)}$, *Lotus* sp. $^{(M)}$, *Melilotus* sp. $^{(M)}$	23.00	4.00	18.04	49.06	38.14	1.29	1.98	55.00	0.44	0.47
MF6	*Eucalyptus* sp.$^{(D)}$, *Trifolium* sp. $^{(S)}$	15.50 ± 1.50	4.00 ± 0.00	17.60 ± 1.32	47.10 ± 0.72	40.23 ± 0.10	1.17 ± 0.02	2.47 ± 0.74	76.50 ± 38.50	0.41 ± 0.12	0.57 ± 0.32
MF7	*Cissus* sp.$^{(S)}$, Fabaceae$^{(S)}$, *Eupatorium* sp. $^{(M)}$	36.00	4.50	17.10	37.38	22.98	1.63	3.45	150.00	1.24	0.24
UE	*Eucalyptus* sp.	16.88 ± 1.01	4.25 ± 0.14	16.15 ± 0.94	41.08 ± 2.98	38.12 ± 1.79	1.08 ± 0.06	2.88 ± 0.40	74.25 ± 20.82	0.34 ± 0.03	0.61 ± 0.19
UC	*Clovers* (*Trifolium repens*, *Melilotus* sp., *Lotus* sp., *Medicago sativa*)	14.00	3.00	16.32	45.36	37.22	1.22	2.66	101.0	0.4	0.36
UL	*Lotus* sp.	20.00	4.00	19.47	42.84	36.52	1.17	2.24	73.00	0.36	0.43

[a] Twenty four honey samples were classified according to botanical origin as multifloral (MF: 1–7) and unifloral (UE: 70% *Eucalyptus* sp. pollen, UC: 20% clovers pollen, UL: 20% *Lotus* sp. pollen). For MF honeys the dominant (D), secondary (S) and minor importance (M) pollen are described. Data were expressed as Mean ± SE. when there was more than one sample for each honey.

(Wilks Λ=0.23; $F_{9,14}$ = 5.36; P = 0.028) for the parameters analyzed. MH had lighter colour, higher mean values of acidity, pH, moisture, fructose, glucose, phenolic compounds, and antioxidant activity than UH (Table 2).

3.4. Antibacterial activity

Power of inhibition varied according to honey concentration, and was significantly greater ($P \leq 0.05$) for 1:2 dilution, as compared with 1:4 dilution (data not showed).

Some honeys (1:4 w/v dilutions) lost their ability to inhibit bacterial growth. Significant differences in antimicrobial capacity of honeys were detected against different bacterial strains ($X^2 = 10.05$, D.F = 4, P = 0.039), while some strains were inhibited by more than one honey samples. For example, *E. coli* strain was inhibited by 70% of the honeys tested −17 samples-, while only 29% −7 samples-inhibited *P. aeruginosa*. Between 58% and 61% of honeys formed inhibition zone for *Salmonella* spp., *S. aureus,* and *B. cereus* strains.

Most 1:2 w/v honey dilutions had antimicrobial capacity for all bacterial strains tested (Table 3). Regardless of botanical origin, the power of inhibition against the different strains of all the samples was not significantly different (F = 1.44; D.F = 4; P = 0.2199). Considering the power of inhibition of individually honeys against each bacterial strain, four honeys -UL, MF3, MF6 and MF7- showed significant differences ($P \leq 0.05$) against different strains (Table 3).

The inhibitory power of honeys varied significantly (F = 35.35; D.F. = 9; $P \leq 0.0001$) depending on their botanical origin (Fig. 2). Three MH had a significantly greater inhibitory power (Fisher LSD test = 4.32; d.f = 423) producing inhibition zones which ranged from 21 mm to 23 mm, while others had low power of inhibition, ranging from 5.9 to 7.9 mm (Fig. 2).

4. Discussion

All honeys tested comply with the standards set by the Codex Alimentarius (2001). Most moisture values were similar to those obtained by Silvano et al. (2014) for honeys with similar geographical origin. In this work, Lotus UH had the highest percentage of moisture which, although it did not exceed the limit established by the Codex Alimentarius (2001), it was higher than the 18% limit allowed by the Argentinean regulations (CAA, 2010). This could be due to region, climate, and environmental conditions at the time of harvest. Similar moisture values for this kind of honeys from the same region have previously been reported (Fangio et al., 2010; Malacalza, Caccavari, Fagúndez, & Lupano, 2005).

