

19. Cosmetic properties of honey. 1. Antioxidant activity

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Abstract

Honey components (sugars, aminoacids, lactic acid) may behave as humectants and also enhance humectant properties in a formulation. Active principles of functional properties in honey vary according to visited flora, type of bee and localities where honey is produced. Studies of honey in dermo-cosmetic applications are scarce. In this work we review the topical use of honey through history, cutaneous interventions with honey, and toxicity. Hydrating power was measured with a corneometer in emulsions prepared with six honey types. Polyphenol contents and antioxidant properties of honey were measured to contrast *Apis mellifera*, imitation honey and pot-honey types of *Tetragonula carbonaria* and three species of *Melipona*, with two methods (DPPH and TEAC). The Australian pot-honey of *Tetragonula carbonaria* was the most active with the DPPH method (IC₅₀ 83.33 mg/ml) and the Brazilian pot-honey of *Melipona fasciculata* was the most active with the TEAC method (307.2 μ moles Trolox equivalents/100g honey) as well as in polyphenol contents (718.7 mg gallic acid/100 g honey), and higher hydrating power of the emulsion (51.23%).

Keywords:

Antioxidant activity, cosmetic properties, DPPH, polyphenols, humectant, hydrating, TEAC

Introduction

The difference between humectant and hydrating properties is sometimes unclear because these terms are often misused. A humectant is a substance that absorbs or helps to retain moisture from the environment in the stratum corneum of the skin –the

outermost layer. A moisturizer (hydrating) is a substance that imparts or restores water to the deep layers of the skin (Pareja, 2002).

Cosmetics only have humectant properties; however, functional cosmetics are able to hydrate

transdermic layers of skin. Honey can also do this. Antibacterial properties of honey explain its use in treating burns, sores and wounds in modern dermatology. Generally speaking, honey uses the osmotic power of sugar as well as its emollient properties to soften the skin, and increase blood circulation (Proserpio, 1981). The presence of hydroxyl groups enhances both humectant and antioxidant activities. Humectant activity is due to presence of small polyol molecules such as glycerin, propylene glycol and sorbitol, commonly used as solvents in cosmetic formulations (Bikowski, 2001; Draelos, 2010). Honey components (sugars, aminoacids, lactic acid) may behave as humectants and also enhance humectant properties in a formulation. The polyphenols present in honey are also rich in hydroxyls, and display antioxidant activity by proton release (acidic hydrogen). For these reasons, humectant and antioxidant properties are explained by the hydroxylated nature of honey.

Physico-chemical and sensory characteristics of European unifloral honeys have been carefully reported (Persano Oddo and Piro, 2004). Therapeutic use of honey is also validated by the antioxidant properties of honey, the composition of active principles such as flavonoids, polyphenols, vitamins, carboxylic acids, and the metallic matrix (Bogdanov, 2012a). As a natural product, honey may also have toxic components and cause allergic reactions (Dutau and Rance, 2009) which can be prevented with routine quality control and test.

Different bees produce different honeys. Several bee species produce and store honey in beeswax combs (*Apis cerana*, *Apis dorsata*, *Apis florea*, *Apis mellifera*) (Michener, 2000) while a great diversity of stingless bees (Meliponini) store honey in cerumen (a mixture of plant resin and beeswax) pots (Camargo and Pedro, 2007; Persano Oddo et al., 2008). Differences in plant type, climate, environmental conditions, botanical source and geographical area affect both cerumen and nectar composition, adding more complexity to active principles of pot-honey (Küçük et al., 2007). The botanical origin of honey impacts on the functional properties due to the presence of plant derived products. However, the entomological origin also explains differences attributed to bee species, as demonstrated for stingless bees (Rodríguez-Malaver et al., 2007) and *Apis dorsata* producing Tualang honey (Krishna Kishorea et al., 2011) compared to *Apis mellifera*.

