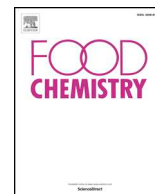




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Eucalyptus honey: Quality parameters, chemical composition and health-promoting properties



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ABSTRACT

Eucalyptus honey is an important unifloral honey commercialized worldwide and much desired by consumers due to the medicinal properties attributed to it because of the plant from which it is produced. In general, eucalyptus honey has been classified as being rich in pollen grains from the eucalyptus tree as well as having physicochemical characteristics that, in a way, have made it stand out from other honeys. Similar to other types of honey, eucalyptus honey can suffer contaminations and adulterations that compromise its quality, safety and authenticity. Thus, detailed knowledge of the composition and properties of this monofloral honeys is of great importance. With this background, the aim of this review is to present and discuss recent data regarding the physicochemical characteristics, chemical and health-promoting properties of eucalyptus honey as well as microbial contamination, authenticity, processing and adulteration.

1. Introduction

Honey is a natural product produced by *Apis mellifera* bees and traditionally used as a sweetener and for therapeutic purposes. Depending on the raw plant material taken by the bees, honey is divided into two groups: honeydew, which is produced from secretions of living parts of plants or excretions of plant-sucking insects; and blossom honey, which is produced from the nectar of flowers (EU Directive, 2002), resulting in honeys with different chemical, sensorial and healthy effects. In general, honey is a supersaturated solution of sugars, which constitute about 95% of its dry weight, with fructose (38%) and glucose (31%) as the principal sugars. Besides water (approximately 20%), there are other minor constituents, such as proteins, enzymes, amino acids, minerals, vitamins, organic acids, and phenolic compounds that contribute towards the honey's quality and health effects

(Santos-Buelga & González-Paramás, 2017).

Honey's composition is related to its botanical and geographical origin and can undergo significant changes depending on storage time and conditions (Santos-Buelga & González-Paramás, 2017). According to botanical origin, floral honeys are classified as poly or multifloral and mono or unifloral. Multifloral means the honey comes from several botanical sources, none of which predominates, while monofloral honeys are made primarily from the nectar of one type of flower and are named according to that plant from which the pollen comes (EU Directive, 2002). The most commercially available floral honeys are multifloral honeys; nevertheless, monofloral honeys have attracted consumers' attention due to the possibility of finding in them the properties of the plants from which they are produced. In fact, it has been suggested that many plants' medicinal properties can be transmitted through honey, making honey a vehicle for transporting said

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properties (Alvarez-Suarez, Tulipani, Romandini, Bertoli, & Battino, 2010a). In this sense, the interest in unconventional types of honey, such as unifloral, has recently increased due to the differentiated nutritional, sensorial and possible therapeutic characteristics of this type. An example of this is eucalyptus honey that has become a popular unifloral honey among consumers due to its aroma and the plant's medicinal properties (Barbosa, Filomeno, & Teixeira, 2016).

Eucalyptus honey is a significant unifloral honey that has been globally commercialized and has been the subject of several studies whose aim was to clarify the relationship between its biological properties and its botanical source. The nectar from the eucalyptus tree (*Myrtaceae* family) – *Eucalyptus* spp. – is the basic raw material for the honey's production. About 800 *Eucalyptus* species are used by bees to produce this honey (Rodríguez Flores, Escuredo Pérez, & Seijo Coello, 2014) and these are important for the ecological stability of the local environments. *Eucalyptus* is widely used for many purposes; hence, these trees nowadays grow in all warm regions around the world (Tavares et al., 2019). This fact has favored extending the production of eucalyptus honey to other geographical areas, thus nowadays this unifloral honey does not only come from Australia, but also from South America (Berriel, 2018a; Ciappini, Vitelleschi, & Calvinõ, 2016; García-Tenesaca et al., 2018; Nascimento et al., 2018; Valdés-Silverio et al., 2018), India (Devi, Jangir, & Appaiah, 2018), North Africa (Chakir, Romane, Marcuzzan, & Ferrazzi, 2016) and Europe (Rodríguez Flores et al., 2014; Rodríguez, Serrano, Galán-Soldevilla, Piva, & Ubera, 2015).

The quality parameters, chemical composition and potential beneficial effects of eucalyptus honey has been widely studied, highlighting important phytochemicals (Yao et al., 2004), nutrient content and health-promoting properties (García-Tenesaca et al., 2018; Scepankova, Saraiva, & Estevinho, 2017; Valdés-Silverio et al., 2018). Notwithstanding, these results have never before been unified in a single report that also exposes the differences that may exist between these honeys according to the *Eucalyptus* species used for its production, as well as its geographical origin. Therefore, considering the significance of eucalyptus honey in the international scenario, this review aims to update and discuss relevant information on this monofloral honey's quality parameters, chemical composition and health-promoting properties, as well as microbial contaminants, adulteration and authenticity. The possible effect of *Eucalyptus* species and geographical origin on its chemical composition and other characteristics will be also analyzed.

2. Pollen analysis and identification

An exhaustive quality control of the hive products, and in particular of honey, must not be based solely on the study of its physico-chemical, microbiological or sensory characteristics, but also on palynological analyses. In that sense, pollen grains and fungal spores present some features (size, type, number or position of apertures or even sporoderm ornamentation) that can be used as taxonomic characteristics in order to identify the botanical and/or geographical origin of a certain honey (Sanchez Reyes & Sánchez Sanchez, 2017). Related to that, pollen grains of the *Myrtaceae* family, to which *Eucalyptus* spp. belongs, are described as isopolar with radial symmetry. In the equatorial vision, these are elliptical; while in the polar vision, they appear as triangular with flat sides. Small in size and trizonocolporate, they present a scabrate surface (Candau, 1987).

The absence of a clear normative regulation that allows one to establish the botanical and geographical origin of nectar honeys by means of palynological criteria has led people to generally consider a honey as monofloral when the percentage of representation of a certain pollen type is greater than 45% (Louveaux, Maurizio, & Vorwohl, 1978). However, some pollen grains, such as those of *Eucalyptus* L'Hér., are over-represented in the pollen spectra, originating in honeys that are considered as monofloral only when the dominant pollen type reaches 70% (Louveaux et al., 1978), 80% (Rodríguez de la Cruz, Sánchez

Reyes, Sánchez Durán, & Sánchez Sánchez, 2013; Rodríguez et al., 2015) or 90% (Bogdanov, Ruoff, & Oddo, 2004). These different criteria may affect the trade of monofloral honeys (Sanchez Reyes & Sánchez Sanchez, 2017). However, although this honey's "main" origin is nectar from the species of the *Eucalyptus* genus, there may be other genera and/or species of *Myrtaceae* that could contribute to its constitution and biological properties, especially in countries like Australia, where these species are abundant and coexist with eucalyptus.

Regarding the palynological analysis itself, diverse methods have been used for both the quantitative (Louveaux et al., 1978) and/or qualitative analysis of this type of honey worldwide (Von Der Ohe, Persano Oddo, Piana, Morlot, & Martin, 2004), the latter being derived from the acetolytic method proposed by Erdtman in 1960 with subsequent modifications made by Hideux (Hideux, 1972), among others. In order to establish eucalyptus honey's main palynological characteristics, regardless of the methodology used, we reviewed studies in which *Myrtaceae* honeys were considered. These studies took place in Europe, South America (Argentina and Brazil), northern Africa (Morocco) and Australia, the latter being the largest producer of this honey (Rodríguez et al., 2015) probably due to the numerous species of the *Myrtaceae* family of apicultural interest (Seijo, Aira, & Méndez, 2003; Sniderman, Matley, Haberle, & Cantrill, 2018).

According to qualitative analysis, it should be noted that this honey type generally presents low pollen richness. A mean number of 6 pollen types were identified in northern Africa (Terrab, Díez, & Heredia, 2003), 10.9/15 pollen types in Australia (Seijo et al., 2003; Sniderman et al., 2018), between 6 and 19 in Argentina (Malacalza, Caccavari, Fagúndez, & Lupano, 2005), 22.6 in Spain and 17.9 in Portugal (Rodríguez de la Cruz et al., 2013; Seijo et al., 2003), belonging to different botanical families. Regarding the latter, the *Leguminosae* family showed the greatest pollen diversity, followed by *Compositae* (Rodríguez de la Cruz et al., 2013; Seijo et al., 2003) and *Labiatae* (Rodríguez de la Cruz et al., 2013), which is the second most important family in the Argentinean Pampean phytogeographic province (Ciappini & Vitelleschi, 2013).

The presence of *Castanea sativa* and the *Ericaceae* family pollen could be geographical indicators of the Iberian Peninsula in comparison to Australian honeys (Seijo et al., 2003). *Cistus ladanifer* pollen is an indicator that the honey was not produced either in the Spanish Northwest (Galicia) or in Portugal (Rodríguez de la Cruz et al., 2013). The frequency of *Echium* as an important minor pollen can be noted in *Myrtaceae* honeys from southern Spain (Rodríguez et al., 2015), while in the Northwest, the presence of the pollen combination of *Castanea sativa*, *Rubus*, *Cytisus*, *Quercus*, *Salix* and *Erica* spp. may be representative for the main flora of apicultural interest in that area (Rodríguez Flores et al., 2014). The presence of *Quercus*, *Plantago* and *Thymelaea* pollen allows one to differentiate Moroccan honeys from other geographical origins (Terrab et al., 2003) and *Citrus* pollen grains are common in eucalyptus honeys from Brazil (Seijo et al., 2003). In the Pampean phytogeographic region in Argentina, eucalyptus pollen was mainly accompanied by *Trifolium* spp., *Lotus*, *Melilotus*, *Helianthus annuus* and *Carduus* spp. (Ciappini & Calvino, 2018). Thus, most *Myrtaceae* morphotypes found in Australian honeys could be a feasible criterion for identifying their geographical origin (Sniderman et al., 2018).

Based on the results derived from the quantitative studies, these honeys present a high number of botanical elements, which are mainly in class III (100,000–500,000) (Rodríguez de la Cruz et al., 2013; Rodríguez et al., 2015; Sniderman et al., 2018) of those defined by Maurizio (Maurizio, 1939), calculated on the basis of number of pollen grains and honeydew elements per unit of weight, usually 10 g of honey. Furthermore, the honeydew elements (HDE) are null (Rodríguez Flores et al., 2014) or low (Rodríguez de la Cruz et al., 2013) and the honeydew index (HDE/P ratio, where P is the total number of pollen grains counted) does not reach the threshold value (3) determined for nectar honeys (Louveaux et al., 1978).

3. Authenticity, processing and adulteration

Honey is a natural product that is subjected many times to possible falsification, especially when talking about different valuable types of honey with medicinal and nutritional properties (Berriel & Perdomo, 2019). Honey authenticity implies two aspects: authenticity in terms of production (without the addition of exogenous substances during the process of obtaining, processing and packaging) and in terms of description (geographical and botanical origin) (Di Bella et al., 2015). The botanical origin of honey has traditionally been established by microscopic analysis of specific pollen (Louveaux et al., 1978), but this requires expertise, which not every research team possesses. Instead, one of the most successful tools for assessing origin authenticity is the use of chemometric information, which was employed in a very recent study (Mădaş et al., 2020).

On the other hand, Serrano et al. (Serrano, Villarejo, Espejo, & Jodral, 2004) analyzed *Citrus* spp. and *Eucalyptus* spp. honeys using the principal factor analysis (PFA) and stepwise discriminant analysis (SDA) of different variables (moisture, hydroxymethylfurfural, diastase, pH, free acidity, lactone acidity, electrical conductivity, glucose, fructose, sucrose, proline, invertase, glucose-oxidase, water activity and insoluble solids). They found that six main factors explain 78.95% of the variance between both types. Of these variables, electrical conductivity and water activity reached the highest discriminant power, allowing for the classification of these types of honeys in 96.6% of cases.

Honey authenticity is generally determined by isotopic (stable isotope methodology) (Berriel & Perdomo, 2019; Bontempo et al., 2017), spectroscopic, chromatographic and electrochemical methods (Trifković, Andrić, Ristivojević, Guzelmerić, & Yesilada, 2017). Spectroscopic methods are non-invasive, involve straightforward sample preparation and minimum consumption of organic solvents and provide information regarding the presence of different classes of compounds, which is useful for food authenticity determination. Fourier transform mid-IR (FT-MIR), near-IR (FT-NIR) and FTIR with attenuated total reflectance (ATR) are useful techniques for determining different food parameters. For instance, the application of mid-FTIR spectroscopy coupled with the SVM (support vector machine) algorithm provided the determination of the botanical origin of Serbian unifloral honey (Lenhardt, Zeković, Dramićanin, & Tešić, 2014).

