

# Functional Properties of Honey, Propolis, and Royal Jelly

M. VIUDA-MARTOS, Y. RUIZ-NAVAJAS, J. FERNÁNDEZ-LÓPEZ, AND J.A. PÉREZ-ÁLVAREZ

**ABSTRACT:** Honey, propolis, and royal jelly, products originating in the beehive, are attractive ingredients for healthy foods. Honey has been used since ancient times as part of traditional medicine. Several aspects of this use indicate that it also has functions such as antibacterial, antioxidant, antitumor, anti-inflammatory, antibrowning, and antiviral. Propolis is a resinous substance produced by honeybees. This substance has been used in folk medicine since ancient times, due to its many biological properties to possess, such as antitumor, antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory effects, among others. Royal jelly has been demonstrated to possess numerous functional properties such as antibacterial activity, anti-inflammatory activity, vasodilative and hypotensive activities, disinfectant action, antioxidant activity, antihypercholesterolemic activity, and antitumor activity. Biological activities of honey, propolis, and royal jelly are mainly attributed to the phenolic compounds such as flavonoids. Flavonoids have been reported to exhibit a wide range of biological activities, including antibacterial, antiviral, anti-inflammatory, antiallergic, and vasodilatory actions. In addition, flavonoids inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility, and the activity of enzyme systems including cyclo-oxygenase and lipoxygenase.

**Keywords:** antimicrobial, functional properties, honey, phenolic compounds antioxidant, propolis

## Introduction

Recent years have seen growing interest on the part of consumers, the food industry, and researchers into food and the ways in which it may help maintain human health. The important role that diet plays in preventing and treating illness is widely accepted. The basic concepts of nutrition are undergoing significant change. The classical concept of "adequate nutrition," that is, a diet that provides nutrients (carbohydrates, proteins, fats, vitamins, and minerals) in sufficient quantities to satisfy particular organic needs, is tending to be replaced by the concept of "optimal nutrition," which includes, besides the above, the potential of food to promote health, improve general well-being, and reduce the risk of developing certain illnesses. This is where functional foods, also known as nutraceuticals, designed foods, therapeutic foods, superfoods, or medicinal foods, play their part (Nagai and Inoue 2004).

The concept of functional food is complex since it covers many different aspects: for example, the term may refer to foods obtained by any procedure, with the particular characteristic that one at least of the components, whether or not a nutrient in itself, affects target functions of the organism, so that it positively and specifically promotes a physiological or psychological effect over and above its traditional nutritive value. This positive effect may arise from a contribution to the maintenance of health and well-being, such as a reduction in the risk of suffering a given illness (Pérez-Álvarez and others 2003).

The market for functional foods is increasing at an annual rate of 15% to 20% (Hilliam 2000). A functional food may be natural or

be obtained by eliminating or modifying one or more of its basic components (Perez-Alvarez and others 2003). Many components, too, may be added to food to make them "functional" among them  $\omega$ -3 fatty acids (Hjaltason and Haraldsson 2006; Jacobsen and Let 2006), vitamins (Baro and others 2003), probiotics (Chaila and others 2005; Salem and others 2006), prebiotics (Brink and others 2005; Malcata and others 2005), symbiotics (D'Antoni and others 2004), fibre (Fernández-Gines and others 2004; Fernández-López and others 2004, 2007), phytochemicals (Wolfs and others 2006), bioactive peptides (Korhonen and Pihlanto 2006; Thoma-Worringer and others 2006), and so on.

Among foods that possess the characteristic of functionality, we may include all those originating in the beehive: honey, propolis, and royal jelly.