The monosaccharide content was within the limits of Argentinean regulations (CAA, 2010), European and Codex standards of 60% minimum for glucose and fructose confirming that all samples were genuine honeys. The fructose/glucose ratio agrees with those one obtained by Silvano et al. (2014). It is an important quality parameter that affects the textural properties of honey, since lower values lead to crystallization, an undesirable process that could be the result of an increase of the liquid phase, making it less appealing to the consumer (Cavia et al., 2002). Most honeys were light amber, which is the characteristic colour of honeys from hills and agricultural zones of the same geographic region (Silvano et al., 2014). The acidity and pH values indicate a good stability and shelf life of honeys, and agree with values previously reported (Baroni et al., 2009; Iurlina & Fritz, 2005).

Most honeys, with the exception of one MH, have similar content of phenolic compounds, which agrees with Meda, Lamien, Romito, Millogo, and Nacoulma (2005) and Pontis, Costa, Silva,

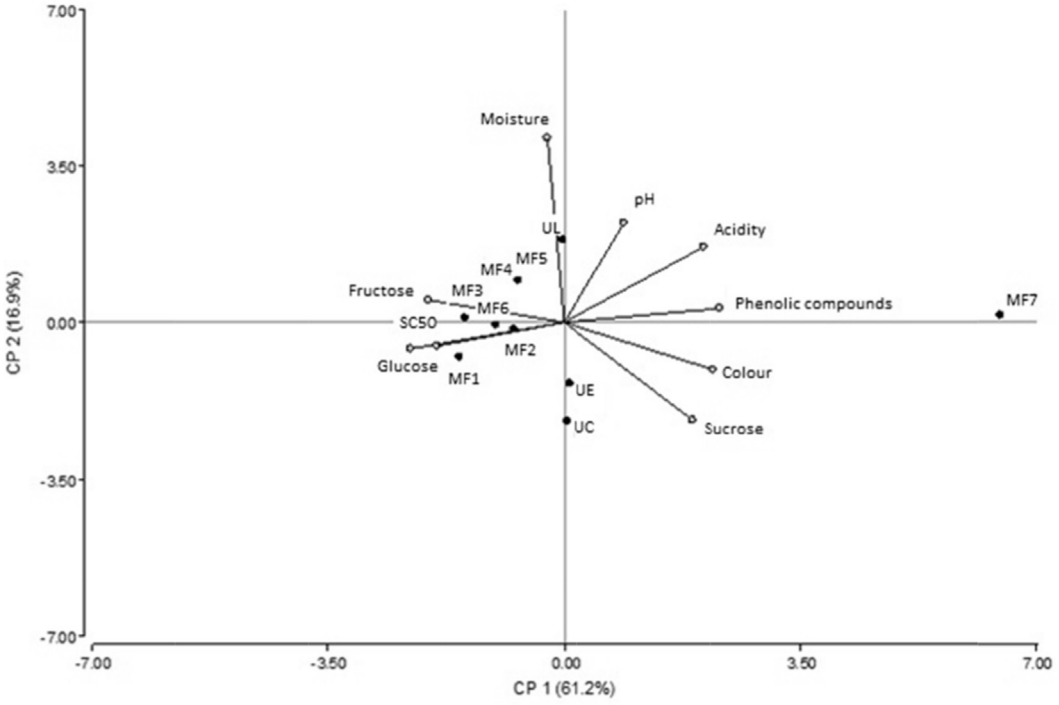

Fig. 1. Biplot of the principal component analysis of honey from different botanical origins. MF (1–7): multifloral honeys, UE: unifloral Eucalyptus honey, UC: unifloral clover honey. UL: unifloral *Lotus* sp. honey.

Table 2
Mean values of physicochemical parameters, sugars, phenolic compounds, and antioxidant activity of honeys classified according to botanical origin.

Botanic origin	Acidity (meq/kg)	pH	Moisture (%)	Fructose (%)	Glucose (%)	Sucrose (%)	Colour (mm Pfund)	Phenolic compounds (mg GAE/g of honey)	SC50 (g/mL)
Unifloral honeys	16.92	4.00	16.73	42.08	37.71	2.74	78.50	0.36	0.54
Multifloral honeys	17.47	4.03	17.30	45.65	40.27	2.41	61.17	0.41	0.53

Table 3
Antimicrobial activity of 1:2 honey dilutions classified according to botanical origins against different bacterial strains.