Nuclear magnetic resonance (NMR) is also a non-invasive method with high reproducibility and sensitivity, allowing for easy and quick data acquisition. Boffo et al. (Boffo, Tavares, Tobias, Ferreira, & Ferreira, 2012) managed to differentiate eucalyptus, citrus and wildflower honeys, using ^1H NMR, hierarchical cluster analysis and principal component analysis. Lolli et al. (Lolli, Bertelli, Plessi, Sabatini, & Restani, 2008) utilized ^1H NMR and heteronuclear multiple-bond correlation (HMBC) spectroscopy and differentiated five botanical types (black locust, chestnut, citrus, eucalyptus and polyfloral) in 72 Italian honey samples. A Brazilian study employed Low Field Nuclear Magnetic Resonance (LF ^1H NMR) to test 80 samples of honey from 8 different botanical sources (eucalyptus, “assa-lipto”, oranges, Barbados cherry, cashew tree, “assa-peixe”, “cipó-uva” and polyfloral) (de Ribeiro, 2014). A close correlation ($p < 0.01$) was established between the LF ^1H NMR analysis and physical and chemical determinations, including water content, water activity, pH and color. Since proteins in honey originate from bees, pollen and nectar, these may be used as tools for botanical origin authentication.

Using electrophoresis (SDS-PAGE), Baroni et al. determined the floral origin of eucalyptus honey (Baroni, Chiabrande, Costa, & Wunderlin, 2002) by identifying protein chemical markers present in the pollen sediment from different origins. The specificity of anti-Eucalyptus pollen antibodies was tested, and the antibodies were found to react with *Eucalyptus* pollen proteins with a different molecular weight. Despite the cross-reaction, the anti-Eucalyptus antibodies bound to three different *Eucalyptus* pollen proteins of ~45, 30, and 20 kDa, which

were not recognized in the other types of pollen in the study. So far, these three proteins can be considered as characteristic of *Eucalyptus* pollen. This immunoblot method could be used for the development of new antibodies from different plants, which could serve as an alternative or complementary method to the usual melissopalynological analysis used to assess honey's floral origin.

The simplest method for eucalyptus honey authenticity determination remains the palynological analysis, because eucalyptus and chestnut honeys have overrepresented pollen grains and are thus denominated as unifloral honeys if they show a pollen frequency of 70% to 90% (Pires, Estevinho, Feás, Cantalapiedra, & Iglesias, 2009).

Honey adulteration can be direct or indirect. The former happens when a substance is added directly to honey and represents a form of economically motivated fraud, while the latter is when honeybees are fed with different sweet substances in order to increase the amount of honey (Zábrodská & Vorlová, 2014). Indirect honey adulteration results from bad apiary management practices (Berriel, 2018a, 2018b). Plants that are sources of substances used for honey adulteration are classified according to their photosynthetic metabolism as C3 or C4 plants. Most honey-contributing plants like rice, wheat and beet are C3 plants, whereas maize and sugarcane are C4 plants. Feeding honeybees with such solutions or sugar syrup has become a serious problem in honey marketing. Optical spectroscopic methods are used for honey adulteration determination, because these are less demanding and have been used successfully in food analysis (Downey, 2016). Near infrared (NIR) spectroscopy, in combination with chemometric methods, was used for the quantitative and qualitative detection of adulteration with beet syrup adulteration (Li, Wen, Yin, Zhou, & Shan, 2013), mixtures of fructose and glucose (Zhu et al., 2010), and HFCS (high fructose corn syrup) (Chen et al., 2011).

In conclusion, it is no longer enough to determine only one indicator or one class of substances, but rather a wide range of quality indicators in order to detect honey adulteration, and new methods and standards for this are mandatory. However, due to the fact that eucalyptus honey comes from a flower with overrepresented pollen, one of the main analyses researchers should use to verify the authenticity of eucalyptus honey could be the melissopalynological analysis.

4. Physicochemical parameters

4.1. Color

According to the values reported from Argentina, Brazil, Ecuador, Morocco and Spain, eucalyptus honey's color ranged from extra light amber (35–50 mm Pfund) to light amber (51–85 mm Pfund) (Chakir et al., 2016; Ciappini et al., 2016; Combarros-Fuentes et al., 2019; García-Tenesaca et al., 2018; McHugh, 2017; Nascimento et al., 2018; Valdés-Silverio et al., 2018). Meanwhile, a group of researchers from Spain described it as light amber honey with a green tone (McHugh, 2017). Although the range of colors described above is the most frequent, samples from the northwest Iberian Peninsula and Morocco had higher values on the Pfund scale, classifying them as amber-colored (86–114 mm Pfund) (Chakir et al., 2016; Rodríguez Flores et al., 2014). These variations in color may be due to several factors. The type of flower from which the honey is produced has a large influence on its color, together with the complex interactions of the different compounds that compose it. The mineral content in honey, closely associated with soil characteristics and therefore geographical region, has also been associated with its color (Alvarez-Suarez et al., 2010b). Indeed, several reports have stated that light-colored honeys usually have a low ash content, whilst dark-colored honeys generally have a higher ash content (Alvarez-Suarez et al., 2010b; García-Tenesaca et al., 2018; Gomes, Dias, Moreira, Rodrigues, & Estevinho, 2010; Machado De Melo, de Almeida-Muradian, Sancho, & Pascual-Maté, 2017). Moreover, the total content of polyphenols, flavonoids and carotenoids were found in high concentrations in darker honeys compared with lighter ones

(Alvarez-Suarez et al., 2010b; García-Tenesaca et al., 2018; Valdés-Silverio et al., 2018). Phytochemicals can also influence the color. These compounds are produced by plants as a protection mechanism against damage caused by biotic and abiotic stress conditions (Mendoza & Silva, 2018) and are transferred from the plant to honey through the nectar collected by bees, depending on the type of plant and the concentration of phytochemicals in the nectar (Bogdanov, Jurendic, Sieber, & Gallmann, 2008). In this way, the concentration can vary significantly between honeys from the same floral origin but from different geographical regions, which also would directly influence the color.

4.2. Carbohydrates

Honey is mainly made up of carbohydrates, which constitute approximately 95% of its dry weight (Santos-Buelga & González-Paramás, 2017) and are produced by honey-bees from nectar sucrose.

Despite there being several reports on sugar content in honey, these are very general and in the case of eucalyptus honey, are scarce and insufficient. In addition to sucrose, in studies from Algeria, Argentina, Brazil, Ethiopia and Spain, several disaccharides have been identified in eucalyptus honey, with isomaltose, maltose and turanose as the most commonly reported ones. An important group of trisaccharides have also been reported, with erlose, melezitose, panose, maltotriose and raffinose as the most commonly reported ones in this honey (Belay, Haki, Birringer, Borck, Lee, & Cho, 2017; Ciappini et al., 2016; Da Costa Leite et al., 2000; De La Fuente, Ruiz-Matute, Valencia-Barrera, Sanz, & Martínez Castro, 2011; Ouchemoukh, Schweitzer, Bachir Bey, Djoudad-Kadji, & Louaileche, 2010; Patrignani et al., 2018) (Table 1). However, the data were very heterogeneous. In most cases, an analysis of only a few sugars (glucose, fructose, maltose and sucrose) was performed instead of a more detailed analysis that allowed for a comparison between the profiles of these types of compounds and other factors that could affect their content, such as geographical and floral origin.

4.3. Hydroxymethylfurfural content and diastase activity

Hydroxymethylfurfural (HMF) is a furanic compound formed by sugar degradation from dehydration of hexoses in acidic medium and, to a lesser extent, as an intermediate in the Maillard reactions (Fallico, Arena, Verzera, & Zappalà, 2006). In fresh honey, HMF can be found in low concentrations or present in trace amounts, so it is considered a good indicator of freshness. According to these regulations, HMF content, determined after processing and mixing in general (except baker's honey), must not exceed 40 mg/kg, whilst honeys of declared origin from tropical climates and blends of these honeys must not exceed 80 mg/kg (EU Directive, 2002).

HMF content in eucalyptus honeys has been reported in South American regions such as Argentina (Ciappini et al., 2016), Brazil (Nascimento et al., 2018) and the Andean regions of Ecuador (García-Tenesaca et al., 2018; Valdés-Silverio et al., 2018), as well as the Euro-Siberian area of the Iberian peninsula (Rodríguez Flores et al., 2014), India (Devi et al., 2018), Morocco (Chakir et al., 2016) and Spain (Combarros-Fuertes et al., 2019). In all these cases, the values of HMF did not exceed those permitted by international standards, as these were mostly relatively low, mainly between 1.5 and 40 mg/kg, indicating acceptable freshness, storage and handling conditions (EU Directive, 2002).

Similarly to the hydroxymethylfurfural content, diastase activity is also used as an indicator of freshness (EU Directive, 2002). According to international standards, diastase activity in honey is measured using the Gothe Scale, where values for honey should generally be no lower than 8 (EU Directive, 2002). Although, as indicated above, diastase activity can be influenced by several factors. In the case of eucalyptus honey, the values reported in samples from different geographical regions ranged between 10 and 65 on the Gothe Scale (Ciappini et al., 2016; Combarros-Fuertes et al., 2019; Devi et al., 2018; García-Tenesaca

et al., 2018; Nascimento et al., 2018; Rodríguez Flores et al., 2014; Valdés-Silverio et al., 2018).

4.4. Moisture and water activity (a_w)

Water content is the second largest constituent of honey and is closely related to several factors, such as the floral and geographical origin of the nectar, soil characteristics, climatic conditions, conditions during harvesting, degree of maturation, manipulation by beekeepers during harvest, extraction, processing methods and storage conditions (Machado De-Melo et al., 2017). The Council of the European Union and de Codex Alimentarius have also established permissible moisture values in honey, which in general should not exceed 20% (EU Directive, 2002).

The moisture content in eucalyptus honeys has been widely reported, showing that despite diverse geographical regions in which it is made, values remain within international standards, ranging between 11 and 20% (Chakir et al., 2016; Ciappini et al., 2016; Combarros-Fuertes et al., 2019; Devi et al., 2018; Nascimento et al., 2018; Rodríguez Flores et al., 2014; Valdés-Silverio et al., 2018).

Water activity (a_w) is a crucial factor that determines the amount of water available for survival and the limitation of the growth of microorganisms that causes food deterioration by fermentation (Abramovič, Jamnik, Burkan, & Kač, 2008). In honey, a_w ranges are typically between 0.49 and 0.65, although in some types of honey, values of up to 0.75 have been reported. Despite the fact that a_w has been documented in several honey types, reports in specific honeys, such as eucalyptus honey, are scarce and so more studies of this type are necessary, however reports found for eucalyptus honey were 0.54 (Ortega-Bonilla, Chito-Trujillo, & Suárez-Ramos, 2016).

4.5. Organic acids, acidity and pH

Low molecular mass aliphatic organic acids, or organic acids, are present in honey at levels between 0.5 and 0.6%. These compounds have been closely related with its organoleptic and physico-chemical properties such as acidity, pH and electrical conductivity (Mato, Huidobro, Simal-Lozano, & Sancho, 2006a) and can also be used as a characterizing indicator of deterioration due to storage and aging or can be used for measuring purity and authenticity (Suárez-Luque, Mato, Huidobro, Simal-Lozano, & Sancho, 2002).

Despite the fact that the content of organic acids has been widely studied in various types of monofloral honeys, studies into the content of these compounds in eucalyptus honey are scarce. The most detailed studies come from eucalyptus honeys from Galicia in Spain. A total of 11 organic acids were reported in these honey through analytical methods such as high-performance liquid chromatography (Suárez-Luque et al., 2002), enzymatic determination (Mato et al., 2006a) and capillary zone electrophoresis with direct ultraviolet detection (Mato, Huidobro, Simal-Lozano, & Sancho, 2006b) (Table 2). However, given the direct effect of organic acids on quality, as well as their potential use for differentiating between honeys according to their botanical and/or geographical origins, more studies are needed to be able to describe their specific profile, as is the case with eucalyptus honey.

pH also has an important effect on conservation due to its capacity to limit and inhibit the growth of microorganisms, making it an important factor for honey stability (Machado De-Melo et al., 2017). In eucalyptus honey, the values of free acidity and pH have been reported in samples from different geographical regions, with recent reports from Brazil (Nascimento et al., 2018), Ecuador (García-Tenesaca et al., 2018; Valdés-Silverio et al., 2018), Portugal (Karabagias, Maia, Karabagias, Gatzias, & Badeka, 2018), Spain (Rodríguez Flores et al., 2014) and Uruguay (Berriel, 2018b), showing pH ranges of 3.6 to 4.6 and free acidity values between 15 and 46 meq/kg. However, although pH value is commonly studied in honey, its acceptable range has yet to be legislated, hence the importance of comparing results with those

Table 1
Sugar profiles reported in eucalyptus honey from different geographical origins.