Honey forms part of traditional medicine in many cultures (Gómez-Caravaca and others 2006), although it is most widely used as sweetener. It is composed of at least 181 components and is basically a solution supersaturated in sugars, the fructose (38%) and glucose (31%) are the most important (Gheldof and others 2002); the moisture content is about 17.7%, total acidity 0.08%, and ashes constitute 0.18% (Nagai and others 2006). In addition, there is a great variety of minor components, including phenolic acids and flavonoids, the enzymes glucose oxidase and catalase, ascorbic acid, carotenoids, organic acids, amino acids, proteins, and  $\alpha$ -tocopherol (Ferrerres and others 1993). The actual composition of honey varies, depending on many factors such as the pollen source, climate, environmental conditions, and the processing it undergoes (Gheldof and others 2002; Azeredo and others 2003).

Propolis is a resinous substance that bees collect from the exudates of plants and which they use to seal holes in the beehive (Marcucci and others 2001). Propolis, too, forms part of traditional medicine, and chemical analysis has pointed to the presence of at least 300 compounds in its composition (Castro 2001). It is mainly composed of resin (50%), wax (30%), essential oils

MS 20080450 Submitted 6/14/2008, Accepted 9/3/2008. Authors are with Grupo Industrialización de Productos de Origen Animal (IPOA), Grupo 1 UMH, Grupo REVIV, Generalitat Valenciana, Dept. de Tecnología Agroalimentaria, Escuela Politécnica Superior de Orihuela, Univ. Miguel Hernández, Ctra. E-03312 Orihuela, Alicante. Direct inquiries to author Fernández-López (E-mail: j.fernandez@umh.es).

(10%), pollen (5%), and other organic compounds (5%) (Gómez-Caravaca and others 2006). Among these organic compounds, we may find phenolic compounds and esters, flavonoids in all their forms (flavonoles, flavones, flavonones, dihydroflavonoles, and chalcones), terpenes, beta-steroids, aromatic aldehydes and alcohols, sesquiterpenes, and stilbene terpenes (Aga and others 1994; Russo and others 2002). As with honey, its composition varies with different factors, such as source of the exudates, climate, and environmental conditions (Chen and Wong 1996; Nieva-Moreno and others 1999). Caffeic acid phenethyl ester (CAPE) is a biologically active ingredient of propolis with several interesting biological properties, including apoptosis (Draganova-Filipova and others 2008), metastasis (Liao and others 2003), and radiation sensitivity (Chen and others 2005) of cancer cells.

Royal jelly is the exclusive food of the queen honeybee (*Apis mellifera*) larva. Chemically royal jelly comprises water (50% to 60%), proteins (18%), carbohydrates (15%), lipids (3% to 6%), mineral salts (1.5%), and vitamins (Nagai and Inoue 2004) together with a large number of bioactive substances such as: 10-hydroxyl-2-decenoic acid (Caparica-Santos and Marcucci 2007) with immunomodulating properties (Ferlat and others 1994), antibacterial protein (Fujiwara and others 1990), fatty acids (Vucevic and others 2007), and peptides (Tokunaga and others 2004). The royal jelly also demonstrated significantly improved the recovery from 5-fluorouracil-induced damage (Suemaru and others 2008).

On the other hand, honey, propolis, and royal jelly may have undesirable effects on health. Some researchers (Choo and others 2008; Gunduz and others 2008) reported intoxication events, such as cardiac dysrhythmias, hypotension, respiratory depression, and altered mental status caused by grayanotoxin present in some honey-made food.

The intrinsic properties of honey affect the growth and survival of microorganisms, in particular, the low pH and high sugar content of undiluted honeys prevent the growth of many species of microorganisms (Iurlina and Fritz 2005). In consequence, honey can be expected to contain a small number and a limited variety of microorganisms. These microorganisms include certain yeasts and spore-forming bacteria; coliforms or yeasts indicative of sanitary or commercial quality, and microorganisms such as *Bacillus cereus*, *Clostridium perfringens*, or *Clostridium botulinum*, which under certain conditions could cause illnesses in humans (Snowdon and Cliver 1996; Küplülü and others 2006; Finola and others 2008). Other researchers found in honey several heavy metals such as Sb, As, Cd(II), and Pb(II), which could cause illness (Rashed and Sultan 2004; Ioannidou and others 2005; Muñoz and Palmero 2006; Pisani and others 2008).

