Journal of Apicultural Research

ISSN: 0021-8839 (Print) 2078-6913 (Online) Journal homepage: http://www.tandfonline.com/loi/tjar20

Physicochemical parameters, chemical composition, antioxidant capacity, microbial contamination and antimicrobial activity of Eucalyptus honey from the Andean region of Ecuador

Luis A Valdés-Silverio, Gabriel Iturralde, Marilyn García-Tenesaca, Jonathan Paredes-Moreta, David A Narváez-Narváez, Maira Rojas-Carrillo, Eduardo Tejera, Pablo Beltrán-Ayala, Francesca Giampieri & José M Alvarez-Suarez

To cite this article: Luis A Valdés-Silverio, Gabriel Iturralde, Marilyn García-Tenesaca, Jonathan Paredes-Moreta, David A Narváez-Narváez, Maira Rojas-Carrillo, Eduardo Tejera, Pablo Beltrán-Ayala, Francesca Giampieri & José M Alvarez-Suarez (2018): Physicochemical parameters, chemical composition, antioxidant capacity, microbial contamination and antimicrobial activity of Eucalyptus honey from the Andean region of Ecuador, Journal of Apicultural Research

To link to this article: https://doi.org/10.1080/00218839.2018.1426349

Published online: 22 Feb 2018.

Journal of Apicultural Research, 2018
https://doi.org/10.1080/00218839.2018.1426349

ORIGINAL RESEARCH ARTICLE

Physicochemical parameters, chemical composition, antioxidant capacity, microbial contamination and antimicrobial activity of *Eucalyptus* honey from the Andean region of Ecuador

Luis A Valdés-Silverio[a], Gabriel Iturralde[b,c], Marilyn García-Tenesaca[d], Jonathan Paredes-Moreta[a], David A Narváez-Narváez[e], Maira Rojas-Carrillo[e], Eduardo Tejera[e], Pablo Beltrán-Ayala[f], Francesca Giampieri[g] and José M Alvarez-Suarez[h]*

[a]*Ingeniería en Biotecnología de los Recursos Naturales, Universidad Politécnica Salesiana, Quito, Ecuador;* [b]*Jardín Botánico de Quito, Quito, Ecuador;* [c]*Carrera de Ingeniería Agroindustrial y Alimentos, Facultad de Ingeniería y Ciencias Agropecuarias, Universidad de Las Américas, Quito, Ecuador;* [d]*Facultad de Ingeniería y Ciencias Agropecuarias, Universidad de Las Américas, Quito, Ecuador;* [e]*Facultad de Ciencias de la Salud, Universidad de Las Américas, Quito, Ecuador;* [f]*Colegio de Administración y Economía, Universidad San Francisco de Quito, Cumbayá, Ecuador;* [g]*Dipartimento di Scienze Cliniche Specialistiche ed Odontostomatologiche (DISCO)-Sez, Biochimica, Facolta di Medicina, Universitá Politecnica delle Marche, Ancona, Italy;* [h]*Escuela de Medicina Veterinaria y Zootecnia, Grupo de Investigación en Biotecnología Aplicada a Biomedicina (BIOMED), Universidad de Las Américas, Quito, Ecuador*

(*Received 3 July 2017; accepted 6 December 2017*)

This study aims to evaluate the pollen profile, physicochemical parameters, chemical composition, antioxidant capacity, microbial contamination and antimicrobial activity of *Eucalyptus* honey from the Andean region of Ecuador. Aerobic mesophiles, molds and yeasts, fecal coliforms and sulfite-reducing clostridia contamination were also assessed. All honey samples met the quality standards required by international legislation, except for hydroxymethylfurfural, for which three samples did not satisfy international standards. Apparent phenolic and flavonoid content, as well as free amino acid and protein content fell within the ranges previously reported for other types of honey of distinct floral and geographical origins. No microbial contamination was observed, while total antioxidant capacity showed evidence of the *Eucalyptus* honey's ability to reduce Fe^{3+} to Fe^{2+} as well as to verify the superoxide radical scavenging activity and metal ion chelating capacity. *Eucalyptus* honey was able to inhibit the growth of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli*, but was not effective against *Candida albicans*.

Parámetros fisicoquímicos, composición química, capacidad antioxidante, contaminación microbiana y actividad antimicrobiana de la miel de *Eucalyptus* de los Andes ecuatorianos

El objetivo de este estudio es evaluar el perfil polínico, los parámetros fisicoquímicos, la composición química, la capacidad antioxidante, la contaminación microbiana y la actividad antimicrobiana de la miel de *Eucalyptus* de los Andes ecuatorianos. También se estimó la contaminación por mesófilos aeróbicos, mohos y levaduras, coliformes fecales y clostridias reductoras de sulfitos. Todas las muestras de miel superaron los criterios de calidad requeridos por la legislación internacional, excepto para el hidroximetilfurfural, según el cual tres muestras no satisficieron los criterios internacionales. El contenido fenólico y flavonoide aparente, así como el contenido de aminoácidos libres y proteínas se encontraron dentro de los rangos previamente descritos en otros tipos de miel de diferentes orígenes florales y geográficos. No se observó contaminación microbiana, mientras que se demostró la capacidad antioxidante de la miel de *Eucalyptus* para reducir Fe^{3+} a Fe^{2+}, así como también se verificó la actividad de absorción del radical superóxido y la capacidad de agente quelante para iones metálicos. La miel de *Eucalyptus* fue capaz de inhibir el crecimiento de *Staphylococcus aureus, Streptococcus pyogenes*, *Pseudomonas aeruginosa* y *Escherichia coli*, pero no fue efectivo contra *Candida albicans*.

Keywords: Ecuadorian honey; *Eucalyptus* honey; physicochemical parameters; microbial contamination; antioxidant capacity

Introduction

Honey is defined as the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants, from secretions of living parts of plants or from excretions of plant-sucking insects on the living parts of plants. These secretions are collected by bees and transformed by combining them with specific substances from their own deposits and leaving them in honey combs to ripen and mature (EU Council, 2002). The composition and quality of the honey depends on several factors, mainly its floral origin and geographical conditions (Acquarone, Buera, & Elizalde, 2007; De-Melo, de Almeida-Muradian, Sancho, & Pascual-Maté, 2018). Sugars are the main compounds present in honey, with fructose (38%) and glucose (31%) being the two most abundant components (Bogdanov, 2017; Bogdanov, Jurendic, Sieber, & Gallmann, 2008).

*Corresponding author. Email: jose.alvarez@udla.edu.ec

However, there are several minor compounds that have also been associated with the quality and beneficial effects of honey, such as polyphenols, enzymes, free amino acids, proteins, minerals and vitamins (Bogdanov, 2017). Current literature has comprehensively shown that honey is an important natural source of bioactive compounds with significant antioxidant properties, which may have applications for human health and nutrition (Bogdanov, 2017; Bogdanov et al., 2008).

Throughout the world, *Eucalyptus* honey is a significant commercial monofloral honey and is highly desired by consumers, who are primarily attracted to the aroma and medicinal properties of the plant (Barbosa, Filomeno, & Teixeira, 2016). The aroma compounds of *Eucalyptus* honey (Alissandrakis, Tarantilis, Pappas, Harizanis, & Polissiou, 2011), it's physicochemical parameters (Moussa, Noureddine, Saad, & Abdelmalek, 2012; Nanda, Kaur, Bera, Singh, & Bakhshi, 2006; Ortega-Bonilla, Chito-Trujillo, & Suárez-Ramos, 2016) and mineral content (González-Miret, Terrab, Hernanz, Fernández-Recamales, & Heredia, 2005) have been studied previously, as well as a variety of bioactive components with potential beneficial effects (Ciappini & Stoppani, 2014; Perna, Intaglietta, Simonetti, & Gambacorta, 2013; Yao, Jiang, Singanusong, Datta, & Raymont, 2004). Notwithstanding these studies, several regions with melliferous potential exist for this unifloral honey where its characteristics and properties remain unknown.