Geographical origin	Eucalyptus species	Analytical method	Compounds	Content (g/100 g)	Reference
Algeria	<i>Eucalyptus</i> spp.	HPAEC-PAD analysis	Fructose	38.7 ± 1.5	Ouchemoukh et al., 2010
			Glucose	30.8 ± 2.3	
			Maltose	1.7 ± 0.8	
			Sucrose	2.1 ± 1.1	
			Isomaltose	0.5 ± 0.7	
			Turanose	0.9 ± 0.5	
			Trehalose	0.1 ± 0.0	
			Erllose	0.2 ± 0.2	
			Raffinose	0.1 ± 0.0	
Argentina	<i>Eucalyptus</i> spp.	HPLC analysis	Fructose	40.5 ± 2.3	Ciappini et al., 2016
			Glucose	34.3 ± 3.4	
			Sacarose	1.0 ± 0.7	
			Maltose	1.7 ± 1.1	
Brazil	<i>Eucalyptus</i> spp.	HPLC analysis	Maltose	2.5 ± 1.5	Da Costa Leite et al., 2000
			Turanose	1.8 ± 1.0	
			Nigerose	1.6 ± 1.0	
			Melibiose	0.1 ± 0.0	
			Sucrose	0.4 ± 0.0	
			Isomaltose	0.6 ± 0.0	
			Maltotriose	0.5 ± 0.0	
			Panose	0.1 ± 0.0	
			Melezitose	0.2 ± 0.0	
Ethiopia	<i>Eucalyptus globulus</i>	HPLC analysis	Fructose	40.3 ± 0.6	Belay et al., 2017
			Glucose	30.6 ± 0.4	
			Sucrose	1.9 ± 0.1	
			Maltose	2.0 ± 0.5	
			Turanose	1.7 ± 0.4	
			Isomaltose	0.2 ± 0.1	
Spain	<i>Eucalyptus</i> spp.	GC analysis	Fructose	37.5 ± 0.2	De La Fuente et al., 2011
			Glucose	28.3 ± 0.4	
			Sucrose	0.1 ± 0.0	
			α,α-Threulose	0.1 ± 0.0	
			α,β-Threulose	0.5 ± 0.0	
			Cellobiose	0.1 ± 0.0	
			Laminaribiose	0.2 ± 0.0	
			Maltulose	1.9 ± 0.2	
			Nigerose	0.8 ± 0.1	
			Turanose	2.2 ± 0.2	
			Maltose	1.8 ± 0.2	
			Kojibiose	1.5 ± 0.1	
			Trehalulose	1.5 ± 0.2	
			Palatinose	0.3 ± 0.0	
			Gentiobiose	0.1 ± 0.0	
			Isomaltose	1.6 ± 0.2	
			6-kestose	0.1 ± 0.0	
			Erllose	0.2 ± 0.0	
			Melezitose	0.1 ± 0.0	
			Panose	0.1 ± 0.0	

Values are reported as means ± standard deviations; HPAEC-PAD – High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection; HPLC – High-performance liquid chromatography; GC – Gas Chromatography.

reported by other authors.

4.6. Ash, minerals and electrical conductivity

The ash content in honey is highly related to its composition of minerals, which in turn can be influenced by factors such as soil, climatic conditions, the chemical composition of nectar involved in its formation, harvesting and extraction techniques (Machado De-Melo et al., 2017). Its content in honey ranges between 0.02 and 0.3% and is composed mainly of minerals, such as potassium, calcium, sodium and magnesium, whilst the least abundant elements are iron, copper, manganese and chlorine (Santos-Buelga & González-Paramás, 2017).

Eucalyptus honeys have been well characterized in terms of their ash content, mineral content and EC. According to reports from Argentina, Brazil, Ecuador, Portugal and Spain, (Ciappini et al., 2016;

Combarros-Fuertes et al., 2019; Karabagias et al., 2018; Nascimento et al., 2018; Rodríguez Flores et al., 2014; Valdés-Silverio et al., 2018), the values reported for ash content and EC were within the recommended limits according to international regulations for blossom honey: ≤ 0.6% and 0.8 mS/cm, respectively (EU Directive, 2002).

5. Microbiological contamination

Honey is a product with minimum types and levels of microorganisms that are attributed to natural properties and industry control (Sereia et al., 2010).

The main microbiological and parasitological contaminants of honey include molds and yeasts, as well the spores of *Bacillus* spp., *Clostridium* spp., *Escherichia coli*, *Staphylococcus aureus*, *Ascosphaera apis*, *Nosema* spp., *Aspergillus flavus*, and *Aspergillus fumigatus*, their counts

Table 2
Principal organic acids reported in eucalyptus honey from different geographical origins.

Geographical origin	Eucalyptus species	Analytical method	Organic acids	Values (mg/kg)	Reference
Spain	<i>Eucalyptus</i> spp.	HPLC analysis	Citric acid	20–6	Suárez-Luque et al., 2002
			Fumaric acid	0.1–0.7	
			Maleic acid	0.1–0.5	
			Malic acid	13–123	
			Succinic acid	12–101	
		Enzymatic determination Capillary zone electrophoresis with direct ultraviolet detection	D-gluconic acid	4.5–8.6	Mato et al., 1997
			Acetic acid	62–325	
			Citric acid	47–49	Mato et al., 2006b
			Formic acid	61–64	
			Gluconic acid	3.7–8.4	
			Lactic acid	29–78	
			Oxalic acid	0–14	
			Pyruvic acid	0–39	

HPLC – High Performance Liquid Chromatography.

being indicative of honey's commercial quality and safety. It was determined that coliforms and *E. coli* had significant positive correlations with *S. aureus*, *P. larvae*, and *M. pluton* (Dümen, Akkaya, Öz, & Sezgin, 2013).

Despite the effect that microbiological contamination has on consumer health and product quality, studies concerning eucalyptus honeys are scarce. The most recent studies have been reported in honey from Ecuador and Portugal (Gomes et al., 2010; Valdés-Silverio et al., 2018).

In eucalyptus honey from Portugal and Spain, researchers studied aerobic mesophiles, molds and yeasts, fecal coliforms and sulphite-reducing clostridia and the antimicrobial effect against fermentative yeasts (Combarros-Fuertes et al., 2019; Gomes et al., 2010). The levels of quantification for the commercial quality parameters (aerobic mesophiles, molds, and yeasts) in the analyzed honey samples were low and all samples were negative, thus respecting sanitary quality (fecal coliforms) and safety (sulphite-reducing clostridia and *Salmonella*).

The microbiological quality of eucalyptus honey from the Andean regions of Ecuador was also studied in samples collected directly from ten beekeepers. 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 significantly lower (< 10 CFU/g of honey). Moreover, for fecal coliforms, sulphite-reducing clostridia and *Salmonella* spp., all samples tested negative (Valdés-Silverio et al., 2018).

6. Chemical composition

6.1. Proteins

Protein content in honey is very low and varies depending on floral composition and origin. The main source of honey proteins are honeybee secretions and flowers (pollen and nectar) (Machado De-Melo et al., 2017).

There are few studies that report protein content of eucalyptus honey. For European eucalyptus honeys, higher protein contents have been found (0.9–1.24 mg prot/g honey) (Erban, Shcherbachenko, Talacko, & Harant, 2018; Rossano et al., 2012) compared to other unifloral honeys, such as orange (0.58–0.66 mg prot/g honey), chestnut (0.59–0.71 mg prot/g honey) (Erban et al., 2018), acacia (0.1 mg prot/g honey) and sunflower (0.4 mg prot/g honey) (Rossano et al., 2012). Both studies showed similar protein content determined by the Bradford Method although the protein isolation method was different. In one case, honey was mixed with 50 mM Tris/HCl, centrifuged and the supernatant was dialyzed (membrane cutoff 3.500 Da) (Rossano et al., 2012). Meanwhile, Erban et al. (2018) dissolved honey samples in pure water and cleaned using PD MidiTrap G-25 columns (GE Healthcare Life Sciences). Analysis conducted on eucalyptus honeys from Latin

America exhibited lower protein contents. For eucalyptus honey samples from Brazil, researchers found 0.7 mg prot/g honey, which was not significantly different from citrus samples (Azeredo, Azeredo, De Souza, & Dutra, 2003). Another study developed in the Andean region of Ecuador showed broad differences in protein content (0.2 to 0.9 mg prot/g honey), depending on the beekeeper rather than the sampling location (Valdés-Silverio et al., 2018). The protein contents were determined using the Bradford Method on honey samples diluted in pure water 50% w/v.

Pollen proteins were proven to be useful markers for discriminating between sunflower and eucalyptus honey through the immunoblot assay (Baroni et al., 2002). These assays demonstrated that after immunoblotting different honey protein extracts with anti-eucalyptus pollen antibodies, a single band of ~38 kDa could be visualized only in honey containing eucalyptus pollen. Additionally, the immunoblotting-banding pattern in eucalyptus honey extracts exhibit different molecular weights than what was obtained in honey extracts. This could be due to the proteolytic activity of honeybee saliva enzymes on pollen proteins.

The eucalyptus protein profile based on SDS-PAGE electrophoresis is similar to that of other honeys from different floral origins, showing the most abundant bands to be between 50 and 75 kDa (Baroni et al., 2002; Di Girolamo, D'Amato, & Righetti, 2012). Therefore, the main honey proteins appear to be derived from honeybees rather than plants. This is supported by the results on protein composition conducted via proteomic tools that did not detect proteins of plant origin in honey samples (Di Girolamo et al., 2012; Erban et al., 2018; Rossano et al., 2012). It is likely that these proteins are present in undetectable amounts because these are degraded by honeybee proteases. Rossano et al. found relevant differences in the proteolytic pattern in eucalyptus honeys depending on geographical origin, which was less important in orange and chestnut honeys (Rossano et al., 2012). According to their results, most proteolytic enzymes predicted in eucalyptus honey could be assigned to serine proteases of *Apis mellifera*.

Regarding the protein composition of eucalyptus honey, the most abundant are Mayor Royal Jelly proteins (MRJPs1-5) together with glucose oxidase, alpha-glucosidase and defensin-1, which is similar to acacia, chestnut and sunflower honeys (Di Girolamo et al., 2012; Erban et al., 2018). Defensin-1 is an antimicrobial peptide synthesized in the salivary glands (Ilyasov, Gaifullina, & Nikolenko, 2012), whilst glucose oxidase activity contributes to antimicrobial properties by maintaining H₂O₂ levels. Eight Mayor Royal Jelly proteins, MRJPs1-7 and MRJP9, were identified in eucalyptus honey in a comprehensive honey proteomic analyses accomplished by Erban et al. (Erban et al., 2018). Other royal jelly-related proteins distinguished in eucalyptus honey are glucose dehydrogenase, proteases, amylases and allergens. The aforementioned proteomic study encompassed 13 different honeys and the eucalyptus honey sample's proteome differed most prominently from

Table 3
Comparison of reported proline content (mg/100 g) in eucalyptus honey samples from different geographical origins.

Geographical origin	Eucalyptus species	Analytical method	Proline content (mg/100 g)	Reference
China	<i>E. robusta</i> Smith.	HPLC analysis previous derivatization	63.2* ± 8.7	Dong, Zheng, & Xu, 2013
	<i>E. exserta</i> F. Muell		56.8 ± 14.6	
	<i>E. citriodora</i> Hook. F		54.8 ± 15.4	
Ecuador	<i>Eucalyptus</i> spp.	Cd-Ninhydrin method	140.8 ± 40.7	García-Tenesaca et al., 2018
Spain	<i>Eucalyptus</i> spp.	HPLC analysis previous derivatization	49.3 ± 16.8	Hermosín et al., 2003
		HPLC method with fluorescence detection	225 [‡] ± 49.6	Bouseta, Scheirman, & Collin, 1996
Turkey	<i>Eucalyptus</i> spp.	UPLC-ESI-MS/MS	130.5* ± 1.6	Kivrak, 2015
		Cd-Ninhydrin method	45.5* ± 1.6	Kivrak, Kivrak, & Karababa, 2017
Tunisia	<i>Eucalyptus</i> spp.	Bogdanov method	102.6 ± 2.1	Boussaid et al., 2015

Values are reported as means ± standard deviations. *convert from mg/kg; [‡]convert from ppm; HPLC – High Performance Liquid Chromatography; UPLC-ESI-MS/MS – Ultra-Performance Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry.

the others. Some proteins such as hexamerin 70a-c, a larval specific lipoprotein and a short chain dehydrogenase/reductase were found only in eucalyptus honey. The authors attribute the differences to the fact that eucalyptus honey was produced by Spanish bees while the rest were produced by Czech bees.

6.2. Amino acids

Amino acids account for around 1% (w/w) of honey. Proline is the predominant amino acid in eucalyptus honey and is an indicator of quality (Bogdanov et al., 2004). Proline content reported for eucalyptus honey samples from different geographical origins varies substantially, but also according to the analytical method employed to determine it. Table 3 summarizes the origin, proline content and analytical method reported for different studies. What is more, phenylalanine, proline, tyrosine and glutamic acid were revealed as the main amino acids in eucalyptus honey, while amounts of serine, methionine and cysteine were low (Hermosín, Chicón, & Cabezudo, 2003; Kivrak, 2015).