The aim of the present study was to study some of the functional properties of some compounds produced by bees.

### Phenolic Compounds

Phenolic compounds in their many forms are the main components responsible for the functional properties associated with many foods, such as antioxidant capacity (Kerem and others 2006; Almaraz and others 2007), antibacterial capacity (Huang and others 2006; Theodori and others 2006), antiviral capacity (Evers and others 2005; Ozcelik and others 2006), anti-inflammatory capacity (Harris and others 2006; Wu and others 2006), cardio-protective effects (Moon and others 2003; Celle and others 2004), and the prevention of enzymatic browning (Chen and others 2000; Jeon and Zhao 2005). Among these foods, we may include honey, propolis, and royal jelly since they contain phenolic compounds collected by the bees from the plants where they gather nectar (Marcucci and others 2001; Fiorani and others 2006).

Phenolic compounds are found mainly in fruits, to which in many cases they contribute color and taste (Belitz and Grosh 1997). Chemically, phenols can be defined as substances that possess an aromatic ring bound with one or more hydrogenated substituents, including their functional derivatives (Marín and others 2001). The presence of phenols in foods may affect their oxidative stability and microbiological safety, hence the interest in their extraction and use as natural antioxidant and antimicrobial substances.

The simplest phenols are liquids or solids with a low fusion point; they have a high boiling point due to the hydrogen bridges they form. Most other phenols are insoluble. They are colorless, but possess a group capable of being colored, which is why they are frequently found colored when oxidized (Marín and others 2001).

The main groups of phenolic compounds present in plants, whether in free form or as glucosides, are derivatives of cinnamic acid, cumarins, and flavonoids (Manthey and Grohmann 2001).

In honey, propolis, and royal jelly, most of the phenolic compounds are in the form of flavonoids (Table 1 and Figure 1), whose concentration depends on various factors, including plant species used by the bees, health of the plant, season, environmental factors, and so on (Küçük and others 2007).

### Antioxidant Capacity

During recent years, functional foods have attracted growing attention because of consumers' increasing concerns about their health, which has spurred greater research effort into such foods. One of their most important properties is their antioxidant capacity, which contributes to the prevention of certain illnesses, including cardiovascular diseases, cancer, and diabetes (Ames and others 1993; Gutteridge and Halliwell 1994). The importance of protecting cell defense systems against the damage caused by oxygen is well known. Free radicals and other oxidative agents are of great importance in the action mechanism of many toxins (Nagai and others 2001). These radicals induce oxidative damage in biomolecules, such as carbohydrates, proteins, lipids, and nucleic acids, which may alter the cell and provoke its death (Diplock and others 1994). The tissues of living organisms possess their own protective agents against oxidative damage, mainly antioxidative enzymatic systems such as superoxide dismutase, catalase, peroxidase, and low molecular weight molecules such as tocopherol, ascorbic acid, and polyphenols (Nagai and others 2001). The undesirable effects of oxidation reactions in foods also have to be taken into account because of the resulting reduction in their shelf life (Johnston and others 2005). These effects include unpleasant odors and flavors (Antony and others 2006; Fernández-López and others 2006), color loss (Thanonkaew and others 2007), and the loss of nutritional values.

Honey and other bee products, such as royal jelly and propolis may be used as functional foods because of their naturally high antioxidant potential. Apart from sugars, honey contains many minor components with antioxidant activity (Gheldof and others 2002), among them amino acids and proteins, carotenes, phenolic compounds and flavonoids, ascorbic acid, organic acids, and Maillard

**Table 1 – Principal flavonoids present in honey, propolis, and royal jelly.**

Group	Compound
Flavonoles	Quercetin, kaempferol, galangin, fisetin
Flavanones	Pinocembrin, naringin, hesperidin
Flavones	Apigenin, acacetin, chrysin, luteolin

Source: Cushnie and Lamb (2005) and Fiorani and others (2006).

reaction products (Al-Mamary and others 2002; Schramm and others 2003; Aljadi and Kamaruddin 2004).