Ecuador is one of the most biodiverse countries in the world, possessing a floral wealth that can be used in the production of honey (Cabrera, 2007). According to the National Beekeeping Health Program run by the Ecuadorian Agency for Agricultural Quality Assurance (AGROCALIDAD), beekeeping activities in Ecuador are primarily concentrated in the central and northern highlands where there is a floral abundance, which allows bees to use different blooms throughout the year and so produce varieties of honey from both specific and non-specific blooms. Despite enjoying an autochthonous flora, the extensive eucalyptus plantations in the country constitute an important melliferous source for honey production; it is therefore not uncommon to find that *Eucalyptus* honey is one of the main monofloral varieties of honey produced in the country (Cabrera, 2007). The main species of *Eucalyptus sp* in Ecuador (*E. globulus*) was initially introduced in 1865 and was extensively planted on the central plateau between the altitudes of 1.800 and 3.300 m, with the best growth occurring in areas between 2.000 and 2.900 m above sea level. To date, no studies have been published on the composition and beneficial effects of different types of Ecuadorian honey; as such, the aim of this study was to confirm the floral origin, as well as determine the physicochemical parameters, antioxidant capacity, microbial contamination and antimicrobial activity of several samples of monofloral *Eucalyptus* honey from Ecuador.

Materials and methods

Honey samples

Samples of honey were collected directly from beekeepers in the province of Pichincha, located in the inter-Andean region of Ecuador. The Pichincha cantons, from where the samples were taken, and their respective districts: Quito (Tumbaco, Chillogallo, Amaguaña and Pintag), Cayambe (Cangahua) and Mejía (Machachi) are shown in Figure 1, while the distribution of beekeepers by districts (parishes) sampled is presented in Table 1. A total of 10 beekeepers were chosen, on the basis of number of apiaries kept (≥20), which made up approximately 50% of total beehives in the province. For each beekeeper, all apiaries were sampled, by taking three samples at random from each apiary. The samples were kept at room temperature and the different analyses were performed within a time period not exceeding three months from the date of collection. Beekeepers were identified as Beekp1 to Beekp10 and results were expressed as the mean values obtained from the analyses of the three samples from each beekeeper.

A solution of artificial honey (1.5 g of sucrose, 7.5 g of maltose, 40.5 g of fructose and 33.5 g glucose in 17 ml of de-ionized water) was included to eliminate any interference that might occur from these compounds in the tests performed (Cooper, Molan, & Harding, 2002).

Pollen content and physicochemical analysis

The botanical origin was confirmed by the traditional qualitative microscopic analysis and frequency determination of the classes of pollen grains in the honey samples following a previously reported protocol (Louveaux, Maurizio, & Vorwohl, 1978; Von Der Ohe, Oddo Livia, Piana, Morlot, & Martin, 2004). The pollen grains' morphology was compared to that in a published pollen catalog (Montoya-Pfeiffer, León-Bonilla, & Nates-Parra, 2014).

The honey samples were tested using the usual physicochemical tests, such as ashes (%), color (mm Pfund), Diastase activity (Schade units), free acidity (meq/kg), pH, electrical conductivity (mS/cm), moisture (%), density (g/ml) and the hydroxymethylfurfural (HMF) test (mg/kg) (AOAC, 1990; EU Council, 2002).

Determination of apparent total phenolic and total flavonoid content

Apparent total phenolic content (ATPC) was determined using the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1998). Aliquots (0.5 ml) of honey solution (1 g/10 ml) were mixed with 2.5 ml of Folin–Ciocalteu reagent (0.2 N) for 5 min and then 2 ml of sodium carbonate solution (0.7 M) was added. The solution was allowed to stand in the dark at room temperature for 2 h and the absorbance was measured

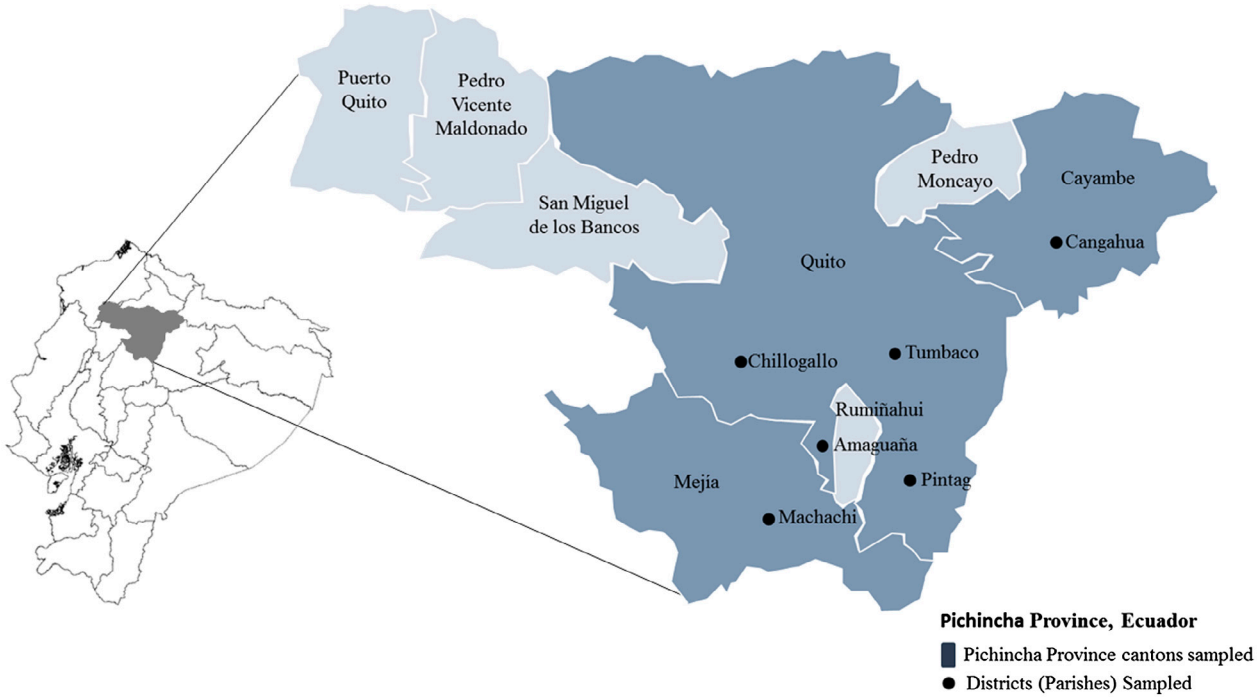

Figure 1. Pichincha cantons sampled and their respective districts.

Table 1. Distribution of beekeepers by districts (parishes) of Pichincha cantons sampled.

Pichincha province	Pichincha cantons sampled	Districts (parishes) sampled	Beekeepers
	Quito	Chilogallo	1
		Tumbaco	2, 3, 4
		Amaguaña	5
		Pintag	6, 7
	Mejia	Machachi	8
	Cayambe	Cangahua	9, 10

spectrophotometrically at 760 nm against the artificial honey blank. Gallic acid was used as a standard to produce the calibration curve (0.5–3.0 mM, R^2 = 0.9954) and TPC was expressed as mg of gallic acid equivalents (GAE) per kg of honey (mg GAE/kg of honey).

Apparent total flavonoid content (ATFC) was determined by previously established methods (Chang, Yang, Wen, & Chern, 2002). Honey samples were diluted in methanol (50% w/v) and 0.25 ml of each was mixed with distilled water (1.25 ml) and a 5% $NaNO_2$ solution (75 µl). The solution was incubated for 6 min, then 150 µl of a 10% $AlCl_3{\cdot}6H_2O$ solution was added and incubated for a further 5 min before 0.5 ml of 1 M NaOH was added. Distilled water was added to give a final volume of 2.5 ml and the absorbance was determined spectrophotometrically at 510 nm against the artificial honey blank. (+)-Catechin was used as a standard to produce the calibration curve (0.037–0.25 mM, R^2 = 0.9916) and the TFC was expressed as (+)-catechin equivalents (CE) per kg of honey (mg of CE/kg of honey).

Determination of protein and free amino acids content

The protein content was determined as previously reported (Azeredo, Azeredo, de Souza, & Dutra, 2003). Honey samples were diluted in distilled water (50% w/v) and 0.1 ml of this solution was added to 5 ml of Coomassie Brilliant Blue reagent. This solution was allowed to stand for 2 min at room temperature, after which its absorbance was measured spectrophotometrically at 595 nm. Bovine serum albumin was used for the calibration curve (10–100 µg/0.1 ml, R^2 = 0.9980) in 0.15 M of sodium chloride and the protein content was expressed as mg of bovine serum albumin (BSA) per 100 grams of honey (BSA/100 g of honey).