In this chapter we review historical aspects of topic uses, cutaneous interventions, polyphenol content and antioxidant properties of honey, compared to honey of *A. mellifera*, imitation honey and pot-honey of *Tetragonula carbonaria* and three species of *Melipona*, as indicated in Table 1. Honey emulsions were prepared with these honeys and the hydrating power was measured besides honey polyphenol content, and antioxidant activity by DPPH and TEAC methods.

Table 1. Honey samples evaluated

Honey	Common name of bees	Bee species	Country	Color
1	Honeybees	<i>Apis mellifera</i>	Venezuela	light amber
2	"imitation"	"McLaus"	Venezuela	light amber
4	Tiúba	<i>Melipona fasciculata</i>	Moura, Brasil	dark amber
6	Isabitto	<i>Melipona aff. fuscopilosa</i>	Venezuela	light amber
5	Jandaíra	<i>Melipona subnitida</i>	Brasil	creamy
3	Carby	<i>Tetragonula carbonaria</i>	Australia	light amber

19.1 Topic use of honey through history

Egyptians, Greeks, Romans and oldest civilizations of the Indus Valley used honey as long as 9000 years. The use of honey as a medicine is referred to in the most ancient written records. Honey was prescribed by the physicians of many ancient civilizations for a

wide variety of diseases (Molan 1999). Even today honey is used in folk medicine, as a traditional therapy for infected leg ulcers, earache, topical treatment of measles, gastric ulcers, wound healing and many other ailments (Bogdanov et al., 2008).

There is a growing interest in the study of honey for its cosmetic properties because it is a natural product that can be substituted for artificial products and used by pharmaceutical and cosmetic industry. Cosmetic use of honey for skin care began –as already mentioned– in ancient times in Egypt, Greece and Rome, especially as a natural moisturizer, due to its emollient, nourishing and antiseptic properties. Honey is used alone or combined with other substances such as milk, eggs, oats, lemon juice, apple, olive oil (Saraf, 2012). In fact, honey was used in formulations of balms, masks, ointments after bathing, facial lotions, among others things. In ancient civilizations honey was considered a food for the gods, and was used like money to pay taxes. According to Pythagoras and Democritus, honey was a source of longevity and intellectual power (Young 2005). At the present time, honey is present in a wide number of cosmetics for different parts of body. The hand cream with honey is very nutritious, which functions to enrich and to protect damaged skin. Some honey based lipsticks are formulated for chapped lip treatment, and shampoo with honey is considered moisturizer of hair fiber (Davis and Perez, 2009; Saraf, 2012).

19.2 Cutaneous interventions with honey and honey products

Ethnopharmacological applications of honey in dermatology have esthetic and medicinal values because honey has systematic uses in specific cultural groups. Belscak et al. (2009) demonstrated that the addition of ascorbic acid enhances the anti-oxidant capacity of fruit teas while the addition of honey causes a decrease in the total phenol content. This may adversely affect the radical scavenging potential of analyzed fruit teas. This observation begs the question of the actual benefit from honey in a hydrating cream. Isla et al. (2011) proposed a nanoemulsion system oil-water-surfactant alone, with and without *Apis mellifera* honey to evaluate the hydrating power with a corneometer –based on the capacitance value. They compared the nanoemulsion hydrating percentages. Later, this experiment was conducted with ten female 20-30 years-old. The base emulsion consisted in magistral proportions of fat phase, aqueous phase, surfactants, conservatives, with aqueous vehicle approximately in a proportion 60:35:4:1. To this base, 15% (w/w) of six honey types (see Table 1) were added with a mixer until honey was completely integrated into the emulsion. This honey emulsion was applied with a syringe in a fixed exposure area of the skin to measure hydrating

power with a corneometer (Derma Unit SSC 3, Köln, Germany). For that purpose 0.1 g of honey emulsion was measured with a syringe, dropped onto the skin, and massaged lightly to help skin of inner forearm absorb the emulsion. Measurements were taken up to two hours after application, at 30, 60, and 120 min. The initial average of hydrating power of the skin was 40.45. Hydrating percentages of the six honey emulsions are shown in Table 2.