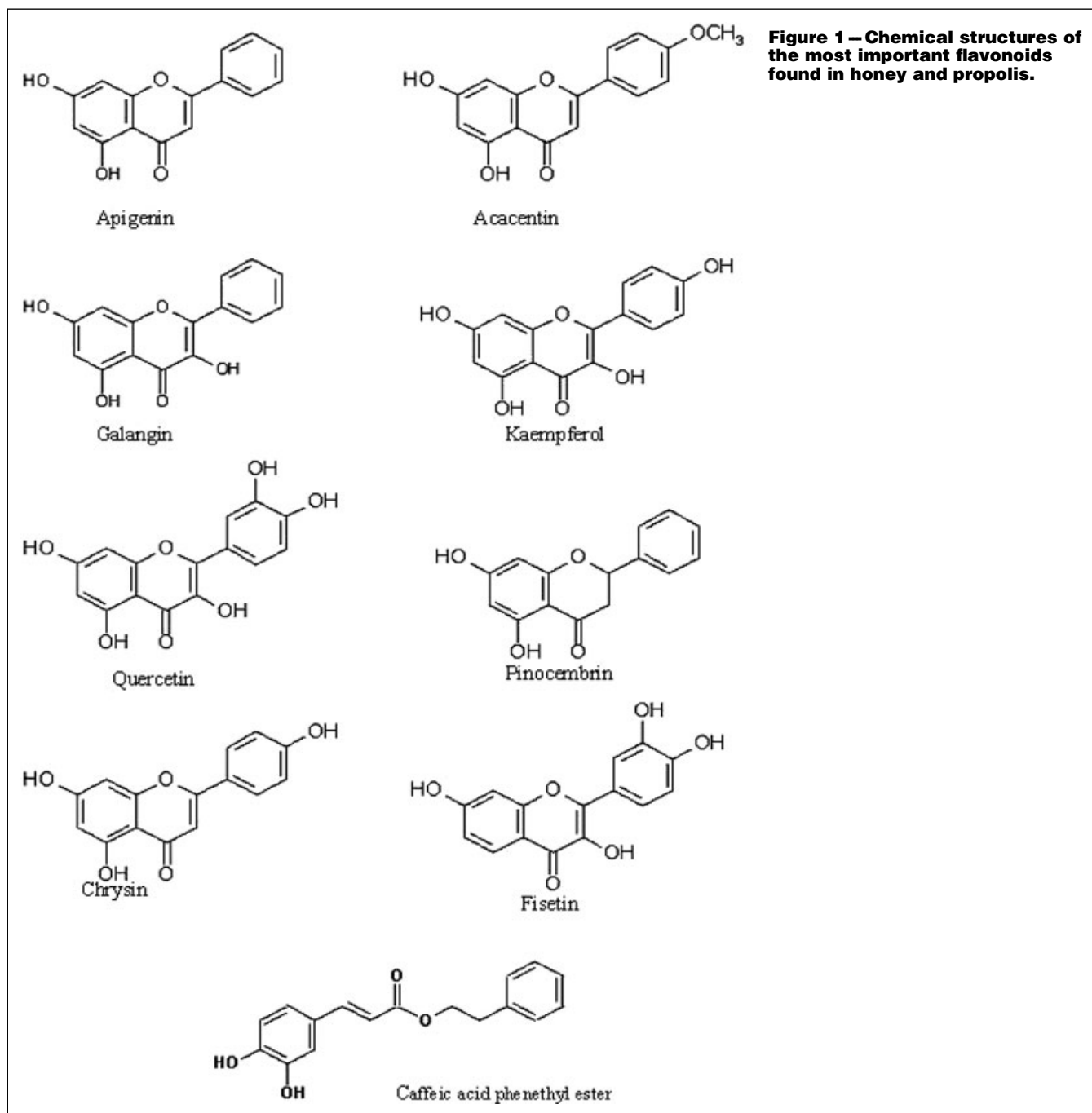
According to Aljadi and Kamaruddin (2004), the antioxidant capacity of honey and propolis is due mainly to the phenolic compounds and flavonoids they contain, and there is a high degree of correlation between these substances and the antioxidant capacity of honey, although a synergic action between several compounds cannot be discounted (Johnston and others 2005; Küçük and others 2007). Propolis, which also shows antioxidant activity, contains amino acids, phenolic acids, flavonoids, terpenes, steroids, aldehydes, and ketones (Borrelli and others 2002).