Free amino acid content was determined using the Cd-ninhydrin method (Doi, Shibata, & Matoba, 1981). Honey samples (1.25 g) were diluted in 25 ml of distilled water and 1 ml of this solution was added to 2 ml of the Cd-ninhydrin reagent. The reaction mixture was incubated in a water bath at 84 °C for 5 min and then cooled in ice, after which the absorbance was deter-

mined spectrophotometrically at 507 nm. L-Leucine was used for the calibration curve (2.4–42 mg/l, R^2 = 0.9935) and the free amino acid content was expressed as mg of L-leucine equivalents (LE) per 100 grams of honey (mg LE/100 g of honey).

Determination of total carotenoid content

Total carotenoid content (TCC) was determined following a previously published method (Ferreira, Aires, Barreira, & Estevinho, 2009). Briefly, 1 gram of honey was shaken (500 $r{\cdot}m^{-1}$) in 10 ml of extraction solution (n-hexane-acetone, 6:4) at room temperature for 10 min and then filtered (Whatman No. 4 filter paper). The absorbance was determined spectrophotometrically at 450 nm. β-carotene was used for the calibration curve (0.25–10 μg/ml, R^2 = 0.9982) and results were expressed as mg of β-carotene equivalent (βcarotE) per kg of honey (mg βcarotE/kg of honey).

Ferric reducing antioxidant power (FRAP) assay, metal ions chelating capacity and the superoxide radical ($O_2^{\bullet-}$) scavenging activity

The FRAP assay was conducted as previously reported (Benzie & Strain, 1996). Honey samples were diluted in distilled water (1:10 w/v), then aliquots of 200 μl were added to 1.8 ml of FRAP reagent, allowed to stand for 5 min at room temperature and the absorbance was determined spectrophotometrically at 593 nm. Trolox was used as a standard (0.03–2.5 mM, R^2 = 0.9902) and TAC was expressed as μmoles of Trolox equivalents (TE) per 100 g of honey (μmol TE/100 g of honey).

The metal ions chelating capacity was determined as previously reported (Wang, Lien, & Yu, 2004). Honey samples were incubated with 0.05 mL of $FeCl_2{\cdot}4H_2O$ (2 mM). The reaction was initiated by adding 5 mM of ferrozine (0.2 ml) and after 10 min of incubation at room temperature; the absorbance was determined spectrophotometrically at 562 nm. Chelating ability results were expressed as percentages and were calculated using the following equation:

$$\mathrm{ChA} = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

in which A_0 was the absorbance of the control and A_1 the absorbance of each honey sample.

The $O_2^{\cdot-}$ scavenging activity of honey was investigated using a modified nitrite method based on the ability of the $O_2^{\cdot-}$ generated by hypoxanhine/xanthine oxidase (HX/XO) to oxidize hydroxylamine to nitrite ion at pH 8.2 (Oyanagui, 1984). The nitrite ion generated was measured spectrophotometrically at 550 nm and results were expressed as the percentages of inhibition (PI) of nitrite ion generation in the presence of honey, calculated by the change in the slope of increasing spectrophotometric absorption (A_m) of the samples compared to the blank (A_b, with distilled H_2O) using the following equation:

$$\mathrm{PI} = [(A_b - A_m)/A_b] \times 100 \quad (2)$$

The results were expressed as the concentration of honey solution required to inhibit the rate of nitrite ion generation by 50% (IC_{50}) (mg/ml).

Microbial contamination

Microbial contamination was determined following a previously reported method (Gomes, Dias, Moreira, Rodrigues, & Estevinho, 2010). Honey samples (10 g) were diluted in 90 ml of peptone water. A general tryptic soy agar media (TSA) was incubated at 37 °C for 48 h and used for the primary screening of bacterial contamination in honey. The presence of aerobic mesophilic bacteria was determined using a standard plate count agar (PCA) incubated at 37 °C for 48 h, while yeasts and molds were studied by using sabouraud dextrose agar (SDA), supplemented with chloramphenicol and potato dextrose agar (PDA) media, both incubated at 25 °C for 96 h. For detecting and enumerating *Enterobacteriaceae,* the violet red bile glucose (VRBG) agar was used as previously described (ISO 21528-2:2006, 2004). For determining sulphite-reducing clostridia, SPS (sulfite-polymixin-sulfadiazine) agar media were used (ISO 15213:2003, 2003). Aliquots of the initial honey suspension (10, 5, 1 and 0.1 ml) were thermally treated at 80 °C for 5 min in a test tube, covered with the SPS agar media and then incubated at 37 °C for 5 days.

Antimicrobial activity

The antimicrobial activity of the honey samples was studied using four ATTC bacterial strains (*Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922) and one fungal strain (*Candida albicans* ATTC 90028).

The minimum active dilution (MAD) of the honey samples was determined by an agar incorporation technique (French, Cooper, & Molan, 2005) in nutrient agar for bacterial strains and Yeast Mold (YM) agar for fungal strains. A dilution series using final honey samples or artificial honey dilutions in the range of 1–20% (v/v) in 1% increments was used for susceptibility assays. The inoculated plates were incubated at 37 °C overnight and the complete growth inhibition was recorded at each inoculation position. The MAD was taken to be the lowest honey dilution at which microbial growth was completely inhibited.

Statistical analyses

The samples were analyzed in triplicate and results were expressed as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics for Windows version 20.0. A multivariate analysis (MANOVA) was done for the physicho-chemical properties

Table 2. Predominance of pollen class in *Eucalyptus* honey from Ecuador.

Samples	Frequency class[a]	Pollen identification (% of frequency and botanical genus)
Beekp1	P	*Eucalyptus sp.* (79.12)
	S	*Brassica napus.* (13.65)
	M	*Poulsenia type* (7.23)
Beekp2	P	*Eucalyptus sp.* (73.24)
	S	*Mimosa c.f. albida* (21.63)
	M	*Persea americana* (5.13)
Beekp3	P	*Eucalyptus sp.* (74.02)
	S	*Mimosa c.f. albida* (11.18
	M	*Trifolium type* (8.16), *Persea americana* (6.64)
Beekp4	P	*Eucalyptus sp.* (71.07)
	S	*Mimosa c.f. albida* (17.13)
	M	*Persea americana* (6.59), *Trifolium type* (5.21)
Beekp5	P	*Eucalyptus sp.* (80.31)
	S	*Mimosa c.f. albida* (12.09)
	M	*Unidentified pollen* (7.6)
Beekp6	P	*Eucalyptus sp.* (72.26)
	S	*Brassica napus* (18.71)
	M	*Weinmannia type* (9.03)
Beekp7	P	*Eucalyptus sp.* (86.32)
	S	*Brassica napus* (7.48)
	M	*Unidentified pollen* (6.20)
Beekp8	P	*Eucalyptus sp.* (80.46)
	S	Asteraceae type (12.02)
	M	*Poulsenia type* (7.52)
Beekp9	P	*Eucalyptus sp.* (71.35)
	S	*Mimosa c.f. albida* (16.16), *Trifolium* type (12.52),
Beekp10	P	*Eucalyptus sp.* (81.52)
	S	*Mimosa c.f. albida* (10.64),
	M	*Poulsenia type* (7.84)

Notes: P = Predominant pollen (more than 45% predominance of a pollen grains specific for a botanical type); S = Secondary pollen (10–45%); M = Minor pollen, Unidentified pollen (5–10%).
[a]Predominance of pollen class.

and the chemical composition. The bonferroni correction was used in both cases for *post hoc* analysis adjusting for multiples groups' comparison. The correlations between the variables were calculated using Pearson's coefficient. In all cases a p-value < 0.05 was considered as statistically significant.