Bacteria	Types of honey									
	UE	UC	UL	MF1	MF2	MF3	MF4	MF5	MF6	MF7
P. aeruginosa	+++ ^{a}	- ^{a}	- ^{a}	+++ ^{a}	++ ^{a}	+++ ^{c}	+++ ^{a}	- ^{a}	+ ^{ab}	+ ^{c}
Salmonella sp.	++ ^{a}	- ^{a}	- ^{a}	+++ ^{a}	+ ^{a}	+++ ^{b}	+++ ^{a}	- ^{a}	- ^{a}	+ ^{b}
E. coli	++ ^{a}	- ^{a}	- ^{ab}	+++ ^{a}	+ ^{a}	+++ ^{b}	+++ ^{a}	- ^{a}	- ^{a}	+ ^{c}
S. aureus	++ ^{a}	- ^{a}	+ ^{bc}	++ ^{a}	++ ^{a}	+ ^{a}	+ ^{a}	- ^{a}	+ ^{b}	+ ^{a}
B. cereus	+ ^{a}	- ^{a}	++ ^{c}	+++ ^{a}	++ ^{a}	+++ ^{b}	+++ ^{a}	- ^{a}	- ^{a}	+ ^{b}

The antibacterial activity was classified as: no sensitive (−) for diameters lower than 8 mm; sensitive (+) for diameters from 8 to 14 mm; very sensitive (++) for diameters from 15 to 19 mm; extremely sensitive (+++) for diameters higher than 20 mm. Values with the same letter indicate that are not significantly different ($P \geq 0.05$) within each column. MF (1–7): multifloral honeys, UE: unifloral Eucalyptus honey, UC: unifloral clover honey. UL: unifloral Lotus sp. honey. A total of 24 honey samples were analyzed in triplicate.

and Flach (2014). A direct correlation between colour, phenolic compounds, and antioxidant activity of honeys was observed, which agrees with Liu et al. (2013) and Bueno-Costa et al. (2016).

Although a MH from northwestern Argentina differed from the rest of the samples in most of the parameters evaluated, the remaining parameters agree with values reported by Silvano et al. (2014) for honeys of the same geographical region. Their botanical composition provides distinctive features, as high antioxidant activity and a fructose/glucose ratio over 1.5%, keeping the honey in liquid physical state, which is an appealing characteristic for consumers.

The results show that MH differ from UH in their physicochemical parameters, confirming that botanical source influences the composition and physicochemical characteristics of honeys, which agrees with previous studies (Alvarez-Suarez et al., 2010; Fangio et al., 2010). Honeys had antimicrobial activity against the bacterial strains tested. The lowest power of inhibition observed in 1:4 honey dilution shows that the lowest concentration of