Several researchers (Cowan 1999; Chen and others 2000; Al-Mamary and others 2002; Yao and others 2003) reported that the composition of honey and so its antioxidant capacity depends on several factors, such as the flower source of the nectar, season, and

environmental factors, such as soil type and climate, genetic factors, and processing methods. In other words, the possible health-related effects due to the antioxidant activity of honey and propolis may well depend on the origin of both (Baltrusaityte and others 2007).

As mentioned previously, the antioxidant activity is basically due to the presence of phenolic compounds and flavonoids, although the exact action mechanism is unknown. Among the mechanisms proposed are free radical sequestration, hydrogen donation, metallic ion chelation, or their acting as substrate for radicals such as superoxide and hydroxyl (Van Acker and others 1996; Al-Mamary and others 2002).

These biophenols may also interfere with propagation reactions (Cotelle and others 1996; Russo and others 2000), or inhibit the enzymatic systems involved in initiation reactions (Hoult and others



1994; You and others 1999). Note that the more hydroxyl groups that flavonoids contain, the more easily they are oxidized (Mayer and others 1998). Furthermore, studies made by Gazzani and others (1998) indicated that some phenolic compounds, in their role as antioxidants, may react more rapidly in the same conditions. It has also been suggested that the organic acids present in honey, such as gluconic, malic, and citric acids, contribute to antioxidant activity through metal chelation and increase the effect of flavonoids by synergy 2 enzymes also present in honey, glucose oxidase, and catalase, also show antioxidant activity through their ability to eliminate oxygen from foods (Rajalakshim and Narasimha 1999). During processing heating may provoke changes in composition due to caramelization of the carbohydrates, Maillard reactions, or fructose decomposition. All these reactions lead to the formation of hydroxymethylfurfural aldehyde, other furfural compounds, and Maillard reaction products that may act as antioxidants (Namiki 1988).

These antioxidant properties have already been assayed in meat products such as minced turkey (McKibben and Engeseth 2002), turkey breast (Antony and others 2006), ready-to-eat ground beef patties (Jonhston and others 2005), and cured pork sausages (Han and Park 2002).

### Anti-Inflammatory Capacity

The inflammatory process is triggered by several chemicals and/or biologicals, including pro-inflammatory enzymes and cytokines, low molecular weight compounds such as eicosanoids or the enzymatic degradation of tissues (Dao and others 2004). According to several studies (Griswold and Adams 1996; Cho and others 2004), the enzyme most related with the inflammatory process is cyclooxygenase-2 (COX-2), an isoform of cyclooxygenase (COX), which catalyses the transformation of arachidonic acid to prostaglandin. The other isoform is cyclooxygenase-1 (COX-1), which regulates homeostasis processes (Dao and others 2004). In the last 30 y, many studies have pointed out the anti-inflammatory properties of honey and propolis (Ali and others 1991; Ali 1995), properties due basically to the presence of flavonoids that inhibit the development of inflammation provoked by a variety of agents (Laslo and Marghitas 2004; Teixeira and others 2005; Mani and others 2006). Among these flavonoids, galangin is of particular interest. This compound is capable of inhibiting cyclooxygenase (COX) and lipo-oxygenase activity, limiting the action of polygalacturonase, and reducing the expression of the inducible isoform of COX-2 (Raso and others 2001; Rossi and others 2002a, 2002b). Another compound, caffeic acid phenethyl ester (CAPE), also present in propolis, shows anti-inflammatory activity through inhibiting the release of arachidonic acid from the cell membrane, which leads to the suppression of COX-1 and COX-2 activity and inhibits the activation of the genic expression of COX-2 (Mirzoeva and Calder 1996). These data were confirmed by the studies of Lee and others (2004).