Amino acids in honey come mainly from pollen, though the amino acid profile of a type of honey is more likely to reveal the characteristics of its floral origin than its protein composition (Cotte et al., 2004; Hermosín et al., 2003). In this regard, lavender and eucalyptus honey samples showed significant differences for tyrosine and phenylalanine content, the former accounting for higher levels (Hermosín et al., 2003; Kivrak, 2015). These aromatic amino acids could be involved in the characteristic aroma of each honey. Furthermore, significant differences in valine contents were detected between eucalyptus honey and thyme, and between orange blossom and rosemary honey samples (Hermosín et al., 2003). However, none of these attempts were able to fully distinguish the botanical origins of honey samples based on their amino acid compositions.

6.3. Phenolic compounds

The phenolic compounds present in honey are secondary metabolites, which are typically biosynthesized by plants for protection against oxidative stress and are passed into honey through the nectar. The variety and amount of these compounds depends on many factors, such as the botanical and geographical origins, as well as climatic features of local production (Santos-Buelga & González-Paramás, 2017). In eucalyptus honey, the content of phenolic compounds in samples from Argentina, Brazil, the Euro-Siberian area of the Iberian peninsula, Ecuador, Italy, India and Mexico, ranged between 11.29 and 83.86 mg gallic acid equivalent/100 g honey, while total flavonoids were between 4.20 and 13.76 mg quercetin equivalent/100 g honey (Ciappini et al., 2016; García-Tenesaca et al., 2018; Perna, Simonetti, Intaglietta, Sofo, & Gambacorta, 2012; Ramos et al., 2018; Rodríguez Flores et al., 2014; Rodríguez, Mendoza, Iturriga, & Castaño-Tostado, 2012; Sant'Ana, Sousa, Salgueiro, Lorenzon, & Castro, 2012; Valdés-Silverio et al., 2018).

Flavonoids and phenolic acids are the two main families of polyphenols that have been identified in honey (Santos-Buelga & González-Paramás, 2017). Flavonoids have a C6–C3–C6 nuclear structure, linking two benzene rings joined by a pyran ring and can be classified into flavones, flavanones and flavonols according to the type of chemical groups present on the ring. The main flavonoids detected in eucalyptus honey from Australia, Brazil, India, Italy, Lithuania and Spain, were catechin, myricetin, quercetin, apigenin, kaempferol, luteolin, rutin, isorhamnetin, pinocembrin and gallochatechin (Devi et al., 2018; Di Marco et al., 2018; Escuredo, Silva, Valentão, Seijo, & Andrade, 2012; Nascimento et al., 2018; Perna et al., 2012; Ramanauskienė, Stelmakiene, Briedis, Ivanauskas, & Jakštas, 2012; Yao et al., 2004). In addition to flavonoids, the phenolic acids in honey can be divided according to chemical structure into: hydroxybenzoic acids, with a C1–C6 nuclear structure derived from benzoic acid, with different hydroxylation and methylation of the aromatic ring; and hydroxycinnamic acids, with nuclear structure C3–C6 and differences in the rings. In the honey samples mentioned above, the most common hydroxybenzoic acids found were gallic acid, benzoic acid and vanillic acid, while the most common hydroxycinnamic acids were caffeic acid, *p*-coumaric acid, ferulic acid and cinnamic acid (Devi et al., 2018; Di Marco et al., 2018; Escuredo et al., 2012; Nascimento et al., 2018; Perna et al., 2012; Ramanauskienė et al., 2012; Yao et al., 2004). It is necessary to highlight that although the phenolic and flavonoid profile in honey may depend on floral origin and geographical area, one should also consider the influence that analytical methods, such as chromatographic conditions, can have on this type of analysis (Alvarez-Suarez, Tulipani, Romandini, Vidal, & Battino, 2009; Santos-Buelga & González-Paramás, 2017).

6.4. Volatile compounds

Honey's aroma and taste are related to volatile organic compounds (VOCs) and other compounds such as sugars, acids, amino acids, tannins and phenolics (Machado De-Melo et al., 2017; Machado, Miguel, Vilas-Boas, & Figueiredo, 2020), which, to a large extent, are determined by their floral origin. In fact, some of VOCs identified in honey have been described as characteristic of the floral source, whereas other compounds, like alcohols, branched aldehydes or furan derivatives, are related to microbial purity or the processing and storage conditions of the honey (Castro-Vázquez, Pérez-Coello, & Cabezudo, 2003).

Eucalyptus honey from different geographical regions has been widely studied in terms of its VOC composition (Table 4). Australian eucalyptus honey, derived from *Eucalyptus leucoxylon* and *Eucalyptus melliodora* species, has been authenticated on the basis of its 8,9-dehydrotheaspyrone and 3-oxo- α -ionone contents (D'Arcy, Rintoul, Catherine, Rowland, & Blackman, 1997). A total of 37 compounds, including norisoprenoids, monoterpenes, benzene derivatives, aliphatic compounds and Millard reaction products, have been identified in this type of honey, allowing one to suggest that the nature of the

Table 4
Principal volatile compounds identified in some eucalyptus honey from different geographical origins.

Geographical origin	Eucalyptus species	Analytical method	Principal volatile compounds	Reference
Australia	<i>Eucalyptus leucoxylon</i> , <i>Eucalyptus melliodora</i>	GC and GC/MS	Levo-butane-2,3-diol, 3-hydroxybutan-2-one (acetoin), hydroxyoleic acid lactone, 3-oxo- α -ionone, dehydrovomifolol, 18-hydroxyoleic acid lactone, levo-butane-2,3-diol, dehydrovomifolol, meso-butane-2,3-diol, 5-(hydroxymethyl)-2-furfural (HMF)	D'Arcy et al., 1997
Greece	<i>Eucalyptus globulus</i>	GC-MS	Octane, isovaleric acid, nonanal, benzoic acid, 1,3-di- <i>tert</i> -butylphenol, nonanoic acid, 4-hydroxyisophorone, hexadecane, 3-oxo- α -ionone, tetradecanoic acid, dehydrovomifolol, palmitic acid, heneicosane, oleic acid, stearic acid, docosane, tricosane, tetracosane (Z)-9-tricosane	Alissandrakis et al., 2011
Italy	<i>Eucalyptus</i> spp.	GC-MS/SPME	Acetoin, nonanoic acid, nonanal, benzylalcohol, 2-Phenylethanol and 4-(3-Hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one	Piasenzotto et al., 2003
	<i>Eucalyptus camaldulensis</i>	GC-MS/DHS GC-MS/SBSE GC-MS/SPME GC-MS/SPME	Nonanal, nonan-1-ol, benzyl alcohol, benzaldehyde, octane Nonan-1-ol, decanal, nonanoic acid, nonanal, octane Nonan-1-ol, octane, nonanoic acid, <i>p</i> -cymene Nonanal, nonanal, nonanoic acid, 5-hexen-2-ol, 2,3-dimethyl-5-hexen-2-ol	Petretto et al., 2017
Portugal	<i>Eucalyptus</i> spp.	GC and GC/MS	Heptane, octane, nonane, decane, linalool, octanoic acid ethyl ester, nonanoic acid ethyl ester, hexanoil acid ethyl ester, decanoic acid ethyl ester, benzaldehyde, octanal, benzeneacetaldehyde, decanal, 1-nonanol, 2-ethyl-1-hexanol, α -pinene, linalool, <i>cis</i> -linalool oxide, hotrienol, <i>para</i> -cymene	Verzera, Campisi, Zappalà, & B. I., 2001 Karabagias et al., 2018
Spain	<i>Eucalyptus</i> spp. <i>Eucalyptus globulus</i> <i>Eucalyptus</i> spp.	GC-MS/SDE GC-MS/SPE GC-MS/SPME	Phenylacetaldehyde, 3-hydroxy-2-butanone, furfural, 3-hydroxy-5-methyl-2-hexanone, nonanoic acid Hydroxymethylfurfural, 6-methyl-2-methoxy-pyrazine, methyl furoate, 3-oxo- α -ionone, 2-methyl butanoic acid Octanoic acid, nonanoic acid, 1-mnanol, phenylacetaldehyde, 3-methyl-butanoic acid	Castro-Vázquez et al., 2006 Castro-Vázquez et al., 2006 Soria, Sanz, & Martínez-Castro, 2009

GC, Gas Chromatography; GC-MS, Gas Chromatography, Mass Spectrometry; SDE, Simultaneous Distillation-Extraction; SPE, Solid Phase Extraction; SPME, Solid Phase Micro Extraction; DHS, Dynamic Headspace.

VOCs in this honey is quite distinctive compared to other honeys, with vomifolol as one of the most abundant VOCs.

In eucalyptus honeys from Spain, Castro-Vázquez et al. (Castro-Vázquez, Díaz-Maroto, Guchu, & Pérez-Coello, 2006) reported the presence of 35 VOCs classified into five groups: terpenes and derivatives; ketones; furan and pyran compounds; norisoprenoids; and other compounds (Table 4). In the Spanish honeys analysed, camphor was one of the most abundant terpenes, 2-hydroxycineol and was the compound with the highest concentration, while 3-hydroxy-2-butanone (acetoin) and other hydroxyketones were proposed as floral markers for eucalyptus honeys from the region (Castro-Vázquez et al., 2006). A similar profile was also reported in Portuguese eucalyptus honey where approximately 44 VOCs were identified (Karabagias et al., 2018). The terpenoid compounds 1,8-cineol and *p*-cymen-8-ol, reported by Castro-Vázquez et al. (2006) in eucalyptus honeys from Spain, were also found in Italian eucalyptus honey and are reported among the major volatile compounds in most eucalyptus species, especially in *Eucalyptus camaldulensis* (Verzera, Campisi, & Zappalà, 2001). Similarly, acetoin and nonanoic acid were also found in eucalyptus honeys from both geographical regions (Italy and Spain) (Castro-Vázquez et al., 2006; Piasenzotto, Gracco, & Conte, 2003), while in *Eucalyptus* spp. honey from Argentina, the principal VOC reported was nonanal, which has been proposed as a possible floral marker for eucalyptus honeys in this region (Patrignani, Fagúndez, Tananaki, Thrasyvoulou, & Lupano, 2018).

According to Verzera et al. (Verzera et al., 2001), eucalyptus honey can be characterized by a high amount of nonanal, nonanoic acid, 5-hexen-2-ol, and 2,3-dimethyl-5-hexen-2-ol. Since borneol and dihydrocarveol have only been identified in this type of honey, these components could be used as markers. Moreover, other authors have indicated dimethylsulfide and trimethylsulfide as markers of eucalyptus honey (Bouseta, Collin, & Dufour, 1992).

Eucalyptus samples from Greece were characterized by the presence of 2-hydroxy-5-methyl-3-hexanone and 3-hydroxy-5-methyl-3-hexanone, as well as exo-2-hydroxycineole and an unknown norisoprenoid. Aside from that, acetoin, nonanal, methyl nonanoate, and dehydrovomifolol were identified in higher concentrations. Norisoprenoids from eucalyptus flower extracts were also identified in honey, as well as 3-Oxo- α -ionone and eucalyptol (Alissandrakis, Tarantilis, Pappas, Harizanis, & Polissiou, 2011).

Other VOCs, such as furan derivatives (furfural and methyl furoate), were also reported in eucalyptus honeys and they are mainly formed by heating or during prolonged storage (Machado De-Melo et al., 2017). Some compounds are produced by Maillard reactions (Castro-Vázquez et al., 2003), including maltol (3-hydroxy-2-methyl-4H-pyran-4-one), 2-methoxy-6-methyl pyrazine and pantolactone, and they have also been identified in eucalyptus honey and serve as indicators of loss of freshness, negatively affecting the honey's sensory properties.

7. Health-promoting properties

7.1. Antioxidant properties

Honey's antioxidant activity (AA) has been widely reported by several authors, and is known as the capacity of honey to reduce oxidative damage within food systems and human health (Scepankova et al., 2017). AA has been related to floral origin and a group of compounds transmitted to honey through pollen and plant nectar, as well as others transmitted by the bee through its saliva (Scepankova et al., 2017). These compounds are flavonoids, phenolic acids and some enzymes (e.g. glucose oxidase), ascorbic acid, carotenoids, organic acids, amino acids, proteins and Maillard reaction products (Alvarez-Suarez et al., 2010a; Machado De-Melo et al., 2017).

To quantify honey's AA, different tests are usually employed, such as 1,1-diphenyl-2-picrylhydrazil (DPPH) assay, Ferric Reducing Antioxidant Power (FRAP) assay, Oxygen Radical Absorbance Capacity

Table 5
Antioxidant activity of eucalyptus honey samples from different geographical locations.