Results and discussion

Pollinic analysis and physicochemical analyses

Pollen analysis was used to confirm the floral origin of the different honey samples. Table 2 shows the most predominant pollen grain classes identified in the samples from each beekeeper. All samples showed a high content of pollen grains specific for *Eucalyptus ssp*. (between 70–90%), which is in accordance with previous reports for this monofloral honey (Ciappini & Vitelleschi, 2013; Gomez, Hernandez, Gomez, & Marin, 1993; Rodríguez Flores, Escuredo Pérez, & Seijo Coello, 2014; Terrab, Díez, & Heredia, 2003). Moreover, secondary pollen grain classes were also identified in the honey samples showing, in some cases, common plant species between the honeys from different beekeepers from the same region. The species *Mimosa c.f. albida* was the secondary vegetal species identified in honey from the district of Tumbaco, Quito canton, while in the district of Pitag, the secondary vegetal specie was *Brassica napus*. It was also found that a group of plant species, including Persea *Americana*, had a low prevalence, as did pollen grains from the genera *Poulsenia, Trifolium*, and *Weinmannia*. These species represented only between 5 and 10% of the total pollen grains identified in the honey samples.

Table 3 shows the physicochemical analysis of the different samples of *Eucalyptus* honey. The moisture content (%) ranged from 11.74 to 19.42%, all of which were within internationally recommended parameters (≤20%) for quality of commercial honey (EU Council, 2002). Since the moisture content in honey is closely linked to environmental and geographical conditions, apiary management and honey storage (Acquarone et al., 2007), the results suggest appropriate handling and storage of the honey. High moisture content may cause fermentation during storage and affect the organoleptic characteristics and biological properties of honey, thus affecting its overall quality (Chirife, Zamora, & Motto, 2006).

The acceptable range of pH values for honey has yet to be legislated, so it is important to compare the present results with those published previously. All samples were found to be acidic, exhibiting pH values ranging between pH 3.1 and pH 4.2 (Table 3), which corresponds to those previously reported in *Eucalyptus* honey from other geographical origins (Gomez et al., 1993; Moussa et al., 2012; Nanda et al., 2006; Ortega-Bonilla

Table 3. Average values of data obtained in physicochemical analysis of *Eucaliptus* honey from Ecuador.

Samples	Color		Moisture (%)	pH	Free acidity (meq/kg)	HMF (mg/kg)	Diastase Index (U Schade)	Electrical conductivity (mS/cm)	Density (g/ml)	Ashes (%)
	mm Pfund	Classification								
Beekp1	46.61 ± 1.51^{a}	Extra Light Amber	19.42 ± 1.81^{a}	3.61 ± 0.04^{a}	42.22 ± 5.84^{a}	9.71 ± 1.52^{a}	19.26 ± 3.30^{a}	0.38 ± 0.02^{a}	1.41 ± 0.01^{a}	0.13 ± 0.01^{a}
Beekp2	48.36 ± 2.33^{a}	Extra Light Amber	12.71 ± 1.52^{b}	4.03 ± 0.02^{a}	37.47 ± 2.63^{a}	4.21 ± 0.02^{a}	21.64 ± 4.52^{a}	0.51 ± 0.01^{b,d}	1.40 ± 0.02^{a}	0.36 ± 0.02^{b,c}
Beekp3	45.62 ± 3.40^{a}	Extra Light Amber	12.93 ± 1.66^{b}	4.12 ± 0.04^{a}	40.71 ± 3.42^{a}	3.46 ± 0.03^{a}	34.57 ± 5.64^{b}	0.52 ± 0.03^{b}	1.40 ± 0.01^{a}	0.38 ± 0.01^{c,e,f}
Beekp4	46.48 ± 2.82^{a}	Extra Light Amber	14.44 ± 1.47^{b}	3.96 ± 0.01^{a}	58.90 ± 4.66^{b}	25.21 ± 2.80^{b}	21.92 ± 4.81^{a}	0.53 ± 0.00^{b}	1.41 ± 0.04^{a}	0.46 ± 0.01^{d,e,f}
Beekp5	45.21 ± 3.46^{a}	Extra Light Amber	13.16 ± 2.30^{b}	4.15 ± 0.02^{a}	41.76 ± 3.73^{a}	8.73 ± 1.21^{a}	36.58 ± 5.46^{b}	0.52 ± 0.02^{b}	1.41 ± 0.03^{a}	0.48 ± 0.06^{d,f}
Beekp6	43.30 ± 2.89^{a}	Extra Light Amber	14.47 ± 1.86^{b}	3.92 ± 0.04^{a}	28.27 ± 2.86^{c}	117.92 ± 12.64^{c}	8.64 ± 1.24^{c}	0.24 ± 0.04^{c}	1.43 ± 0.01^{a}	0.45 ± 0.03^{d,e,f}
Beekp7	41.96 ± 3.21^{a}	Extra Light Amber	11.82 ± 1.47^{b}	4.20 ± 0.02^{a}	229.63 ± 3.61^{c,d}	83.32 ± 4.61^{d}	10.23 ± 1.87^{c}	0.49 ± 0.01^{d,f}	1.46 ± 0.03^{b}	0.42 ± 0.01^{c,e,f}
Beekp8	46.11 ± 2.70^{a}	Extra Light Amber	12.56 ± 2.13^{b}	3.93 ± 0.03^{a}	27.74 ± 3.51^{c}	172.53 ± 15.85^{e}	9.87 ± 2.83^{c}	0.78 ± 0.03^{e}	1.40 ± 0.01^{a}	0.43 ± 0.01^{c,d,e,f}
Beekp9	44.53 ± 3.53^{a}	Extra Light Amber	12.79 ± 1.82^{b}	3.97 ± 0.01^{a}	42.51 ± 2.56^{a}	40.70 ± 5.63^{f}	22.82 ± 3.83^{a}	0.52 ± 0.01^{b}	1.41 ± 0.02^{a}	0.36 ± 0.01^{b,c}
Beekp10	43.57 ± 1.81^{a}	Extra Light Amber	11.74 ± 1.79^{b}	4.11 ± 0.01^{a}	36.88 ± 3.14^{a,d}	11.74 ± 1.56^{a}	36.49 ± 3.51^{b}	0.51 ± 0.00^{b,d,f}	1.40 ± 0.05^{a}	0.37 ± 0.02^{b,c,e}

Notes: Data are means ± standard deviation. Mean values within a column sharing the same letter are not significantly different. Sample was analyzed in triplicate.

et al., 2016; Rodríguez Flores et al., 2014). The acidity of honey affects the growth and proliferation of several microorganisms, which can affect organoleptic characteristics, such as texture, flavor, odor, stability and shelf-life (Terrab, Díez, & Heredia, 2002). In most of the samples tested, the values for free acidity were within the established limits (50 meq/kg) (Codex Alimentarius, 2001; EU Council, 2002) and similar to other *Eucalyptus* honey from other geographical origins (Moussa et al., 2012; Nanda et al., 2006; Ortega-Bonilla et al., 2016). Only one sample yielded values higher than those established (Table 3). Moreover, it is important to note that, in general, honey is naturally acidic regardless of its geographical origin (Acquarone et al., 2007). However, high acidity values are indicative of fermentative processes of different sugars that convert to organic acids and can be used as an indicator of the freshness of honey (Subramanian, Umesh Hebbar, & Rastogi, 2007). All the samples showed electrical conductivity values within the established range, i.e., between 0.24 and 0.78 mS/cm, and not exceeding 0.8 mS/cm. Furthermore, the total ash content, which did not exceed 0.6%, suggests that all the honey samples were from floral nectar (Codex Alimentarius, 2001; EU Council, 2002).

Results of the color analysis on the samples of honey are also presented in Table 3. All samples were classified as having an extra light amber shade. The color of honey can be influenced by several factors, such as mineral content, TPC and TCC; light-colored honeys have been associated with low concentrations of these compounds, while dark-colored honey usually presents a higher content (Al et al., 2009; Gomes et al., 2010).

HMF could be found in low quantities or was completely absent in fresh honey but increased during processing and/or aging, influenced by several factors, such as temperature, pH, storage conditions and floral source (Fallico, Arena, Verzera, & Zappalà, 2006). Honey samples from three beekeepers (Beekp 6, 7 and 8) had HMF values outside the allowable range for honey with declared origin from tropical climates (lower than 80 mg/kg), in accordance with international standards for quality (EU Council, 2002), suggesting that these samples might have been exposed to temperature abuse during processing and/or were subject to poor storage practices (EU Council, 2002). All other honey samples were within the international limits (≥80 mg/kg) (EU Council, 2002) and are in agreement with the values previously reported in *Eucalyptus* honeys from other geographical regions (Moussa et al., 2012; Nanda et al., 2006; Terrab et al., 2003). The content of diastase depends on the honey's freshness as well as its geographic and floral origin. The diastase index can be used as a marker of aging and temperature abuse in honey, but should be interpreted with care, as the diastase content of fresh unheated honey can vary greatly depending on its origin and processing conditions (Fallico et al., 2006). According to the results presented here, all samples were within the established limits, in line with international regulations for quality, which state diastase should not be less than 8 Schade units (EU Council, 2002) and with the diastase values previously reported for this type of honey in other geographical regions (Nanda et al., 2006; Ortega-Bonilla et al., 2016; Terrab et al., 2003).