Table 2. Average of hydrating percentages of six honey emulsions

Honey	Bee species	Time after emulsion application (min)		
		30	60	120
1	<i>Apis mellifera</i>	51.90	52.20	49.93
2	imitation honey	48.23	50.08	50.03
4	<i>Melipona fasciculata</i>	46.93	48.98	51.23
6	<i>Melipona aff. fuscopilosa</i>	45.30	47.65	47.93
5	<i>Melipona subnitida</i>	46.88	48.80	49.53
3	<i>Tetragonula carbonaria</i>	46.20	48.60	47.28

The sugar composition of honey confers hygroscopic properties, fixing water molecules. This provides a clear and protective film –known as occlusive film– that helps to maintain skin hydration, without being a fatty substance. In some cases it acts as an astringent, where the chemical compounds tend to shrink or constrict tissues, usually locally, after topical, medicinal application (Ahshawat et al., 2008). In addition, honey nourishes internal epithelial tissues and activates superficial circulation; thus preventing dry skin, wrinkles and impurities. Honey can be used externally due to its antimicrobial and antiseptic properties, and it helps healing in wounds and superficial burns (Olaitan et al., 2007). All honeys contain vitamin E and K, thiamine, riboflavin, and mineral elements like iron, cooper, manganese, phosphorous, calcium and sulfur. Those ingredients can also be related to the hydrating properties of honey (Ciulu et al., 2011; Tuberoso et al., 2012) and surfactive actions (Miñana and Goncalves, 2011).

19.3 Polyphenol contents of honey

A wide range of phenolic constituents in honey are known: quercetin, caffeic acids, caffeic acid phenethyl ester (CAPE), acacetin, kaempferol, galangin, chrysin, acacetin, pinocembrin, pinobanksin and apigenin. They have promising effect in the treatment of some chronic diseases due to their antioxidant activity. In general, most of the phenolic compounds found in honey are in the form of flavonoids (Küçük et al., 2007) and confer beneficial functional properties.

Different plants have diverse spectra of phenolic compounds and cause variation in the total phenolic content of honey (Blum, 1996). For example, for *A. mellifera* honeys, total phenolic content of honey from northeastern Brazil varied from 10.21 to 108.5 mg gallic acid equivalents (GAE)/100g honey (Tavares et al, 2011), similar to flavonoid content of honey from Burkina Faso (32.59-114.75 mg of GAE/100 g honey) (Meda et al., 2005). However, substantial differences were observed in honey from Chile, with total phenolic content varying from 0.0 to 8.83 mg/100 g of honey (Muñoz and Copaja, 2007), honey samples of different floral origins from Poland with 21.7-75.3 mg GAE/100 g of honey (Socha et al., 2009); whereas honey samples from Slovenia varied between 44.8 and 241 mg GAE/100 g of honey (Bertoncelj et al., 2007).

The total polyphenol content was analyzed by spectrometry at 765 nm using Folin-Ciocalteu (Sigma-Aldrich, USA) reagent (Singleton et al., 1999). In this method, 100 μ L of honey was mixed with 500 μ L of Folin-Ciocalteu's reagent diluted 1/10 with water, and 400 μ L of sodium carbonate (Sigma, Steinheim, Germany) 7.5% (w/v) were added. Absorbance at 765 nm was recorded after 10 min of reaction at 37°C, against a blank with MQ water instead of ethanolic extract. The polyphenol concentration was estimated with a calibration curve using a solution of 0.1 g/L of gallic acid (Sigma, Steinheim, Germany) as standard (0, 0.25, 0.05 and 0.1 g/L). The results are presented in Table 3.