Geographical origin	Eucalyptus species	Analytical method	Values	Reference
Argentina	<i>Eucalyptus</i> spp.	OH radical-scavenging activity FRAC assay	0.52–2.03 mg QE/100 g honey 2.5–98.1 mg AAE/100 g honey	Ciappini & Stoppani, 2014
Australia	<i>Eucalyptus</i> spp.	DPPH assay	14.4 ± 0.7%	Marceau & Yaylayan, 2009
Brazil	<i>Eucalyptus</i> spp.	DPPH assay	2.6–17.2 mg QEA/100 g honey	Bueno-Costa et al., 2016
	<i>Eucalyptus</i> spp.	TEAC assay	8.2–111.5 mg TEA/100 g honey	
	<i>Eucalyptus</i> spp.	ORAC assay	3.4–18.5 µmol TE/g honey	Nascimento et al., 2018
	<i>Eucalyptus</i> spp.	DPPH (EC ₅₀)	25.4–105.3 mg/ml honey	
	<i>Eucalyptus</i> spp.	FRAP assay	0.4–2.1 µmol TE/g honey	
	<i>Eucalyptus</i> spp.	DPPH (EC ₅₀)	10.2–21.5 mg/mL honey	Sant'Ana et al., 2012
	<i>Eucalyptus</i> spp.	FRAP assay	229.2–388.5 µmol Fe(II)/100 g honey	
	<i>Eucalyptus</i> spp.	TEAC assay	306.2–700.6 µmol TE/100 g honey	
Ecuador	<i>Eucalyptus</i> spp.	FRAP assay	33.9–79.4 µmol TE/100 g honey	Valdés-Silverio et al., 2018
	<i>Eucalyptus</i> spp.	Chelating metal ions capacity	22.2–38.2% honey solution	
	<i>Eucalyptus</i> spp.	O ₂ ^{•-} RSA IC ₅₀	3.3–5.6 mg/ml honey	
	<i>Eucalyptus</i> spp.	FRAP assay	142.9 µmol TE/100 g honey	García-Tenesaca et al., 2018
	<i>Eucalyptus</i> spp.	DPPH assay	44.3 µmol TE/100 g honey	
	<i>Eucalyptus</i> spp.	O ₂ ^{•-} RSA IC ₅₀	4.9 mg/ml honey	
	<i>Eucalyptus</i> spp.	Chelating metal ions capacity	26.2% honey solution	
	<i>Eucalyptus</i> spp.	TBARS assay IC ₅₀	17.8 mg/mL honey	
Italy (Sicily)	<i>Eucalyptus globulus</i>	DPPH assay	180.6–194.3 µmol TE/100 g	Attanzio et al., 2016
	<i>Eucalyptus</i> spp.	FRAP assay	62.8–75.2 mg AAE/100 g	
	<i>Eucalyptus</i> spp.	TEAC assay	220.6–225.3 µmol TE/100 g	
Italy (Sardinia)	<i>Eucalyptus</i> spp.	DPPH assay	25.0–63.0 mmol/kg	
	<i>Eucalyptus</i> spp.	FRAP assay	1.4–3.0 mmol/kg	
Italy (Southern Italy)	<i>Eucalyptus</i> spp.	DPPH assay	54.3–78.7%	Perna et al., 2012
	<i>Eucalyptus</i> spp.	FRAP assay	152.6–881.3 µmol(FeII)	
	<i>Eucalyptus</i> spp.	TEAC assay	58.4–60.4%	
Mexico	<i>Eucalyptus globulus</i>	DPPH assay	200.0–255.0 µmol Trolox/kg	Rodríguez et al., 2012
	<i>Eucalyptus</i> spp.	TEAC assay	1779.5–2927.4 µmol Trolox/kg	
	<i>Eucalyptus</i> spp.	FRAP assay	1480.8–2476.7 µmol FeSO ₄ /kg	
Portugal	<i>Eucalyptus</i> spp.	DPPH assay (EC ₅₀)	0.1–0.2 g/mL	Karabagias et al., 2018
Turkey	<i>Eucalyptus</i> spp.	DPPH assay	24.43 mg/mL	Gül & Pehlivan, 2018
	<i>Eucalyptus</i> spp.	FRAP assay	0.0041*10 ⁻⁵ mg/100g	

DPPH, 1,1-diphenyl-2-picrylhydrazyl radical assay; FRAP, Ferric Reducing Antioxidant Power; TEAC, Trolox Equivalent Antioxidant Capacity assay; ORAC, Oxygen Radical Absorption Capacity; TBARS, Thiobarbituric Acid Reactive Substances; O₂^{•-} RSA, Superoxide Radical Scavenging Activity; TE, Trolox Equivalents; QE, Quercetin Equivalents; QEA, Quercetin Equivalents Antioxidant; EC₅₀, Minimum concentration required for the antioxidant to reduce the initial concentration of DPPH by 50%; AAE, Ascorbic Acid Equivalent.

(ORAC) and TEAC (Trolox-equivalent antioxidant capacity) assay (Alvarez-Suarez et al., 2009; Džugan, Tomczuk, Sowa, & Grabek-Lejko, 2018), or Copper Reduction Assay (CUPRAC) as a variant of the FRAP assay, employing Cu instead of Fe (Pascual-Maté, Osés, Fernández-Muiño, & Sancho, 2018). Despite all these assays, there is no official method for determining honey's antioxidant activity and one determination requires more methods to be used. Using a diversity of methods, large amounts of data regarding AA in eucalyptus honey from different geographical regions has been reported (Table 5).

As shown in Table 5, different AA values were obtained for eucalyptus honeys from different geographical origins, even if the same methods of determination were used. Honeys from Argentina and Australia (Ciappini & Stoppani, 2014; Marceau & Yaylayan, 2009) exhibit lower antioxidant activity compared to Brazilian eucalyptus honeys, where several ranges of AA values have been registered (Bueno-Costa et al., 2016; Nascimento et al., 2018; Sant'Ana et al., 2012). Using the FRAP assay, different values were obtained, which were significant between samples and originated from different regions of Brazil. Different studies concerning eucalyptus honey from Ecuador (García-Tenesaca et al., 2018; Valdés-Silverio et al., 2018) using DPPH and FRAP assays, chelating metal ions capacity and O₂^{•-} Radical Scavenging Activity demonstrated very high antioxidant activity for this honey type. For eucalyptus honeys from Mexico (Rodríguez et al., 2012), the antioxidant activity was similar to Cuban honey (Alvarez-Suarez et al., 2010b) and higher than Slovakian honeys (Zalibera et al., 2008).

In eucalyptus honey from Italy (Sicily, Sardinia and southern Italy) (Attanzio, Tesoriere, Allegra, Livrea, & Livrea, 2016; Ciulu et al., 2018; Perna et al., 2012), the values of DPPH, FRAP and ABTS were similar for all regions, with slightly higher values for samples collected in

southern Italy (Perna et al., 2012) and similar values to Argentinian honeys (Ciappini & Stoppani, 2014).

7.2. Antibacterial properties

The antimicrobial activity of honey has been considered one of its most relevant biological properties (Scepankova et al., 2017). The main factors related to this are the high molarity caused by honey's high concentration of sugar, acidity and particularly hydrogen peroxide (Alvarez-Suarez et al., 2010a), which is formed from the oxidation of glucose by the action of the enzyme glucose oxidase during honey maturation (Scepankova et al., 2017). However, other components of honey have also been related to its antimicrobial activity, known as non-peroxide antibacterial activity, such as lysozyme, phenolic acids and flavonoids (Alvarez-Suarez et al., 2010a), as well as the antimicrobial peptide Defensin-1 (Alvarez-Suarez et al., 2009).

The antibacterial capacity of eucalyptus honey has been widely reported in samples from different geographical regions (Table 6) (Aumeeruddy et al., 2019; Combarros-Fuertes et al., 2019; Coniglio, Laganà, Faro, & Marranzano, 2018; García-Tenesaca et al., 2018; Leyva-Jimenez, Lozano-Sanchez, Borrás-Linares, & de la Cadiz-Gurrea, 2019; Roshan, Rippers, Locher, & Hammer, 2017; Valdés-Silverio et al., 2018). This activity has been tested against several types of microorganisms (gram positive, gram negative and yeast), the most common being *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

The most common techniques for this type of study have been traditional microbiology techniques, such as the Kirby-Bauer Test and methods to determine the minimum inhibitory concentrations (MIC)

Table 6
Antimicrobial activity of eucalyptus honeys from different geographical regions.

Geographical origin	Eucalyptus species	Analytical method	Analyzed strains	Results	Reference
Australia	<i>E. marginata</i>	Kirby-Bauer Test	<i>S. aureus</i> ATCC 25923	18.6 mm inhibition zone (IZ) using 50% w/v honey	Roshan et al., 2017
		MIC and MBC methods (broth microdilution method)	<i>S. aureus</i> ATCC 25923, NCTC 10442 and <i>S. epidermidis</i> ATCC 11047 <i>E. faecalis</i> ATCC 29212 <i>E. faecalis</i> ATCC 51299 <i>A. baumannii</i> ATCC 7844, <i>E. coli</i> ATCC 25922, <i>P. aeruginosa</i> ATCC 27853 and <i>S. typhimurium</i> ATCC 13311	32% w/v honey for both methods 16% for MIC and 32% for MBC 16% w/v honey for both methods	
	<i>E. macrorrhyncha</i>	MIC (agar dilution method)	<i>Alcaligenes faecalis</i> , <i>Citrobacter freundii</i> , <i>E. coli</i> , <i>Enterobacter aerogenes</i> , <i>Klebsiella pneumoniae</i> , <i>Mycobacterium phlei</i> , <i>Salmonella californica</i> , <i>Salmonella enteritidis</i> , <i>Salmonella typhimurium</i> , <i>Shigella sonnei</i> , <i>S. aureus</i> and <i>S. epidermidis</i> <i>Serratia marcescens</i> <i>Candida albicans</i> <i>S. aureus</i> ATCC 25923	5% w/v honey 20% honey > 20% honey 20.0 mm IZ using 50% w/v honey 8% w/v honey for both methods 4% w/v honey for both methods 32% w/v honey for both methods 16% w/v honey for both methods	Lusby, Coombes, & Wilkinson, 2005
		Kirby-Bauer Test	<i>S. aureus</i> ATCC 25923		Roshan et al., 2017
		MIC and MBC methods (broth microdilution method)	<i>S. aureus</i> ATCC 29213, ATCC 25923 and NCTC 10442 <i>S. epidermidis</i> ATCC 11047 <i>E. faecalis</i> ATCC 29212 and ATCC 51299 <i>A. baumannii</i> ATCC 7844, <i>E. coli</i> ATCC 25922, <i>P. aeruginosa</i> ATCC 27853 and <i>S. typhimurium</i> ATCC 13311		
		Kirby-Bauer Test	<i>S. aureus</i> ATCC 25923		Roshan et al., 2017
		MIC and MBC methods (broth microdilution method)	<i>S. aureus</i> ATCC 29213 <i>S. aureus</i> ATCC 25923 <i>S. aureus</i> NCTC 10442 <i>S. epidermidis</i> ATCC 11047 <i>E. faecalis</i> ATCC 29212 and ATCC 51299 <i>E. faecalis</i> ATCC 29212 and ATCC 51299, <i>A. baumannii</i> ATCC 7844, <i>E. coli</i> ATCC 25922, <i>P. aeruginosa</i> ATCC 27853 and <i>S. typhimurium</i> ATCC 13311		Roshan et al., 2017 Roshan et al., 2017
		Kirby-Bauer Test	<i>S. aureus</i> ATCC 25923		
		MIC and MBC methods (broth microdilution method)	<i>S. aureus</i> ATCC 29213 and ATCC 25923 <i>S. aureus</i> NCTC 10442 and <i>A. baumannii</i> ATCC 7844 <i>S. epidermidis</i> ATCC 11047 <i>E. faecalis</i> ATCC 29212 and ATCC 51299, <i>A. baumannii</i> ATCC 7844, <i>E. coli</i> ATCC 25922, <i>P. aeruginosa</i> ATCC 27853 and <i>S. typhimurium</i> ATCC 13311		
		Kirby-Bauer Test	<i>S. aureus</i> ATCC 25923		
Ecuador	<i>E. wandoo</i>	Kirby-Bauer Test	<i>S. aureus</i> ATCC 25923	18.3 mm IZ using 50% w/v of honey	Valdés-Silverio et al., 2018
		MIC and MBC methods (broth microdilution method)	<i>S. aureus</i> ATCC 29213 and ATCC 25923 <i>S. aureus</i> NCTC 10442 and <i>A. baumannii</i> ATCC 7844 <i>S. epidermidis</i> ATCC 11047 <i>E. faecalis</i> ATCC 29212 and ATCC 51299, <i>E. coli</i> ATCC 25922, <i>P. aeruginosa</i> ATCC 27853 and <i>S. typhimurium</i> ATCC 13311	8% w/v of honey for both methods 16% w/v of honey for both methods 16% for MICs and 32% for MBC 32% of honey for both methods	
	<i>Eucalyptus</i> sp.	Minimum active dilution (MAD) by agar incorporation technique	<i>S. aureus</i> ATCC 25923 <i>S. pyogenes</i> ATCC 19615 <i>P. aeruginosa</i> ATCC 27853 <i>E. coli</i> ATCC 25922 <i>Candida albicans</i> ATCC 90028 <i>S. aureus</i> CAMP <i>Klebsiella pneumoniae</i> KPC 609803	6% w/v of honey 9% w/v of honey 14% w/v of honey 11% w/v of honey > 20% w/v of honey ~ 45% of inhibition of biofilm formation and ~ 65% of destruction of the preformed biofilm using 20% w/v of honey ~ 60% of inhibition of biofilm formation and ~ 75% of destruction of the preformed biofilm using 20% w/v of honey 8.0–9.0 mm IZ 8.0–9.0 mm IZ 10.0–11.0 mm of IZ 8.0–9.0 mm of IZ > 25.0 mm of IZ > 1.0 mm of IZ	García-Tenesaca et al., 2018
		Inhibition of biofilm formation and remove preformed biofilm assays			
		Kirby-Bauer Test	<i>E. coli</i> ATCC 25922 <i>P. aeruginosa</i> ATCC 27853 <i>S. aureus</i> ATCC 25923 <i>Enterococcus faecalis</i> ATCC 11700 <i>S. aureus</i> subsp. <i>aureus</i> ATCC 9144 <i>P. aeruginosa</i> ATCC 27853		Leyva-Jimenez, Lozano-Sanchez, Borrás-Linares, de la Cadiz-Gurrea, & L., & Mahmoodi-Khaledi, E., 2019
		Kirby-Bauer Test	<i>S. aureus</i> subsp. <i>aureus</i> ATCC 9144 <i>P. aeruginosa</i> ATCC 27853		Comiglio et al., 2018
		Kirby-Bauer Test			
		Kirby-Bauer Test			
		Kirby-Bauer Test			
		Kirby-Bauer Test			