Phytochemical and nutrient content

Polyphenols are a group of phytochemicals that have been to be closely related to the appearance and functional properties of honey (Bogdanov, 2017; Bogdanov et al., 2008) and have been also proposed as potential floral markers for eucalyptus honey (Martos, Ferreres, & Tomás-Barberán, 2000; Perna et al., 2013; Yao et al., 2004). The phytochemical content of the different honey samples was determined and is shown in Table 4, while the correlation matrix for quantitative determination is shown in Table 5**.** The ATPC and ATFC content demonstrate significant differences among the various samples; however, the average values were within those previously reported in monofloral *Eucalyptus* honey from other geographical zones (Ciappini & Stoppani, 2014; Rodríguez Flores et al., 2014; Serrano, Villarejo, Espejo, & Jodral, 2004). In relation to ATFC results, several authors have reported that sugars in honey can interfere with the ATFC assay (Denni & Mammen, 2012; Sancho et al., 2016), so this interference must be corrected in this assay. According to Sancho et al. (2016), total flavonoid content in neutral media must be determined on extracts instead of on honeys. Our assay can overestimate the actual flavonoid content. Therefore, our results should not be considered completely accurate in confirming the actual flavonoid content in the samples analyzed; nevertheless we think that they can serve to give an approximation of the actual content.

TCC was also analyzed and the results are shown in Table 4. The levels of TCC in monofloral *Eucalyptus* honey from Ecuador range between 0.28 and 0.43 mg βCarotE/100 g of honey, which corresponds to those previously reported by other authors in honey from different floral and geographical origins (Alvarez-Suarez et al., 2010; Ferreira et al., 2009; Gomes et al., 2010). A significant correlation was found between ATFC vs. ATPC ($p < 0.01$), ATFC vs. FRAP ($p < 0.01$) and ATFC vs. TCC ($p < 0.01$), as well as a correlation between ATPC vs. FRAP, ATPC vs. TCC and ATPC vs. Prot ($p < 0.01$). ATFC showed a correlation vs. MiChec and $O_2^{\bullet -}$ RSA ($p < 0.05$), suggesting that these compounds could be partly responsible for the antioxidant properties of the samples. TCC was also correlated with Prot and MiChec ($p < 0.05$), while MiChec showed a negative correlation vs. $O_2^{\bullet -}$ RSA (Table 5).

In addition to phytochemical content, the presence of protein and free amino acid content was also ana-

Table 4. Average values of data obtained in chemical analysis, antioxidant capacity and radical scavenging activities of *Eucalyptus* honey from Ecuador.

	Antioxidant and radical scavenging activities							
Samples	FRAP assay (μmol TE/100 g of honey	Chelating metal ions capacity (%)	$O_2^{\bullet -}$ scavenging activity IC_{50} (mg/ml)	Apparent total phenol content (mg GAE/100 g of honey)	Apparent total flavonoid content (mg of CE/100 g of honey)	Total carotenoid content (mg βCarotE/ 100 g of honey)	Free amino acids (mg LE/100 g of honey)	Protein content (mg BSA/100 g of honey)
Beekp1	50.48 ± 3.03^{a}	32.25 ± 2.30^{a,b,c}	4.32 ± 0.23^{a,c}	66.22 ± 8.82^{a,c}	2.69 ± 0.06^{a,c,f,h}	0.30 ± 0.02^{a}	66.59 ± 5.49^{a,b}	41.15 ± 4.25^{a}
Beekp2	72.29 ± 3.54^{b,d,e,g}	29.51 ± 1.52^{a,b}	3.28 ± 0.19^{b}	100.95 ± 4.51^{b}	2.43 ± 0.04^{a,b}	0.33 ± 0.03^{a}	62.08 ± 3.09^{a,b}	84.55 ± 6.72^{b,e}
Beekp3	61.08 ± 4.03^{c,g}	36.41 ± 3.24^{a,b}	4.66 ± 0.13^{a,c}	68.99 ± 3.70^{a,c,d}	2.84 ± 0.09^{a,c,f}	0.38 ± 0.03^{b}	84.17 ± 6.23^{c,e}	55.57 ± 6.14^{c,f}
Beekp4	61.93 ± 3.18^{c,g}	22.17 ± 2.85^{d}	4.89 ± 0.22^{a,c,d}	82.88 ± 4.73^{c,d,e}	2.39 ± 0.05^{b}	0.32 ± 0.03^{a}	42.17 ± 4.64^{d}	70.86 ± 4.15^{d,f}
Beekp5	73.28 ± 4.87^{b,d,e}	27.52 ± 3.13^{a,b}	5.63 ± 0.36^{c,d}	99.10 ± 8.22^{b}	3.35 ± 0.06^{d}	0.43 ± 0.06^{b}	64.59 ± 3.69^{a,b}	90.57 ± 8.95^{b,e}
Beekp6	79.38 ± 4.18^{b,d,e}	38.20 ± 3.74^{c}	3.52 ± 0.27^{b}	82.57 ± 6.06^{c,d,e}	3.61 ± 0.06^{e,d}	0.42 ± 0.06^{b}	46.25 ± 4.66^{d}	83.92 ± 6.56^{b,e}
Beekp7	70.01 ± 3.99^{b,d,g}	31.41 ± 1.85^{a,b,c}	4.76 ± 0.31^{a,c}	100.30 ± 5.55^{b}	2.72 ± 0.05^{a,f,h}	0.36 ± 0.03^{a,b}	75.15 ± 4.54^{a,c}	53.85 ± 4.42^{c,f}
Beekp8	33.85 ± 3.10^{f}	28.20 ± 2.76^{a,b}	3.41 ± 0.14^{b}	49.39 ± 45.88^{f}	0.47 ± 0.06 ^{g}	0.30 ± 0.04^{a}	71.63 ± 7.46^{a,b}	66.04 ± 3.77^{c,d,f}
Beekp9	62.29 ± 5.60^{c,g}	26.41 ± 1.84^{b,d}	5.62 ± 0.29^{d}	60.89 ± 2.92^{a,c}	2.59 ± 0.29^{a,b,c,f}	0.32 ± 0.03^{a}	89.56 ± 7.73^{c,e}	74.87 ± 4.83^{d,e,f}
Beekp10	66.85 ± 4.01^{c,d,g}	27.20 ± 2.64^{a,b,d}	4.58 ± 0.36^{a,c}	60.78 ± 9.24^{a,c}	2.76 ± 0.15^{a,c,f,h}	0.28 ± 0.03^{a}	41.87 ± 2.97^{d}	20.94 ± 3.74^{g}

Notes: Data are means ± standard deviation. Mean values within a column sharing the same letter are not significantly different. Sample was analyzed in triplicate.

Table 5. Correlation matrix (Pearson's correlation coefficients) for quantitative determinations in *Eucalyptus* honey from Ecuador.