Table 3. Polyphenols content of six honeys of different entomological origin

Honey types	Polyphenols mg GAE/100 g honey
<i>Apis mellifera</i>	502.8 \pm 5.1
imitation honey	529.1 \pm 6.6
<i>Melipona aff. fuscopilosa</i>	534.9 \pm 5.9
<i>Melipona fasciculata</i>	718.7 \pm 3.1
<i>Melipona subnitida</i>	550.5 \pm 5.2
<i>Tetragonula carbonaria</i>	518.2 \pm 6.4

The polyphenol content varied between 502.8 and 718.7 mg GA/100 g honey. The highest polyphenol content was observed in the *Melipona fasciculata* honey, while the remaining honeys contained 500-550 mg GA/100 g honey. These are high values compared to previously reported data on total polyphenol contents of pot-honeys. For example, the polyphenol content of honey from *Tetragonula carbonaria* in Australia was 48.53-63.43 mg

GAE/100g honey (Persano Oddo et al., 2008), *Tetragonisca fiebrigi* in Argentina varied between 144.22-431.20 mg GAE/100g honey, while in Paraguay it varied between 125.17 and 176.50 mg GAE/100g honey (Vit et al., 2009). In another study, honeys from different species of stingless bees from Perú had polyphenol contents in the range of 99.7 to 464.9 mg GAE/100g honey (Rodríguez-Malaver et al., 2009). The relationship between phenolics and functional properties considering several foods –antioxidant capacity, antibacterial capacity, antiviral capacity, anti-inflammatory capacity, cardio-protective effects and the prevention of enzymatic browning– has been demonstrated (Ojofeitimi et al., 2004).

19.4 Antioxidant activity as a functional property of honey

Honey is a hygroscopic sugary matrix with antibacterial, antifungal and nurturing properties for the skin. Anti-inflammatory, immunomodulating, antitumor and prebiotic functions of honey have been studied and vary according to its botanical origin (Bogdanov, 2012b). The antioxidant activity of honey has been reviewed recently (Erejuwa et al., 2012) and several methods are used for that purpose: 1. DPPH (named after the reagent 2,2-diphenyl-1-picrylhydrazyl), 2. FRAP (Ferric Reducing Antioxidant Power), 3. ORAC (Oxygen Radical Absorbance Capacity), 4. RSA (Radical Scavenging Activity), 5. TEAC (Trolox Equivalent Antioxidant Capacity). Several authors use DPPH and TEAC methods to measure antioxidant activity of honey. Sant'Ana et al. (2012) measured antioxidant activity of 21 monofloral honeys from Brazil using DPPH, FRAP and TEAC methods. That group found strong correlations between the results obtained by the three methods ($-0,6684 \leq r \leq -0,8410$, $P < 0,05$) and similarity among FRAP, TEAC, and DPPH analyses. Similar results are observed by Rodríguez et al. (2012) for Mexican honeys and Chang et al. (2011) for sixteen different unifloral honeys from China. In these works the total polyphenol contents were highly correlated to their antioxidant capacity values. Below we compare the antioxidant activity measured by the DPPH and the TEAC methods.

19.4.1 Antioxidant activity by the DPPH method

The antioxidant activity of six honeys was measured by the scavenging activity of DPPH free radicals. This method is based on the absorbance reduction of the radical anion DPPH[•] by antioxidants and was developed by Brand-Williams et al. (1995), modified

by Díaz et al. (2011). Triplicates of honey dilutions 100 mg/ml were prepared. Water dilutions of honey (25-50-75-100 µg/mL) were mixed with 2.8 mL of 2, 2-diphenyl-1-picrylhydrazyl DPPH 60 µM (Sigma Aldrich, Canadá), incubated for 30 minutes in the dark, using ascorbic acid (176 µg/mL) as a positive control, and absorbance was measured at 517 nm. In Table 4 results are expressed as the concentration of the extracts necessary to inhibit the initial DPPH concentration by 50% (IC₅₀).