(continued on next page)

Table 6 (continued)

Geographical origin	Eucalyptus species	Analytical method	Analyzed strains	Results	Reference
Mauritius	<i>Eucalyptus</i> spp.	Kirby-Bauer Test	<i>E. coli</i> (clinical isolate) and <i>E. coli</i> ATCC 25922 <i>Proteus</i> spp (clinical isolate) and <i>P. mirabilis</i> ATCC 12453 <i>Pseudomonas</i> spp (clinical isolate) and <i>P. aeruginosa</i> ATCC 27853 <i>Klebsiella</i> spp (clinical isolate) <i>Streptococcus</i> spp (clinical isolate) <i>S. epidermidis</i> ATCC 35984 and <i>S. epidermidis</i> ATCC 14990	22.3 and 28.0 mm IZ for both strains respectively 11.7 mm IZ and No IZ for both strains respectively 10.7 and 9.7 mm IZ for both strains respectively 11.3 mm IZ 8.0 mm IZ 10.0 and 11.3 mm IZ for both strains respectively 0.1 g/mL for both strains	Aumeeruddy et al., 2019
Spain	<i>Eucalyptus</i> spp.	Disk-plate diffusion method	<i>S. aureus</i> and MRSA: methicillin resistant <i>S. aureus</i> (clinical isolate) <i>S. pyogenes</i> , <i>E. coli</i> and <i>P. aeruginosa</i> (clinical isolate)	0.2 g/mL for both strains	Combarros-Fuertes et al., 2019

ATCC, American Type Culture Collection; IZ, Inhibition Zone; MIC, Minimum Inhibitory Concentration; MBC, Minimum Bactericidal Concentration; NCTC, National Collection of Type Cultures.

and minimum Bactericidal Concentration (MBC), as well as the assay to determine the honey's capacity to inhibit biofilm formation and remove the preformed biofilm assays. A wide range of eucalyptus honey concentrations has been studied and concentrations ranged between 1 and 50%, showing different effects according to the microorganism at hand. According to these results from various studies, *S. aureus* (gram positive) could be identified as one of the most sensitive and *P. aeruginosa* (gram negative) one of the most resistant to this type of honey. However, other types of bacteria have also demonstrated significant sensitivity to this type of honey, such as *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Klebsiella pneumoniae*, which are microorganisms with relevant clinical importance.

Although the results obtained are relevant and contribute to the study of this biological property of eucalyptus honey, more specific studies are needed to understand the biological mechanisms by which this honey exerts this kind of activity that goes beyond what has been previously mentioned here. An example could be those molecular mechanisms in this type of activity that have already been demonstrated in other honeys, for instance in Manuka honey (Jenkins, Burton, & Cooper, 2014).

8. Conclusions

Eucalyptus honey has become a commercially attractive product due to its typical physicochemical, sensory and therapeutic characteristics. In this review, we highlighted its chemical composition, as well as particular characteristics related to the species of *Eucalyptus* from which the honey originates, as well as its geographical origin. Special attention has been given to the quality parameters of this honey, as well as to the contents of specific chemical compounds, such as its protein profile and content of volatile compounds. Another aspect that was treated with special attention was its antimicrobial activity, which is considered one of the main biological effects attributed to this honey. The results suggest that geographical region and eucalyptus species could influence the honey's chemical composition. Moreover, the presence of some compounds in eucalyptus honey, such as phenolic compounds and proteins, suggest their correlation with its possible beneficial health effects, justifying the importance of further research, especially on its protein profile.

Authors contributions

J.M. Alvarez-Suarez and O. Bobis conceived the idea of the review article and designed the contents. O. Bobis and A. Ramona Moise collected the data and wrote the sections about volatile compounds, antioxidant properties, microbiological contamination, and authenticity, processing and adulteration. I. Ballesteros collected the data and wrote the section about proteins and amino acids. E. Sánchez Reyes, S. Sánchez Durán and J. Sánchez-Sánchez collected the data and wrote section about the pollen analysis and identification. S. Cruz Quintana collected the data and wrote the antimicrobial properties section. F. Giampieri collected the data and wrote the phenolic compounds sections. J.M. Alvarez Suarez collected the data and wrote the introduction and quality parameters section. M. Battino conducted a critical review of manuscript. J.M. Alvarez Suarez and Isabel Ballesteros corrected the final version of the manuscript. All authors read and approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- Abramovič, H., Jamnik, M., Burkan, L., & Kač, M. (2008). Water activity and water content in Slovenian honeys. *Food Control*, 19(11), 1086–1090. <https://doi.org/10.1016/j.foodcont.2007.11.008>.
- 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. <https://doi.org/10.1016/j.lwt.2010.10.002>.
- Alvarez-Suarez, J. M., Tulipani, S., Díaz, D., Estevez, Y., Romandini, S., Giampieri, F., et al. (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. <https://doi.org/10.1016/j.fct.2010.06.021>.
- Alvarez-Suarez, J. M., Tulipani, S., Romandini, S., Bertoli, E., & Battino, M. (2010). Contribution of honey in nutrition and human health: A review. *Mediterranean Journal of Nutrition and Metabolism*, 3(1), 15–23. <https://doi.org/10.1007/s12349-009-0051-6>.
- Alvarez-Suarez, J. M., Tulipani, S., Romandini, S., Vidal, A., & Battino, M. (2009). Methodological aspects about determination of phenolic compounds and *in vitro* evaluation of antioxidant capacity in the honey: A review. *Current Analytical Chemistry*, 5(4), 293–302. <https://doi.org/10.2174/157341109789077768>.
- Attanzio, A., Tesoriere, L., Allegra, M., Livrea, M. A., & Livrea, M. A. (2016). Monofloral honeys by Sicilian black honeybee (*Apis mellifera* spp. *sicula*) have high reducing power and antioxidant capacity. *Heliyon*, 2(11), e00193. <https://doi.org/10.1016/j.heliyon.2016.e00193>.
- Aumeeruddy, M. Z., Aumeeruddy-Elalfi, Z., Neetoo, H., Zengin, G., Blom van Staden, A., Fibrich, B., et al. (2019). Pharmacological activities, chemical profile, and physico-chemical properties of raw and commercial honey. *Biocatalysis and Agricultural Biotechnology*, 18, 101005. <https://doi.org/10.1016/j.cbab.2019.01.043>.
- Azeredo, L. D. C., 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. [https://doi.org/10.1016/S0308-8146\(02\)00261-3](https://doi.org/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), 1–33. <https://doi.org/10.3390/molecules21121671>.
- Baroni, M. V., Chiabrandi, G. A., Costa, C., & Wunderlin, D. A. (2002). Assessment of the floral origin of honey by SDS-page immunoblot techniques. *Journal of Agricultural and Food Chemistry*, 50(6), 1362–1367. <https://doi.org/10.1021/jf0111214i>.
- Belay, A., Haki, G. D., Birringer, M., Borck, H., Lee, Y. C., Cho, C. W., et al. (2017). Sugar profile and physicochemical properties of Ethiopian monofloral honey. *International Journal of Food Properties*, 20(11), 2855–2866. doi: 10.1080/10942912.2016.1255898.
- Berriel, V. (2018b). Carbon stable-isotope and physicochemical data as a possible tool to differentiate between honey-production environments in Uruguay. *Foods*, 7(86), <https://doi.org/10.3390/foods7060086www.mdpi.com/journal/foods>.
- Berriel, V., & Perdomo, C. H. (2019). Differentiating pasture honey from eucalyptus honey based on carbon isotopic data in Uruguay. *Heliyon*, 5(3), <https://doi.org/10.1016/j.heliyon.2019.e01228>.
- Berriel, V. (2018a). Effect of energetic incentive of bees with carbohydrates on the 13C isotopic composition of honeys from Uruguay. *Revista del Laboratorio Tecnológico del Uruguay*, 16, 47–50. <https://doi.org/10.26461/16.04>.
- Boffo, E. F., Tavares, L. A., Tobias, A. C. T., Ferreira, M. M. C., & Ferreira, A. G. (2012). Identification of components of Brazilian honey by 1H NMR and classification of its botanical origin by chemometric methods. *LWT - Food Science and Technology*, 49(1), 55–63. <https://doi.org/10.1016/j.lwt.2012.04.024>.
- Bogdanov, S., Ruoff, K., & Oddo, L. P. (2004). Physico-chemical methods for the characterisation of unifloral honeys: A review. *Apidologie*, 35, 4–17. <https://doi.org/10.1051/apido:2004047>.
- Bontempo, L., Camin, F., Ziller, L., Perini, M., Nicolini, G., & Larcher, R. (2017). Isotopic and elemental composition of selected types of Italian honey. *Measurements*, 98, 283–289. <https://doi.org/10.1016/j.measurement.2015.11.022>.
- Bouseta, A., Collin, S., & Dufour, J. P. (1992). Characteristic aroma profiles of unifloral honeys obtained with a dynamic headspace GC-MS system. *Journal of Apicultural Research*, 31(2), 96–109. <https://doi.org/10.1080/00218839.1992.11101268>.
- Bouseta, A., Scheirman, V., & Collin, S. (1996). Flavor and free amino acid composition of lavender and eucalyptus honeys. *Journal of Food Science*, 61(4), 683–687. <https://doi.org/10.1111/j.1365-2621.1996.tb12181.x>.
- Boussaid, A., Chouaibi, M., Rezig, L., Missaoui, R., Donsí, F., Ferrari, G., & Hamdi, S. (2015). Physicochemical, rheological, and thermal properties of six types of honey from various floral origins in Tunisia. *International Journal of Food Properties*, 18(12), 2624–2637. <https://doi.org/10.1080/10942912.2014.1001072>.
- Bueno-Costa, F. M., Zambiasi, R. C., Bohmer, B. W., Clavas, F. C., Padilha da Silva, W., Zanusso, J. T., & Dutra, I. (2016). Antibacterial and antioxidant activity of honeys from the state of Rio Grande do Sul, Brazil. *LWT - Food Science and Technology*, 65, 333–340. <https://doi.org/10.1016/j.lwt.2015.08.018>.
- Candau, P. (1987). Myrtaceae. In U. de Sevilla (Ed.), *Atlas polínico de Andalucía Occidental* (p. 450). Retrieved from <https://www.worldcat.org/title/atlas-polinico-de-andalucia-occidental/oclc/715541597?referer=di&ht=edition>.
- Castro-Vázquez, L., Díaz-Maroto, M. C., Guchu, E., & Pérez-Coello, M. S. (2006). Analysis of volatile compounds of eucalyptus honey by solid phase extraction followed by gas chromatography coupled to mass spectrometry. *European Food Research and Technology*, 224(1), 27–31. <https://doi.org/10.1007/s00217-006-0284-2>.
- Castro-Vázquez, L., Pérez-Coello, M. S., & Cabezudo, M. D. (2003). Analysis of volatile compounds of rosemary honey. Comparison of different extraction techniques. *Chromatographia*, 57(3–4), 227–233. <https://doi.org/10.1007/BF02491721>.
- Chakir, A., Romane, A., Marcazzan, G. L., & Ferrazzi, P. (2016). Physicochemical properties of some honeys produced from different plants in Morocco. *Arabian Journal of Chemistry*, 9, S946–S954. <https://doi.org/10.1016/j.arabjoc.2011.10.013>.
- Chen, L., Xue, X., Ye, Z., Zhou, J., Chen, F., & Zhao, J. (2011). Determination of Chinese honey adulterated with high fructose corn syrup by near infrared spectroscopy. *Food Chemistry*, 128(4), 1110–1114. <https://doi.org/10.1016/j.foodchem.2010.10.027>.
- Ciappini, C., & Calvino, A. (2018). An holistic view to develop descriptive sheets for Argentinean clover and eucalyptus unifloral honeys. *Current Nutrition & Food Science*, 14. <https://doi.org/10.2174/1573401314666180723161102>.
- 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. <https://doi.org/10.2478/jas-2014-0010>.
- Ciappini, M. C., & Vitelleschi, M. S. (2013). Palynological characteristics of eucalyptus (*Eucalyptus* spp.) and clover (*Trifolium* spp.) honeys from Argentinean pampean phytoecographic province. *Revista de La Facultad de Ciencias Agrarias*, 45(1), 247–258. Retrieved from http://revista.fca.uncu.edu.ar/images/stories/pdfs/2013-01/T45_01_20_Ciappini.pdf.
- Ciappini, M., Vitelleschi, M., & Calvino, A. (2016). Chemometrics classification of Argentine clover and eucalyptus honeys according to palynological, physicochemical, and sensory properties. *International Journal of Food Properties*, 19(1), 111–123. <https://doi.org/10.1080/10942912.2015.1020436>.
- Ciulu, M., Serra, R., Caredda, M., Salis, S., Floris, I., Pilo, M. I., et al. (2018). Chemometric treatment of simple physical and chemical data for the discrimination of unifloral honeys. *Talanta*, 190, 382–390. <https://doi.org/10.1016/j.talanta.2018.08.025>.
- Combarros-Fuertes, P., Estevinho, L. M., Dias, L. G., Castro, J. M., Tomás-Barberán, F. A., Tornadijo, M. E., et al. (2019). Bioactive components and antioxidant and antibacterial activities of different varieties of honey: A screening prior to clinical application. *Journal of Agricultural and Food Chemistry*, 67(2), 688–698. <https://doi.org/10.1021/acs.jafc.8b05436>.
- Coniglio, M. A., Laganà, P., Faro, G., & Marranzano, M. (2018). Antimicrobial potential of Sicilian honeys against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Journal of AOAC International*, 101(4), 956–959. <https://doi.org/10.5740/jaoacint.17-0450>.
- Cotte, J. F., Casabianca, H., Giroud, B., Albert, M., Lheritier, J., & Grenier-Loustalot, M. F. (2004). Characterization of honey amino acid profiles using high-pressure liquid chromatography to control authenticity. *Analytical and Bioanalytical Chemistry*, 378(5), 1342–1350. <https://doi.org/10.1007/s00216-003-2430-z>.
- D'Arcy, B. R., Rintoul, G. B., Catherine, Y., Rowland, C. Y., & Blackman, A. J. (1997). Composition of Australian honey extractives. 1. Norisoprenoids, monoterpenes, and other natural volatiles from Blue Gum (*Eucalyptus leucocylon*) and Yellow Box (*Eucalyptus melliodora*) honeys. *Journal of Agricultural and Food Chemistry*, 45(5), 1834–1843. <https://doi.org/10.1021/JF960625+>.
- Da Costa Leite, J. M., Trugo, L. C., Costa, L. S. M., Quintero, L. M. C., Barth, O. M., Dutra, V. M. L., et al. (2000). Determination of oligosaccharides in Brazilian honeys of different botanical origin. *Food Chemistry*, 70(1), 93–98. [https://doi.org/10.1016/S0956-7135\(99\)00115-2](https://doi.org/10.1016/S0956-7135(99)00115-2).
- De La Fuente, E., Ruiz-Matute, A. I., Valencia-Barrera, R. M., Sanz, J., & Martínez Castro, I. (2011). Carbohydrate composition of Spanish unifloral honeys. *Food Chemistry*, 129(4), 1483–1489. <https://doi.org/10.1016/j.foodchem.2011.05.121>.
- Devi, A., Jangir, J., & Appaiah, K. A. (2018). Chemical characterization complemented with chemometrics for the botanical origin identification of unifloral and multifloral honeys from India. *Food Research International*, 107, 216–226. <https://doi.org/10.1016/j.foodres.2018.02.017>.
- Di Bella, G., Lo Turco, V., Potorti, A. G., Bua, G. D., Fedè, M. R., & Dugo, G. (2015). Geographical discrimination of Italian honey by multi-element analysis with a chemometric approach. *Journal of Food Composition and Analysis*, 44, 25–35. <https://doi.org/10.1016/j.jfca.2015.05.003>.
- Di Girolamo, F., D'Amato, A., & Righetti, P. G. (2012). Assessment of the floral origin of honey via proteomic tools. *Journal of Proteomics*, 75(12), 3688–3693. <https://doi.org/10.1016/j.jprot.2012.04.029>.
- Di Marco, G., Gismondi, A., Panzarella, L., Canuti, L., Impei, S., Leonardi, D., et al. (2018). Botanical influence on phenolic profile and antioxidant level of Italian honeys. *Journal of Food Science and Technology*, 55(10), 4042–4050. <https://doi.org/10.1007/s13197-018-3330-8>.
- Dong, R., Zheng, Y., & Xu, B. (2013). Floral origin identification and amino acid profiles of Chinese unifloral honeys. *International Journal of Food Properties*, 16(8), 1860–1870. <https://doi.org/10.1080/10942912.2011.613510>.
- Downey, G. (2016). *Advances in food authenticity testing*. Woodhead Publishing, p. 798,