Chrysin, another flavonoid present in both honey and propolis, also shows anti-inflammatory activity (Kim and others 2002; Ko and others 2003). Its action mechanism is related with the suppression of the pro-inflammatory activities of COX-2 and inducible nitric oxide synthase (iNOS) (Cho and others 2004), as first suggested by Jiang and others (1998). Woo and others (2005) also described the relationship between the anti-inflammatory capacity of chrysin and COX-2 synthesis, although for these researchers the cellular and molecular mechanisms by means of which chrysin inhibited COX-2 synthesis were not clear.

### Inhibition of Enzymatic Browning in Fruits and Vegetables

Enzymatic browning seriously affects the quality of foods. The action of polyphenol oxidase is responsible for this process in fruits and vegetables, while in crustaceans it prevents melanosis (Aubourg and others 2007), provoking the appearance of brown colors, unpleasant smells, and generally unfavorable effects on the nutritional value of foods (Chen and others 2000). The browning reactions induced by this enzyme have traditionally been countered by using chemical substances such as citric acid, ascorbic acid, and sulfites. However, the high cost, restricted action periods, and potential health hazards of some of these products limit their use in food (Jeon and Zhao 2005). Sulfites are the most potent substances in preventing browning but their use may induce asthma attacks or anaphylactic reactions in susceptible subjects (Taylor and others 1986; Sapers 1993). For this reason, there is a search for natural substances that have the same effect, while not inducing harmful reactions.

Honey has been used since ancient times as sweetener, while more modern studies have suggested it may also be a food protector (Osztmianski and Lee 1990; Chen and others 2000). As mentioned previously, honey has a wide number of substances that may act in this way, including ascorbic acid, small peptides, flavonoids,  $\alpha$ -tocopherol, and enzymes such as glucose oxidase, catalase, and peroxidase (Ferreas and others 1993; Jeon and Zhao 2005). Therefore, honey may be a natural alternative to sulfites for controlling enzymatic browning during fruit and vegetable processing and for obtaining juices and preserves (McLellan and others 1995; Lee 1996).

### Antiviral Properties

Propolis and its derivatives have the capacity to inhibit virus propagation. Several *in vitro* studies have shown the effect of propolis on the DNA and RNA of different viruses, among them Herpes simplex type 1, Herpes simplex type 2, adenovirus type 2, vesicular stomatitis virus, and poliovirus type 2. The effects observed involve a reduction in viral multiplication and even a virucidal action (Amoros and others 1992a).

It has also been claimed that various propolis fractions affect the replication of viruses such as vaccinia virus and the virus responsible for Newcastle disease (Maksimova and others 1985). Substances isolated from propolis have also been seen to have antiviral activity. For example, isopentyl ferulate inhibits the infectious activity of Hong Kong virus A (Serkedjieva and others 1992). In studies by Critchfield and others (1996), it was seen that characteristic honey flavonoids, like chrysin, acacetin, and apigenin, inhibit the activation of HIV-1 in latent models of infection through a mechanism that probably includes inhibition of viral transcription. Two of the flavonoids present in propolis (chrysin and campherol) have also been studied and were seen to be very active in the inhibition of replications of several herpes viruses, adenovirus, and rotavirus (Cheng and Wong 1996), while other flavonoids, which are responsible for antioxidant activity (galangin and acacetin) had no effect on these viruses (Debiaggi and others 1990; Amoros and others 1992b). However, other studies have pointed to the antiviral effect of galangin on herpes simple virus (HSV) and Coxsackie b virus (Meyer and others 1997). Flavonoids such as quercetin and rutin, which are found in both honey and propolis (Yao and others 2004; Orsolich and Basic 2005), show antiviral activity against HSV, syncytial virus, poliovirus, and Sindbis virus (Selway 1986; Middleton and Chithan 1993). The action mechanisms proposed for these compounds are related with the inhibition of viral polymerase and the binding of viral nucleic acid or viral capsid proteins (Selway 1986).