Table 4. DPPH inhibition of honeys

Honey types	% inhibition DPPH	IC ₅₀ (mg/mL)
<i>Apis mellifera</i>	16	-
imitation honey	21	-
<i>Tetragonula carbonaria</i>	71	83.33
<i>Melipona fasciculata</i>	44	-
<i>Melipona subnitida</i>	15	-
<i>Melipona aff. fuscopilosa</i>	18	-
ascorbic acid	89	97

These results show a good free radical scavenging activity for the *Tetragonula carbonaria* honey with 71% inhibition of DPPH at the concentration of 100 mg/mL, compared to the positive control ascorbic acid with 89% inhibition of DPPH. Considering this result, a concentration curve in order to determine the inhibitory concentration by 50% (IC₅₀). The water dilutions of honey (25-50-75-100 µg/mL) in triplicate were mixed with 2.8 mL 2, 2-diphenyl-1-picrylhydrazyl DPPH 60 µM, incubated for 30 minutes in the dark, using ascorbic acid (176 µg/mL) as positive control, and absorbance was recorded at 517 nm with a spectrophotometer (Sigma Aldrich, USA). Results were expressed as the concentration of the extracts to inhibit the necessary initial DPPH concentration by 50% (IC₅₀). The results in Figure 1 show that the DPPH free radical sequestering activity is concentration dependent. The *T. carbonaria* honey IC₅₀ of 83.33 mg/ml means very good antioxidant properties by this method.

Indeed, *Tetragonula angustula* honey had a high content of non-aromatic organic acids and aromatic substances such as flavonoids and polyphenols (Persano Oddo et al., 2008). Therefore, we may suggest they influence observed antioxidant activity. Our results are higher than DPPH depletion of 48.03 ± 12.58 equivalents of ascorbic acid previously reported by Persano Oddo et al. (2008), both in the range of *Rhododendron* honey of *Apis mellifera* from Turkey with averages between 36.11 and 90.73 % inhibition DPPH (Silici et al., 2010).

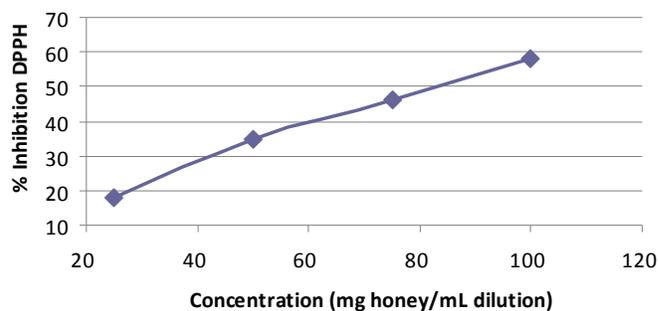


Figure 1. DPPH free radical sequestering activity of *Tetragonula carbonaria* honey

In DPPH assay, antioxidants reduce the free radical 2,2-diphenyl-1-picrylhydrazyl. In the presence of an antioxidant, the purple color of DPPH[•] fades and the change of absorbance can be followed spectrophotometrically at 515 nm (Schlesier et al. 2002). The scavenging activity of honey for DPPH[•] has been evaluated by several authors following the methodology described by others (Meda et al., 2005; Khalil et al., 2012). The DPPH analysis is a quick and simple test; it guarantees reliable results and needs only a UV-vis spectrophotometer, which likely explains its widespread use in antioxidant screening. However, interpretation is complicated when the test compounds have spectra that overlap DPPH at 515 nm. Carotenoids, present in honey, can interfere. Despite the abovementioned limitations, DPPH[•] is stable, commercially available, and does not have to be generated before assay unlike ABTS^{•+} as the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) is known. For these reasons it could be considered an easy and useful spectrophotometric method with regard to screening/measuring antioxidant capacity in honey (Alvarez-Suarez et al., 2009).

19.4.2 Total antioxidant activity by the TEAC method

TEAC has been measured in honeys from Malaysia (Aljadi y Kamaruddin, 2004), Slovenia (Bertoncelj et al. 2007), Brazil (Lianda et al., 2012), Italy (Perna et al., 2012), Mexico (Rodríguez et al., 2012), and southern Africa (Serem and Bester, 2012), among others. This assay measures total radical scavenging capacity based on the ability of a compound to scavenge the stable ABTS radical cation (ABTS^{•+}). ABTS^{•+} was produced by reacting ABTS with potassium persulphate (K₂S₂O₈) (Schlesier et al. 2002). The TEAC assay is considered an easy and accurate method for measuring the radical scavenging ability of honey by hydrogen-donation reactions (Alvarez-Suarez et al. 2009).