- doi: 10.1016/C2014-0-01962-4.
- Dümen, E., Akkaya, H., Öz, G. M., & Sezgin, F. H. (2013). Microbiological and parasitological quality of honey produced in Istanbul. *Turkish Journal of Veterinary and Animal Sciences*, 37(5), 602–607. <https://doi.org/10.3906/vet-1301-46>.
- Dżugan, M., Tomczuk, A., Sowa, P., & Grabek-Lejko, D. (2018). Antioxidant activity as biomarker of honey variety. *Molecules*, 23, 2069. <https://doi.org/10.3390/molecules23082069>.
- Erbani, T., Shcherbachenko, E., Talacko, P., & Harant, K. (2018). The unique protein composition of honey revealed by comprehensive proteomic analysis: Allergens, venom-like proteins, antibacterial properties, royal jelly proteins, serine proteases, and their inhibitors. *Journal of Natural Products*, 82(5), 1216–1226. <https://doi.org/10.1021/acs.jnatprod.8b00968>.
- Escuredo, O., Silva, L. R., Valentão, P., Seijo, M. C., & Andrade, P. B. (2012). Assessing Rubus honey value: Pollen and phenolic compounds content and antibacterial capacity. *Food Chemistry*, 130(3), 671–678. <https://doi.org/10.1016/j.foodchem.2011.07.107>.
- EU Directive. (2002). European Commission Council Directive 2001/110/EC of 20 December 2001 relating to honey. Official Journal of the European Communities, 10–47. Retrieved from <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:010:0047:0052:EN:PDF>.
- 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. <https://doi.org/10.1007/s00769-006-0128-6>.
- García-Tenesaca, M., Navarrete, E. S., Iturralde, G. A., Villacrés Granda, I. M., Tejera, E., Beltrán-Ayala, P., et al. (2018). Influence of botanical origin and chemical composition on the protective effect against oxidative damage and the capacity to reduce in vitro bacterial biofilms of monofloral honeys from the andean region of Ecuador. *International Journal of Molecular Sciences*, 19(1), <https://doi.org/10.3390/ijms19010045>.
- 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. <https://doi.org/10.1016/j.fct.2009.11.029>.
- Gül, A., & Pehlivan, T. (2018). Antioxidant activities of some monofloral honey types produced across Turkey. *Saudi Journal of Biological Sciences*, 25(6), 1056–1065. <https://doi.org/10.1016/j.sjbs.2018.02.011>.
- Hermosin, I., Chicón, R. M., & Cabezedo, M. D. (2003). Free amino acid composition and botanical origin of honey. *Food Chemistry*, 83(2), 263–268. [https://doi.org/10.1016/S0308-8146\(03\)00089-X](https://doi.org/10.1016/S0308-8146(03)00089-X).
- Hideux, M. (1972). Techniques d'étude du pollen an MED: Effets comparés des différents traite-ments physicochimiques. *Micron*, 3, 1–31.
- Ilyasov, R. A., Gaifullina, L. R., & Nikolenko, A. G. (2012). Review of the expression of antimicrobial peptide defensin in honey bees *Apis mellifera*. 56(1), 115–124. doi: 10.2478/v10289-012-0013-y.
- Jenkins, R., Burton, N., & Cooper, R. (2014). Proteomic and genomic analysis of methicillin-resistant staphylococcus aureus (MRSA) exposed to manuka honey in vitro demonstrated down-regulation of virulence markers. *Journal of Antimicrobial Chemotherapy*, 69(3), 603–615. <https://doi.org/10.1093/jac/dkt430>.
- Karabagias, I. K., Maia, M., Karabagias, V. K., Gatzias, I., & Badeka, A. V. (2018). Characterization of eucalyptus, chestnut and heather honeys from Portugal using multi-parameter analysis and chemo-calculus. *Foods*, 7(12), <https://doi.org/10.3390/foods7120194>.
- Kivrak, I. (2015). Free amino acid profiles of 17 Turkish unifloral honeys. *Journal of Liquid Chromatography and Related Technologies*, 38(8), 855–862. <https://doi.org/10.1080/10826076.2014.976712>.
- Kivrak, S., Kivrak, I., & Karababa, E. (2017). Characterization of Turkish honeys regarding of physicochemical properties, and their adulteration analysis. *Food Science and Technology*, 37(1), 80–89. <https://doi.org/10.1590/1678-457X.07916>.
- Lenhardt, L., Zeković, I., Dramićanin, T., Tešić, M., Milojković-Opsenica, D., & Dramićanin, M. D. (2014). Authentication of the botanical origin of unifloral honey by infrared spectroscopy coupled with support vector machine algorithm. *Physica Scripta*, T162. doi: 10.1088/0031-8949/2014/T162/014042.
- Leyva-Jimenez, F. J., Lozano-Sanchez, J., Borrás-Linares, I., Cadiz-Gurrea, M. de la L., & Mahmoodi-Khaledi, E. (2019). Potential antimicrobial activity of honey phenolic compounds against Gram positive and Gram negative bacteria. *LWT*, 101, 236–245. doi: 10.1016/j.lwt.2018.11.015.
- Li, S. F., Wen, R. Z., Yin, Y., Zhou, Z., & Shan, Y. (2013). Qualitative and quantitative detection of beet syrup adulteration of honey by near-infrared spectroscopy: A feasibility study. *Guang Pu Xue Yu Guang Pu Fen Xi/Spectroscopy and Spectral Analysis*, 33(10), 2637–2641. [https://doi.org/10.3964/j.issn.1000-0593\(2013\)10-2637-05](https://doi.org/10.3964/j.issn.1000-0593(2013)10-2637-05).
- Lolli, M., Bertelli, D., Plessi, M., Sabatini, A. G., & Restani, C. (2008). Classification of Italian honeys by 2D HR-NMR. *Journal of Agricultural and Food Chemistry*, 56(4), 1298–1304. <https://doi.org/10.1021/jf072763c>.
- Louveau, J., Maurizio, A., & Vorwohl, G. (1978). Methods of Melissopalynology. *Bee World*, 59(4), 139–157. <https://doi.org/10.1080/0005772x.1978.11097714>.
- Lusby, P. E., Coombes, A. L., & Wilkinson, J. M. (2005). Bactericidal activity of different honeys against pathogenic bacteria. *Archives of Medical Research*, 36(5), 464–467. <https://doi.org/10.1016/j.armed.2005.03.038>.
- Machado De-Melo, A. A., de Almeida-Muradian, L. B., Sancho, M. T., & Pascual-Maté, A. (2017). Composition and properties of *Apis mellifera* honey: A review. *Journal of Apicultural Research*, 57(1), 5–37. <https://doi.org/10.1080/00218839.2017.1338444>.
- Machado, A. M., Miguel, M. G., Vilas-Boas, M., & Figueiredo, A. C. (2020). Honey volatiles as a fingerprint for botanical origin – A review on their occurrence on monofloral honeys. *Molecules*, 25, 374 doi: 10.3390/molecules25020374.
- Mádaş, M. N., Märgitaş, L. A., Dezmierean, D. S., Bobiş, O., Abbas, O., Danthine, S., et al. (2020). Labeling regulations and quality control of honey origin: A review. *Food Reviews International*, 36(3), 1–26. <https://doi.org/10.1080/87559129.2019.1636063>.
- Malacalza, N. H., Caccavari, M. A., Fagúndez, G., & Lupano, C. E. (2005). Unifloral honeys of the province of Buenos Aires, Argentina. *Journal of the Science of Food and Agriculture*, 85(8), 1389–1396. <https://doi.org/10.1002/jsfa.2105>.
- Marceau, E., & Yaylayan, V. A. (2009). Profiling of α -Dicarbonyl content of commercial honeys from different botanical origins: Identification of 3,4-Dideoxyglucosone-3-ene (3,4-DGE) and related compounds. *Journal of Agricultural and Food Chemistry*, 57(22), 10844. <https://doi.org/10.1021/jf903341t>.
- Mato, I., Huidobro, J. F., Sánchez, M. P., Muniategui, S., Fernández-Muiño, M. A., & Sancho, M. T. (1997). Enzymatic determination of total D-gluconic acid in honey. *Journal of Agricultural and Food Chemistry*, 45(9), 3550–3553. <https://doi.org/10.1021/jf970012c>.
- Mato, I., Huidobro, J. F., Simal-Lozano, J., & Sancho, M. T. (2006b). Rapid determination of nonaromatic organic acids in honey by capillary zone electrophoresis with direct ultraviolet detection. *Journal of Agricultural and Food Chemistry*, 54(5), 1541–1550. <https://doi.org/10.1021/jf051757i>.
- Mato, I., Huidobro, J. F., Simal-Lozano, J., & Sancho, M. T. (2006a). Analytical methods for the determination of organic acids in honey. *Critical Reviews in Analytical Chemistry*, 36(1), 3–11. <https://doi.org/10.1080/10408340500451957>.
- Maurizio, A. (1939). Untersuchungen zur quantitativen pollenlyse des honing. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene*, 30, 27–69.
- McHugh, J. C. (2017). Sensory studies. In *Pediatric Electromyography: Concepts and clinical applications* (p. 31–48). doi: 10.1007/978-3-319-61361-1_4.
- Mendoza, N., & Silva, E. M. E. (2018). Introduction to phytochemicals: Secondary metabolites from plants with active principles for pharmacological importance. *Phytochemicals – Source of Antioxidants and Role in Disease Prevention*. <https://doi.org/10.5772/intechopen.78226>.
- Nascimento, K. S., Gasparotto Sattler, J. A., Lauer Macedo, L. F., Serna González, C. V., Pereira de Melo, I. L., da Silva Araújo, E., et al. (2018). Phenolic compounds, antioxidant capacity and physicochemical properties of Brazilian *Apis mellifera* honeys. *LWT – Food Science and Technology*, 91, 85–94. <https://doi.org/10.1016/j.lwt.2018.01.016>.
- Ortega-Bonilla, R. A., Chito-Trujillo, D. M., & Suárez-Ramos, C. A. (2016). Physicochemical characteristics of commercial eucalyptus honeys from Southwest Casanare. *Corpoica Cienc Tecnol Agropecuaria, Musquera (Colombia)*, 17(1), 73–80.
- Ouchemoukh, S., Schweitzer, P., Bachir Bey, M., Djoudad-Kadji, H., & Louaileche, H. (2010). HPLC sugar profiles of Algerian honeys. *Food Chemistry*, 121(2), 561–568. <https://doi.org/10.1016/j.foodchem.2009.12.047>.
- Pascual-Maté, A., Osés, S. M., Fernández-Muiño, M. A., & Sancho, M. T. (2018). Métodos analíticos en mieles. *Journal of Apicultural Research*, 57(1), 38–74. <https://doi.org/10.1080/00218839.2017.1411178>.
- Patrignani, M., Ciappini, M. C., Tananaki, C., Fagúndez, G. A., Thrasylvoulou, A., & Lupano, C. E. (2018). Correlations of sensory parameters with physicochemical characteristics of Argentinean honeys by multivariate statistical techniques. *International Journal of Food Science and Technology*, 53(5), 1176–1184. <https://doi.org/10.1111/ijfs.13694>.
- Patrignani, M., Fagúndez, G. A., Tananaki, C., Thrasylvoulou, A., & Lupano, C. E. (2018). Volatile compounds of Argentinean honeys: Correlation with floral and geographical origin. *Food Chemistry*, 246, 32–40. <https://doi.org/10.1016/j.foodchem.2017.11.010>.
- Perna, A., Simonetti, A., Intaglietta, I., Sofo, A., & Gambacorta, E. (2012). Metal content of southern Italy honey of different botanical origins and its correlation with polyphenol content and antioxidant activity. *International Journal of Food Science and Technology*, 47(9), 1909–1917. <https://doi.org/10.1111/j.1365-2621.2012.03050.x>.
- Petretto, G. L., Urgeghe, P. P., Mascia, I., Fadda, C., Rourke, J. P., & Pintore, G. (2017). Stir bar sorptive extraction coupled with GC/MS applied to honey: Optimization of method and comparative study with headspace extraction techniques. *European Food Research and Technology*, 243(5), 735–741. <https://doi.org/10.1007/s00217-016-2787-9>.
- Piaseznott, L., Gracco, L., & Conte, L. (2003). Solid phase microextraction (SPME) applied to honey quality control. *Journal of the Science of Food and Agriculture*, 83(10), 1037–1044. <https://doi.org/10.1002/jsfa.1502>.
- Pires, J., Estevinho, M. L., Feás, X., Cantalapiedra, J., & Iglesias, A. (2009). Pollen spectrum and physico-chemical attributes of heather (*Erica spp.*) honeys of north Portugal. *Journal of the Science of Food and Agriculture*, 89(11), 1862–1870. <https://doi.org/10.1002/jsfa.3663>.
- Ramanauskiene, K., Stelmakiene, A., Briedis, V., Ivanauskas, L., & Jakštas, V. (2012). The quantitative analysis of biologically active compounds in Lithuanian honey. *Food Chemistry*, 132(3), 1544–1548. <https://doi.org/10.1016/j.foodchem.2011.12.007>.
- Ramos, O. Y., Salomón, V., Libonatti, C., Cepeda, R., Maldonado, L., & Basualdo, M. (2018). Effect of botanical and physicochemical composition of Argentinean honeys on the inhibitory action against food pathogens. *LWT – Food Science and Technology*, 87, 457–463. <https://doi.org/10.1016/j.lwt.2017.09.014>.
- Ribeiro, R. de O. R., Mársico, E. T., Carneiro, C. da S., Monteiro, M. L. G., Conte Júnior, C. A., Mano, S., et al. (2014). Classification of Brazilian honeys by physical and chemical analytical methods and low field nuclear magnetic resonance (LF 1H NMR). *LWT – Food Science and Technology*, 55(1), 90–95. doi: 10.1016/j.lwt.2013.08.004.
- Rodrigues 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. <https://doi.org/10.1080/10942912.2013.790050>.
- Rodríguez de la Cruz, D., Sánchez Reyes, E., Sánchez Durán, S., & Sánchez Sánchez, J. (2013). Análisis palinológicos de mieles monoflorales comerciales. *Botanica Complutensis*, 37, 171–180.