Variable	Color	ATFC	ATPC	FRAP	TCC	AA	Prot	MiChec	$O_2^{\bullet-}$RSA
Color	–	−0.146	−0.049	−0.176	−0.104	−0.076	0.149	−0.193	−0.171
ATFC		–	0.490**	0.840**	0.517**	−0.183	0.107	0.326*	0.339*
ATPC			–	0.683**	0.507**	−0.148	0.458**	0.085	0.097
FRAP				–	0.543**	−0.244	0.279*	0.207	0.191
TCC					–	0.119	0.498*	0.349*	0.123
AA						–	0.079	0.170	0.246
Prot							–	−0.035	−0.039
FeChec								–	−0.301*
$O_2^{\bullet-}$ RSA									–

Notes: Color; ATFC, apparent total flavonoid content; ATPC, apparent total phenolic content; FRAP, ferric reducing antioxidant power assay; TCC, total carotenoid content; AA, free amino acid content; Prot, total protein content; MiChec, metal ions chelating capacity; $O_2^{\bullet-}$ RSA, superoxide radical ($O_2^{\bullet-}$) scavenging activity. 95% confidence interval.
*Significant at $p < 0.05$.
**Significant at $p < 0.01$.

lyzed (Table 4). The protein and free amino acid content of honey has been associated with two main factors: the presence of certain enzymes introduced by bees from their salivary glands and pharynx, as well as floral sources, nectar and pollen (da Silva, Gauche, Gonzaga, Costa, & Fett, 2016). The levels of total proteins and free amino acids in the samples of honey were within the range previously reported by other authors in honey from different floral and geographical origins (Alvarez-Suarez et al., 2010; Azeredo et al., 2003; Iglesias, de Lorenzo, Polo, Martín-Álvarez, & Pueyo, 2004; Pérez, Iglesias, Pueyo, González, & de Lorenzo, 2007). It was, however, not possible to draw a comparison with other types of *Eucalyptus* honey because no reports have been published of such analyses.

FRAP assay, metal ions chelating capacity and superoxide radical ($O_2^{\bullet-}$) scavenging activity

Antioxidant activity in honey was analyzed using three different methods and the results are shown in Table 4. A solution of artificial honey was included in the study to evaluate the contribution of the predominant sugars to the assayed activities. (Cooper et al., 2002). Artificial honey showed significantly lower antioxidant activity (FRAP values, metal ions chelating capacity and $O_2^{\bullet-}$ scavenging activity) than all other analyzed honey samples ($p < 0.05$) (data not shown), which led to the inference that the main sugars in honey did not contribute significantly to their antioxidant capacity. The antioxidant capacity analysis demonstrated the capacity of *Eucalyptus* honey to reduce Fe^{3+} to Fe^{2+} (Ciappini & Stoppani, 2014), as well as its $O_2^{\bullet-}$ scavenging activity and metal ions chelating capacity. The antioxidant capacity of honey has been closely associated with its floral origin, due to differences in the content of bioactive compounds such as polyphenols, carotenoids and enzyme activities (Bogdanov, 2017; Bogdanov et al., 2008), which directly influences its biological properties, such as the prevention of diseases related to oxidative stress (Bogdanov et al., 2008). The FRAP values in monofloral *Eucalyptus* honeys from Ecuador were within the ranges previously reported in different monofloral honeys, indicating a similarity in its properties compared with other types of monofloral honey (Estevinho, Pereira, Moreira, Dias, & Pereira, 2008; Gomes et al., 2010; Meda, Lamien, Romito, Millogo, & Nacoulma, 2005). Moreover, *Eucalyptus* honey was able to react with $O_2^{\bullet}$, demonstrating its ability to block the radical, as well as chelate metal ions, thereby exhibiting significant antioxidant mechanisms. The analysis of artificial honey showed that honey sugars have low $O_2^{\bullet-}$ scavenging activity and chelating metal ions compared to the honey samples ($p < 0.05$), demonstrating their low contribution to the antioxidant capacity of honey. The results were in agreement with those previously reported in monofloral honey from Cuba where the $O_2^{\bullet-}$ scavenging activity of five monofloral honey samples varied between 3.8 and 5.2 mg/ml (Alvarez-Suarez et al., 2012). On the other hand, the ability of *Eucalyptus* honey to block other radicals, such as $OH^{\cdot}$ (Ciappini & Stoppani, 2014), has been demonstrated, thus showing its broad antioxidant potential. The radical scavenging activity of honey could be attributed, in part, to the presence of several bioactive compounds performing the scavenging activity of free radicals, such as polyphenols and carotenoids (Del Rio et al., 2013).

Microbial contamination

Microbial contamination of the honey samples is presented in Table 6. Honey is subject to microbial contamination from various sources, such as nectar, pollen, microbiological flora from the honey bees' digestive tract, environmental factors (dust, air, soil etc.), and handling and processing (Gomes et al., 2010). The most commonly occurring microbial contamination involves molds and yeasts, as well the spores of *Bacillus spp*. and *Clostridium spp*. (Snowdon & Cliver, 1996). For this reason, their presence was taken into account when determining the microbiological quality of the honey. In all analyzed honey samples, the level of aerobic mesophiles (molds and yeast) was low. In samples where growth had occurred, the number of cfu/g of honey was signifi-

Table 6. Microbial studies in *Eucalyptus* honey from Ecuador

Honey samples	Aerobic mesophiles (cfu/g)	Molds and yeasts (cfu/g)	Fecal coliforms (MPN)	*Salmonella* (in 25 g)	Sulphite-reducing clostridia (in 0.01 g)
Beekp1	<10	<10	<1	Negative	Negative
Beekp2	<10	<10	<1	Negative	Negative
Beekp3	<10	<10	<1	Negative	Negative
Beekp4	<10	<10	<1	Negative	Negative
Beekp5	<10	<10	<1	Negative	Negative
Beekp6	<10	<10	<1	Negative	Negative
Beekp7	<10	<10	<1	Negative	Negative
Beekp8	<10	<10	<1	Negative	Negative
Beekp9	<10	<10	<1	Negative	Negative
Beekp10	<10	<10	<1	Negative	Negative

Notes: cfu/g: colony-forming units per gram of honey (cfu/g of honey); MPN: Most Probable Number (MPN).

Table 7. Minimum Active Dilution (MAD) of *Eucalyptus* honey against the different microorganisms.

Strain	*Eucalyptus honey* (%)
Staphylococcus aureus	6
Streptococcus pyogenes	9
Escherichia coli	11
Pseudomonas aeruginosa	14
Candida albicans	>20

Note: Each value is expressed as mean ± standard deviations (SD) (n = 3).

cantly lower (<10 CFU/g of honey). Moreover, for fecal coliforms, sulphite-reducing clostridia and *Salmonella sp.*, all samples tested negative. The results were consistent with those previously published for commercial honey from Portugal, where low or negative growths of these types of microorganisms were reported (Gomes et al., 2010).

Antimicrobial activity

The antimicrobial activity of honey has been considered to be one of its most relevant biological properties (Bogdanov et al., 2008). Here, the antimicrobial capacity of *Eucalyptus* honey was determined against four bacterial strains (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli*) and one fungal strain (*Candida albicans*) using the agar incorporation technique.

The results demonstrated that *Eucalyptus* honey was able to inhibit microbial growth (Table 7), with MAD values ranging between 1 and 20%. *S. aureus* proven to be the most sensitive to *Eucalyptus* honey, with a MAD mean value of 6% v/v, followed by *S. pyogenes* (MAD: 9% v/v), *E. coli* (MAD: 11% v/v) and lastly *P. aeruginosa*, with the highest MAD value (14% v/v). Moreover, *Eucalyptus* honey was unable to affect the growth of *C. Albicans,* yielding a high MAD (>20%), compared to those found in the three bacteria strains, signifying this honey's low effectiveness against this particular pathogen. The results were in accordance with those previously reported by others authors using similar bacteria strains and methods for the determination of the antibacterial activity of honey, who demonstrated that *S. aureus* was the most sensitive bacteria to different types of monofloral honey (Agbaje, Ogunsanya, & Aiwerioba, 2006; Alvarez-Suarez et al., 2010; Cooper et al., 2002; Mundo, Padilla-Zakour, & Worobo, 2004; Osés et al., 2016). Pathogen susceptibility to honey has also been demonstrated in other studies using honey from different geological regions and of dissimilar floral origins (Basualdo, Sgroy, Finola, & Marioli, 2007; da Silva, Aazza, Faleiro, Miguel, & Neto, 2016; Estevinho et al., 2008; Mundo et al., 2004; Taormina, Niemira, & Beuchat, 2001). These yielded results similar to those obtained in this study, despite being carried out under distinct experimental conditions. The antimicrobial activity of honey has previously been related to its osmotic properties, hydrogen peroxide content and low pH, as well as bee defensin-1 peptide (Alvarez-Suarez, Giampieri, & Battino, 2013) and polyphenol content (Daglia, 2012).