In Table 5 the TEAC values of honey showed a different order of antioxidant activity than DPPH. Here the most active honey is *Melipona fasciculata*, followed by fake honey and *Melipona aff. fuscopilosa*, half of the higher TEAC value is for *Melipona subnitida*, and lower values were observed for *Tetragonula carbonaria* and *Apis mellifera*.

Table 5. TEAC of honeys

Honey types	μ moles Trolox equivalents/100g (TEAC)
<i>Apis mellifera</i>	113.0 \pm 2.1
imitation honey	225.3 \pm 1.3
<i>Tetragonula carbonaria</i>	127.4 \pm 5.0
<i>Melipona fasciculata</i>	307.2 \pm 4.4
<i>Melipona subnitida</i>	164.3 \pm 7.4
<i>Melipona aff. fuscopilosa</i>	221.6 \pm 6.3

The moisturizing power of the honey emulsions (Table 2) was related to the antioxidant activity of honey measured as Trolox equivalents based on the scavenging ability of free radicals ($-\text{OH}^\cdot$, $\text{O}_2^{\cdot-}$, H^+) in Table 5. Therefore some of the honey components contributing to moisturizing power are also contributing to antioxidant activity (e.g. the presence of molecules with hydroxyls such as polyphenols, flavonoids, and especially sugars that are the major components of honey). For this reason, the emulsion of fake honey also exhibited a high hydrating power compared with genuine honeys.

Several authors proposed that the antioxidant capacity of honey is due mainly to the phenolic compounds and flavonoids they contain, and there is a high correlation between polyphenols and honey antioxidant capacity, demonstrated in honeys from different floral, geographic and entomological origins (Alzahrani et al. 2012). However, the exact mechanism of action is unknown. Free radical sequestration, hydrogen donation, metallic ion chelation, or their action as substrate for radicals such as superoxide and hydroxyl, interference with propagation reactions, or inhibition of the enzymatic systems involved in initiation reactions have been proposed as steps contributing to antioxidant activity (Al-Waili et al. 2011). In this sense, all bioactivities related to honey can be attributed to its antioxidant activity explained by polyphenol and flavonoid content. Polyphenols in honey contain hydroxyl groups that increase humectant properties of these components, but also are related to antioxidant activity (Khalil et al. 2011). Further investigation is warranted into the relationship between honey antioxidant activity and its humectant and hydrating properties, especially related to different herbal

(Socha et al., 2009) and floral origins (Tavares et al., 2011; Serem et al., 2012).

On the other hand, the average 65-80% sugar composition of honey confers strong hygroscopic properties, fixing water molecules and acting as a protective film. In addition, honey is an aliment of internal epithelial tissues and an activator of superficial circulation. Thus, prevent dry skin, wrinkles and impurities. Micronutrients of honey also add to hydrating properties of skin.

19.5 Honey toxicity

Toxicity of honey is not a major concern for honey consumers, but the industry has to survey the quality of honey in dermocosmetic. Microorganisms and toxic residues in honey used as an ingredient of a drug or cosmetic can affect the shelf-stability and safety of the final product (Bogdanov, 2006). Therefore product specifications for honey derivatives such as humectant creams are needed (Snowdon, 1999). Contamination with pesticides and veterinary antibiotics is a challenging problem that needs to be fully addressed (Al-Waili et al., 2012). Antibiotics endanger human health by increasing drug-resistant adaptations in microorganisms. To ensure human food safety, antibiotics are not allowed in the management of bees used for honey production, and residue control is achieved with multimethods (Bohm et al., 2012).

Honey allergy seems relatively uncommon; allergies reported can involve reactions varying from cough to anaphylaxis (Sirnik et al., 1978). In this study it was reported that patients allergic to pollen are rarely allergic to honey, although there is one reported case of combined honey pollen allergy (Bousquet et al., 1984). The incidence of honey allergy, reported in a group of 173 food allergy patients was 2.3%. In this study the honey allergy is explained by the presence of components of bee origin.

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