- Rodríguez, B. A., Mendoza, S., Iturriga, M. H., & Castaño-Tostado, E. (2012). Quality parameters and antioxidant and antibacterial properties of some Mexican honeys. *Journal of Food Science*, 77(1), <https://doi.org/10.1111/j.1750-3841.2011.02487.x>.
- Rodríguez, L., Serrano, S., Galán-Soldevilla, H., Piva, G., & Ubera, J. L. (2015). Sensory analysis integrated by palynological and physicochemical determinations plays a key role in differentiating unifloral honeys of similar botanical origins (*Myrtaceae* honeys from southern Spain). *International Journal of Food Science and Technology*, 50(7), 1545–1551. <https://doi.org/10.1111/ijfs.12802>.
- Roshan, N., Rippers, T., Locher, C., & Hammer, K. A. (2017). Antibacterial activity and chemical characteristics of several Western Australian honeys compared to manuka honey and pasture honey. *Archives of Microbiology*, 199(2), 347–355. <https://doi.org/10.1007/s00203-016-1308-3>.
- Rossano, R., Larocca, M., Polito, T., Perna, A. M., Padula, M. C., Martelli, G., & Riccio, P. (2012). What are the proteolytic enzymes of honey and what they do tell us? A fingerprint analysis by 2-D zymography of unifloral honeys. *PLoS ONE*, 7(11), e49164. <https://doi.org/10.1371/journal.pone.0049164>.
- Sanchez Reyes, E., & Sanchez Sanchez, J. (2017). Botanical classification. In *Bee Products – Chemical and Biological Properties* (pp. 3–19). doi: 10.1007/978-3-319-59689-1_1.
- Sant'Ana, L. D. O., Sousa, J. P. L. M., Salgueiro, F. B., Lorenzon, M. C. A., & Castro, R. N. (2012). Characterization of monofloral honeys with multivariate analysis of their chemical profile and antioxidant activity. *Journal of Food Science*, 77(1). doi: 10.1111/j.1750-3841.2011.02490.x.
- Santos-Buelga, C., & González-Paramás, A. M. (2017). Chemical composition of honey. In *Bee Products – Chemical and Biological Properties* (pp. 43–82). https://doi.org/10.1007/978-3-319-59689-1_3.
- Scepankova, H., Saraiva, J. A., & Estevinho, L. M. (2017). Honey health benefits and uses in medicine. In *Bee products – Chemical and biological properties* (pp. 83–96). doi: 10.1007/978-3-319-59689-1_4.
- Seijo, M. C., Aira, M. J., & Méndez, J. (2003). Palynological differences in the pollen content of Eucalyptus honey from Australia, Portugal and Spain. *Grana*, 42(3), 183–190. <https://doi.org/10.1080/00173130310011784>.
- Sereia, M. J., de Toledo, V. A. A., Marchini, L. C., Alves, E. M., Faquinello, P., & de Toledo, T. C. S. O. A. (2010). Microorganisms in organic and non organic honey samples of Africanized honeybees. *Journal of Apicultural Science*, 54(1), 49–54.
- 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. <https://doi.org/10.1016/j.foodchem.2004.01.031>.
- Sniderman, J. M. K., Matley, K. A., Haberle, S. G., & Cantrill, D. J. (2018). Pollen analysis of Australian honey. *PLoS ONE*, 13(5), e0197545. <https://doi.org/10.1371/journal.pone.0197545>.
- Soria, A. C., Sanz, J., & Martínez-Castro, I. (2009). SPME followed by GC-MS: A powerful technique for qualitative analysis of honey volatiles. *European Food Research and Technology*, 228(4), 579–590. <https://doi.org/10.1007/s00217-008-0966-z>.
- Suárez-Luque, S., Mato, I., Huidobro, J. F., Simal-Lozano, J., & Sancho, M. T. (2002). Rapid determination of minority organic acids in honey by high-performance liquid chromatography. *Journal of Chromatography A*, 955(2), 207–214. [https://doi.org/10.1016/S0021-9673\(02\)00248-0](https://doi.org/10.1016/S0021-9673(02)00248-0).
- Tavares, A., Beiroz, W., Fialho, A., Frazão, F., Macedo, R., Louzada, J., & Audino, L. (2019). Eucalyptus plantations as hybrid ecosystems: Implications for species conservation in the Brazilian Atlantic forest. *Forest Ecology and Management*, 433, 131–139. <https://doi.org/10.1016/j.foreco.2018.10.063>.
- 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. <https://doi.org/10.1046/j.1365-2621.2003.00715.x>.
- Tričković, J., Andrić, F., Ristivojević, P., Guzelmerić, E., & Yesilada, E. (2017). Analytical methods in tracing honey authenticity. *Journal of AOAC International*, 100(4), 827–839. <https://doi.org/10.5740/jaoacint.17-0142>.
- Valdés-Silverio, L. A., Iturralde, G., García-Tenesaca, M., Paredes-Moreta, J., Narváez-Narváez, D. A., Rojas-Carrillo, M., et al. (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*, 57(3), 382–394. <https://doi.org/10.1080/00218839.2018.1426349>.
- Verzera A, Campisi S, & Zappalà M, B. I. (2001). SPME-GC-MS analysis of honey volatile components for the characterization of different floral origin. *American Laboratory*, 33(15), 18–21.
- Von Der Ohe, W., Persano Oddo, L., Piana, M. L., Morlot, M., & Martin, P. (2004). Harmonized methods of melissopalynology. *Apidologie*, 35(Suppl. 1), S18–S25. <https://doi.org/10.1051/apido:2004050>.
- Yao, L., Jiang, Y., D'Arcy, B., Singanusong, R., Datta, N., Caffin, N., & Raymont, K. (2004). Quantitative High-Performance Liquid Chromatography analyses of flavonoids in Australian Eucalyptus honeys. *Journal of Agricultural and Food Chemistry*, 52(2), 210–214. <https://doi.org/10.1021/jf034990u>.
- Záborská, B., & Vorlová, L. (2014). Adulteration of honey and available methods for detection – A review. *Acta Veterinaria Brno*, 83, S85–S102. <https://doi.org/10.2754/avb201483S10S85>.
- Zalibera, M., Staško, A., Šlebodavá, A., Jančovičová, V., Čermáková, T., & Brezová, V. (2008). Antioxidant and radical-scavenging activities of Slovak honeys: An electron paramagnetic resonance study. *Food Chemistry*, 110, 512–521. <https://doi.org/10.1016/j.foodchem.2008.02.015>.
- Zhu, X., Li, S., Shan, Y., Zhang, Z., Li, G., Su, D., & Liu, F. (2010). Detection of adulterants such as sweeteners materials in honey using near-infrared spectroscopy and chemometrics. *Journal of Food Engineering*, 101(1), 92–97. <https://doi.org/10.1016/j.jfoodeng.2010.06.014>.