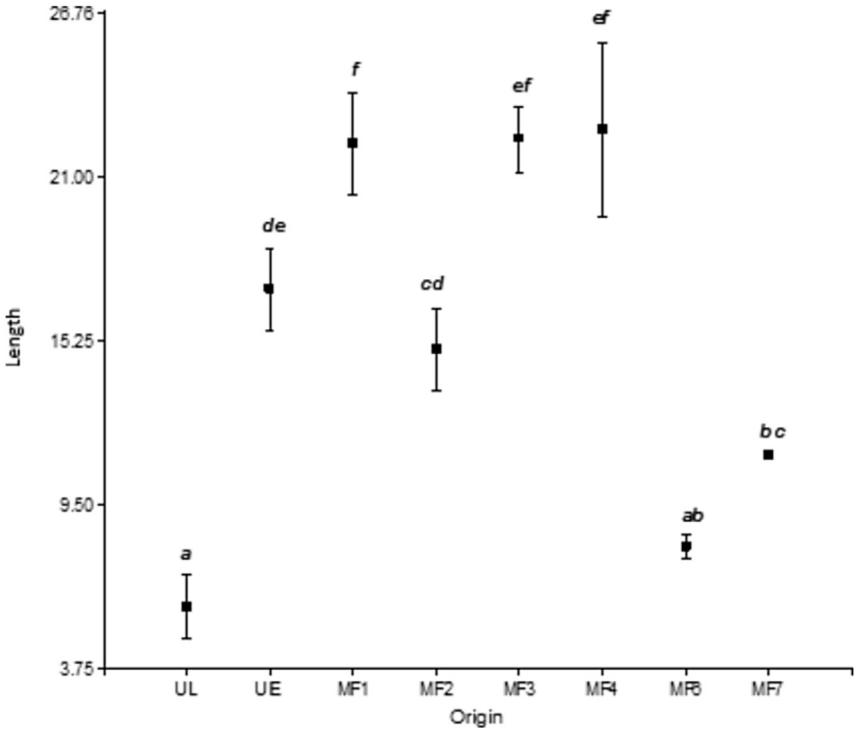

Fig. 2. Mean values (mean ± SE) of bacterial inhibition zones (mm) of honeys classified according to botanical origins. MF (1–7): multifloral honeys, UE: unifloral Eucalyptus honey, UC: unifloral clover honey. UL: unifloral *Lotus* sp. honey. Means with the same letter indicates no significantly differences ($P \geq 0.05$).

antimicrobial components decreased the inhibition, which agrees with previous studies (Adeleke, Olaitan, & Okpekpe, 2006;; Fangio et al., 2010;; Mandal, Debmandal, Pal, & Saha, 2010). Few honeys (1:4 dilution) had antimicrobial activity against P. *aeruginosa*, indicating that this bacteria is less sensitive to low concentration honey. However, Liu et al. (2013), who evaluated the antimicrobial activity of diluted honey, found that P. *aeruginosa* was not inhibited by the honeys studied.

All the honeys used in this study had antimicrobial activity because of the presence of hydrogen peroxide, since the removal of hydrogen peroxide by catalase eliminated the bacteriostatic activities of the honeys. Similar results were obtained by Baltrušaitytė et al. (2007) and Alnaimat, Wainwright, and Al (2012). Our work shows that the microorganisms tested were scarcely or not inhibited by UH or MH when they had dominant or secondary species from the Fabaceae family in their botanical composition. MH featuring similar botanical composition and content of phenolic compounds had greater antimicrobial activity. This could suggest a relation between honey botanical source, phenolic compounds, and peroxide content. Isla et al. (2011) reported that the antimicrobial activity of honey from Northwestern Argentina resulted from the presence of hydrogen peroxide and phenolic compounds. In the present study, MH with the greatest inhibition power against all bacterial strains showed a botanical association of eucalyptus and blueweed, eucalyptus being the dominant pollen. Eucalyptus UH also had good antimicrobial activity against bacterial strains and similar content of phenolic compounds compared with the MH mentioned that had the greatest antimicrobial activity. This relation between antimicrobial activity, botanical source, and phenolic compounds might be associated to the composition of eucalyptus and blueweed nectars, whose components could also affect the content of hydrogen peroxide. Honey non-peroxide factors include phenolic acids, which might play a role in antibacterial activities (Wahdan, 1998). However, its contribution to antibacterial activity may be smaller than that of hydrogen peroxide (Weston, 2000). Although the content of peroxide in honeys used in this study was not quantified, honeys containing highest concentrations of peroxide are likely to have a high inhibitory effect or else, other natural substances present in the honeys may optimize the action of hydrogen peroxide. Furthermore phenolic compounds of honey are necessary intermediates giving oxidative action of hydrogen peroxide; phenolic/hydrogen peroxide induces oxidative stress, which contributes to both antioxidant and bacteriostatic honey activity (Brudzynski, Abubaker, & Miotto, 2012). The botanical mixture of eucalyptus and blueweed in MH of our study may lend a characteristic distribution pattern of phenolic compounds that enhances the hydrogen peroxide antimicrobial activity.

5. Conclusions

Specific honey samples such as eucalyptus and blueweed, were shown capable of inhibiting the growth of all bacterial strains, both spoilage microorganisms and foodborne pathogens. Thus, they could be used as food preservatives under appropriate conditions. Studies are needed to further knowledge about the relationship between hydrogen peroxide content and nectar composition, which could affect honey antimicrobial activity. In this way, the efficacy of honey as an inhibitor of microbial growth in food systems could be identified.

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