Conclusions

The results here exposed constitute the first report on investigation of Ecuadorian *Eucalyptus* honey's floral origin, phytochemical composition, antioxidant capacity, quality and safety parameters, as well as its antimicrobial capacity. These results demonstrate that *Eucalyptus* honey from Ecuador meets the quality parameters established by international regulations and contains bioactive compounds (phenolic, flavonoid and carotenoid), which fall within the range previously reported for different types of honey, and specifically for *Eucalyptus* honey. The results also demonstrate that *Eucalyptus* honey from Ecuador possesses significant biological properties, such as antioxidant and antimicrobial capacity and may therefore be used as a natural source of such compounds displaying these properties.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Universidad de Las Américas (UDLA), Quito, Ecuador [grant number VET.JMA.17.01, 2017]; and Universidad Politécnica Salesiana (UPS), Quito, Ecuador.

ORCID

Marilyn García-Tenesaca http://orcid.org/0000-0003-4317-5751
David A Narváez-Narváez http://orcid.org/0000-0003-2332-9522
Eduardo Tejera http://orcid.org/0000-0002-1377-0413
Francesca Giampieri http://orcid.org/0000-0002-8151-9132
José M Alvarez-Suarez http://orcid.org/0000-0001-8509-1498

References

Acquarone, C., Buera, P., & Elizalde, B. (2007). Pattern of pH and electrical conductivity upon honey dilution as a complementary tool for discriminating geographical origin of honeys. *Food Chemistry, 101*(2), 695–703. doi:10.1016/j.foodchem.2006.01.058

Agbaje, E.O., Ogunsanya, T., & Aiwerioba, O.I.R. (2006). Conventional use of honey as antibacterial agent. *Annals of African Medicine, 5*(2), 78–81.

Al, M.L., Daniel, D., Moise, A., Bobis, O., Laslo, L., & Bogdanov, S. (2009). Physico-chemical and bioactive properties of different floral origin honeys from Romania. *Food Chemistry, 112*(4), 863–867. doi:10.1016/j.foodchem.2008.06.055

Alissandrakis, E., Tarantilis, P.A., Pappas, C., Harizanis, P.C., & Polissiou, M. (2011). Investigation of organic extractives from unifloral chestnut (*Castanea sativa* L.) and eucalyptus (*Eucalyptus globulus* Labill.) honeys and flowers to identification of botanical marker compounds. *LWT - Food Science and Technology, 44*(4), 1042–1051. doi:10.1016/j.lwt.2010.10.002

Alvarez-Suarez, J.M., Giampieri, F., & Battino, M. (2013). Honey as a source of dietary antioxidants: Structures, bioavailability and evidence of protective effects against human chronic diseases. *Current Medicinal Chemistry, 20*(5), 621–638. doi:10.2174/09298671380499935 8.

Alvarez-Suarez, J.M., Giampieri, F., Damiani, E., Astolfi, P., Fattorini, D., Regoli, F., … Battino, M. (2012). Radical-scavenging activity, protective effect against lipid peroxidation and mineral contents of monofloral cuban honeys. *Plant Foods for Human Nutrition, 67*(1), 31–38. doi:10.1007/s11130-011-0268-7

Alvarez-Suarez, J.M., Tulipani, S., Díaz, D., Estevez, Y., Romandini, S., Giampieri, F., … Battino, M. (2010). Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. *Food and Chemical Toxicology, 48*(8–9), 2490–2499. doi:10.1016/j.fct.2010.06.021

AOAC. (1990). *Official Methods of Association of Official Analytical Chemists (AOAC) International* (15th ed.). Arlington, VA: Author.

Azeredo, L.D., Azeredo, M.A.A., de Souza, S.R., & Dutra, V.M.L. (2003). Protein contents and physicochemical properties in honey samples of *Apis mellifera* of different floral origins. *Food Chemistry, 80*(2), 249–254. doi:10.1016/S0308-8146(02)00261-3

Barbosa, L.C.A., Filomeno, C.A., & Teixeira, R.R. (2016). Chemical variability and biological activities of Eucalyptus spp. essential oils. *Molecules, 21*(12). doi:10.3390/molecules21121671

Basualdo, C., Sgroy, V., Finola, M.S., & Marioli, J.M. (2007). Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Veterinary Microbiology, 124*(3–4), 375–381. doi:10.1016/j.vetmic.2007.04.039

Benzie, I.F.F., & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*(1), 70–76. doi:10.1006/abio.1996.0292

Bogdanov, S. (2017). Honey as nutrient and functional food. *Book of Honey*. Bee-hexagon.

Bogdanov, S., Jurendic, T., Sieber, R., & Gallmann, P. (2008). Honey for Nutrition and Health: A Review. *Journal of the American College of Nutrition, 27*(6), 677–689.

Cabrera, J. (2007). *Perspectivas de la Apiterapia en Ecuador*. Paper presented at the Primer Simposio Internacional de Apicultura, Universidad Central del Ecuador, Quito.

Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis, 10*(3), 178–182. doi:docview/1282132177?accountid=33194

Chirife, J., Zamora, M.C., & Motto, A. (2006). The correlation between water activity and % moisture in honey: Fundamental aspects and application to Argentine honeys. *Journal of Food Engineering, 72*(3), 287–292. doi:10.1016/j.jfoodeng.2004.12.009

Ciappini, M.C., & Stoppani, F.S. (2014). Determination of antioxidant capacity, flavonoids, and total phenolic content in eucalyptus and clover honeys. *Journal of Apicultural Science, 58*(1), 103–111. doi:10.2478/jas-2014-0010

Ciappini, M.C., & Vitelleschi, M.S. (2013). Palynological characteristics of eucalyptus (*Eucalyptus* sp.) and clover (*Trifolium* sp.) honeys from Argentinean Pampean Phytogeographic Province. *Revista de la Facultad de Ciencias Agrarias, 45*(1), 247–258.

Codex Alimentarius, C. (2001). Revised codex standart for honey. *Codex STAN, 12*, 1981.

Cooper, R.A., Molan, P.C., & Harding, K.G. (2002). The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. *Journal of Applied Microbiology, 93*(5), 857–863. doi:10.1046/j.1365-2672.2002.01761.x

da Silva, C.I., Aazza, S., Faleiro, M.L., Miguel, M.d.G, & Neto, L. (2016). The antibacterial, anti-biofilm, anti-inflammatory and virulence inhibition properties of Portuguese honeys. *Journal of Apicultural Research, 55*(4), 292–304. doi:10.1080/00218839.2016.1243294

da Silva, P.M., Gauche, C., Gonzaga, L.V., Costa, A.C.O., & Fett, R. (2016). Honey: Chemical composition, stability and authenticity. *Food Chemistry, 196*, 309–323. doi:10.1016/j.foodchem.2015.09.051

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology, 23*(2), 174–181. doi:10.1016/j.copbio.2011.08.007

Del Rio, D., Rodriguez-Mateos, A., Spencer, J.P.E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (Poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants and Redox Signaling, 18*(14), 1818–1892. doi:10.1089/ars.2012.4581

De-Melo, A.A.M., de Almeida-Muradian, L.B., Sancho, M.T., & Pascual-Maté, A. (2018). Composition and properties of *Apis mellifera* honey: A review. *Journal of Apicultural Research, 57*(1), 5–37. doi:10.1080/00218839.2017.1338444

Denni, M., & Mammen, D. (2012). A critical evaluation on the reliability of two aluminum chloride chelation methods for quantification of flavonoids. *Food Chemistry, 135*(3), 1365–1368. doi:10.1016/j.foodchem.2012.05.109

Doi, E., Shibata, D., & Matoba, T. (1981). Modified colorimetric ninhydrin methods for peptidase assay. *Analytical Biochemistry, 118*(1), 173–184. doi:10.1016/0003-2697(81)90175-5

Estevinho, L., Pereira, A.P., Moreira, L., Dias, L.G., & Pereira, E. (2008). Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food and Chemical Toxicology, 46*(12), 3774–3779. doi:10.1016/j.fct.2008.09.062

EU Council. (2002). Council directive 2001/11 O/EC of 20 December 2001 relating to honey. *Official Journal of European Communities, L10*, 47–52.

Fallico, B., Arena, E., Verzera, A., & Zappalà, M. (2006). The European food legislation and its impact on honey sector. *Accreditation and Quality Assurance, 11*(1–2), 49–54. doi:10.1007/s00769-006-0128-6

Ferreira, I.C.F.R., Aires, E., Barreira, J.C.M., & Estevinho, L.M. (2009). Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry, 114*(4), 1438–1443. doi:10.1016/j.foodchem.2008.11.028

French, V.M., Cooper, R.A., & Molan, P.C. (2005). The antibacterial activity of honey against coagulase-negative staphylococci. *Journal of Antimicrobial Chemotherapy, 56*(1), 228–231. doi:10.1093/jac/dki193

Gomes, S., Dias, L.G., Moreira, L.L., Rodrigues, P., & Estevinho, L. (2010). Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. *Food and Chemical Toxicology, 48*(2), 544–548. doi:10.1016/j.fct.2009.11.029

Gomez, M.E.M., Hernandez, E.G., Gomez, J.Y.M., & Marin, J.L.M. (1993). Physicochemical analysis of Spanish commercial *Eucalyptus* honeys. *Journal of Apicultural Research, 32*(3–4), 121–126. doi:10.1080/00218839.1993.11101296

González-Miret, M.L., Terrab, A., Hernanz, D., Fernández-Recamales, M.Á., & Heredia, F.J. (2005). Multivariate correlation between color and mineral composition of honeys and by their botanical origin. *Journal of Agricultural and Food Chemistry, 53*(7), 2574–2580. doi:10.1021/jf048207p

Iglesias, M.T., de Lorenzo, C., Polo, M.D.C., Martín-Álvarez, P.J., & Pueyo, E. (2004). Usefulness of amino acid composition to discriminate between honeydew and floral honeys. Application to honeys from a small geographic area. *Journal of Agricultural and Food Chemistry, 52*(1), 84–89. doi:10.1021/jf030454q

ISO 15213:2003. (2003). *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions*. Switzerland: International Standards Organization.

ISO 21528-2:2006. (2004). *Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of Enterobacteriaceae – Part 2: Colony-count method*. Retrieved from http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=34566

Louveaux, J., Maurizio, A., & Vorwohl, G. (1978). Methods of melissopalynology. *Bee World, 59*(4), 139–157. doi:10.1080/0005772X.1978.11097714

Martos, I., Ferreres, F., & Tomás-Barberán, F.A. (2000). Identification of flavonoid markers for the botanical origin of eucalyptus honey. *Journal of Agricultural and Food Chemistry, 48*(5), 1498–1502. doi:10.1021/jf991166q

Meda, A., Lamien, C.E., Romito, M., Millogo, J., & Nacoulma, O.G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry, 91*(3), 571–577. doi:10.1016/j.foodchem.2004.10.006

Montoya-Pfeiffer, P.M., León-Bonilla, D., & Nates-Parra, G. (2014). Catálogo de polen en mieles de *Apis mellifera* provenientes de zonas cafeteras en la Sierra Nevada de Santa Marta, Magdalena, Colombia. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales, 38*(149), 364–384. doi:10.18257/raccefyn.61

Moussa, A., Noureddine, D., Saad, A., & Abdelmalek, M. (2012). Influence of temperature on the inhibitory potency of *Eucalyptus* honey against Candida albicans. *Asian Pacific Journal of Tropical Disease, 2*(Supplement 2), S567–S570. doi:10.1016/S2222-1808(12)60222-9

Mundo, M.A., Padilla-Zakour, O.I., & Worobo, R.W. (2004). Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *International Journal of Food Microbiology, 97*(1), 1–8. doi:10.1016/j.ijfoodmicro.2004.03.025

Nanda, V., Kaur, A., Bera, M.B., Singh, B., & Bakhshi, A.K. (2006). Palynological studies and application of response surface methodology to establish the quality attributes in *Eucalyptus* honey. *Acta Alimentaria, 35*(4), 409–422. doi:10.1556/AAlim.35.2006.4.5

Ortega-Bonilla, R.A., Chito-Trujillo, D.M., & Suárez-Ramos, C.A. (2016). Physicochemical characteristics of commercial *Eucalyptus* honeys from Southwest Casanare. *Corpoica Ciencia y Tecnologia Agropecuaria, 17*(1), 73–80. doi:10.21930/rcta.vol17_num1_art:462

Osés, S.M., Pascual-Maté, A., de la Fuente, D., de Pablo, A., Fernández-Muiño, M.A., & Sancho, M.T. (2016). Comparison of methods to determine antibacterial activity of honeys against Staphylococcus aureus. *NJAS - Wageningen Journal of Life Sciences, 78*(Supplement C), 29–33. doi:10.1016/j.njas.2015.12.005

Oyanagui, Y. (1984). Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. *Analytical Biochemistry, 142*(2), 290–296. doi:10.1016/0003-2697(84)90467-6

Pérez, R.A., Iglesias, M.T., Pueyo, E., González, M., & de Lorenzo, C. (2007). Amino acid composition and antioxidant capacity of spanish honeys. *Journal of Agricultural and Food Chemistry, 55*(2), 360–365. doi:10.1021/jf062055b

Perna, A., Intaglietta, I., Simonetti, A., & Gambacorta, E. (2013). A comparative study on phenolic profile, vitamin C content and antioxidant activity of Italian honeys of different botanical origin. *International Journal of Food Science and Technology, 48*(9), 1899–1908. doi:10.1111/ijfs.12169

Rodríguez Flores, M.S., Escuredo Pérez, O., & Seijo Coello, M.C. (2014). Characterization of eucalyptus globulus honeys produced in the Eurosiberian area of the Iberian Peninsula. *International Journal of Food Properties, 17*(10), 2177–2191. doi:10.1080/10942912.2013.790050

Sancho, M.T., Pascual-Maté, A., Rodríguez-Morales, E.G., Osés, S.M., Escriche, I., Periche, A., & Fernández-Muiño, M.A. (2016). Critical assessment of antioxidant-related parameters of honey. *International Journal of Food Science and Technology, 51*(1), 30–36. doi:10.1111/ijfs.12988

Serrano, S., Villarejo, M., Espejo, R., & Jodral, M. (2004). Chemical and physical parameters of Andalusian honey: Classification of *Citrus* and *Eucalyptus* honeys by discriminant analysis. *Food Chemistry, 87*(4), 619–625. doi:10.1016/j.foodchem.2004.01.031

Singleton, V.L., Orthofer, R., & Lamuela-Raventós, R.M. (1998). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.

Snowdon, J.A., & Cliver, D.O. (1996). Microorganisms in honey. *International Journal of Food Microbiology, 31*(1–3), 1–26. doi:10.1016/0168-1605(96)00970-1

Subramanian, R., Umesh Hebbar, H., & Rastogi, N.K. (2007). Processing of honey: A review. *International Journal of Food Properties, 10*(1), 127–143. doi:10.1080/10942910600981708

Taormina, P.J., Niemira, B.A., & Beuchat, L.R. (2001). Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of

antioxidant power. *International Journal of Food Microbiology, 69*(3), 217–225. doi:10.1016/S0168-1605(01)00505-0

Terrab, A., Díez, M.J., & Heredia, F.J. (2002). Characterisation of Moroccan unifloral honeys by their physicochemical characteristics. *Food Chemistry, 79*(3), 373–379. doi:10.1016/S0308-8146(02)00189-9

Terrab, A., Díez, M.J., & Heredia, F.J. (2003). Palynological, physico-chemical and colour characterization of Moroccan honeys: I. River red gum (*Eucalyptus camaldulensis* Dehnh) honey. *International Journal of Food Science and Technology, 38*(4), 379–386. doi:10.1046/j.1365-2621.2003.00715.x

Von Der Ohe, W., Oddo Livia, P., Piana, M.L., Morlot, M., & Martin, P. (2004). Harmonized methods of melissopalynology. *Apidologie, 35*(Suppl. 1), S18–S25.

Wang, B.-J., Lien, Y.-H., & Yu, Z.-R. (2004). Supercritical fluid extractive fractionation – study of the antioxidant activities of propolis. *Food Chemistry, 86*(2), 237–243. doi:10.1016/j.foodchem.2003.09.031

Yao, L., Jiang, Y., Singanusong, R., Datta, N., & Raymont, K. (2004). Phenolic acids and abscisic acid in Australian Eucalyptus honeys and their potential for floral authentication. *Food Chemistry, 86*(2), 169–177. doi:10.1016/j.foodchem.2003.08.013