Up to now, we have referred to the separate actions of the phenolic compounds present in honey or propolis and how the flavonoids show antiviral activity. However, the individual components may also act synergistically. Indeed, some studies have pointed to such synergism. For example, Amorós and others (1992b) and Cushine and Lamb (2005) mention the synergistic effect of apigenin and campherol on HSV, which would explain why honey and propolis present greater antiviral activity than their individual components.

### Anti-Ulcerous Properties

Another of the functional properties of both honey and propolis is their anti-ulcerous capacity. Several studies describe such activity in honey (Gurbuz and others 2000; Bruschi and others 2003) and, once again, this ability has been attributed to the presence of phenolic compounds, particularly the flavonoids (Gracioso and others 2002; Batista and others 2004; Hiruma-Lima and others 2006). The action mechanism of these compounds varies: according to Speroni and Ferri (1993), flavonoids increase the mucosal content of prostaglandins, which enhances the protective effect on the gastric mucosa, thus preventing ulceration. Vilegas and others (1999) also mention how the flavonoids increase the mucosal content of prostaglandins and have an important inhibitory effect on acid secretions, preventing the formation of peptic ulcers. Other researchers (for example, Martin and others 1998) argue that ulcers are related with reactive oxygen species, flavonoids inhibiting lipid peroxidation, which considerably increases glutathione peroxidase activity (Martin and others 1998; Young and others 1999; Duarte and others 2001).

Some studies demonstrate the anti-ulcerous activity of campherol and quercetin, both of which are found in honey (Leite and others 2001; Yao and others 2004; Orsolich and Basic 2005; Fiorani and others 2006). Similarly, Kanaze and others (2003) attribute this activity to the flavonoids hesperitin and naringin, which are found in honey made from orange blossom (Ferrerres and others 1993; Ferreres and others 1994).

One theory suggests that the anti-ulcerous capacity of honey and propolis is due to flavonoids but acting jointly with other substances such as esters, terpenes, saponins, gums, and mucilage. This idea is supported by Borrelli and Izzo (2000), Zhu and others (2002), Al-Howiriny and others (2003), and Osadebe and Okoye (2003), among others. All these substances are found to a greater or lesser extent in both honey and propolis (Echigo and others 1986; Sahnler and Kaftanoglu 2005; Teixeira and others 2005).

### Antibacterial Properties

The antibacterial capacity of honey, first reported in 1980, is currently being revised. Two main theories have been proposed to explain this capacity: one is that it is due to the action of the hydrogen peroxide in honey that is produced by glucose oxidase in the presence of light and heat (Dustmann 1979), and the other is that it is the nonperoxide activity, which is independent of both light and heat, that inhibits microbial growth (Bogdanov 1984; Roth and others 1986). This nonperoxide activity, which remains unaltered even during long storage times, depends on the flower source of the nectar used and so not all honeys possess this activity (Molan and Russell 1988).

The major components of honey are sugars, which themselves possess antibacterial activity due to the osmotic effect they have (Molan 1992), although studies carried out to test this antimicrobial activity use concentrations at which the sugars are not osmotically active. It is also well known that honey contains lysozyme, a powerful antimicrobial agent (Bogdanov 1997).

Other researchers attribute the antimicrobial capacity of honey to a combination of properties, such as its low pH and high osmolarity (Yatsunami and Echigo 1984), or to the presence of certain volatile substances, although this has not been studied in great depth (Obaieseki-Ebor and others 1983; Toth and others 1987). The antimicrobial activity of both honey and propolis is basically against Gram-positive bacteria (Marcucci and others 2001). Burdock (1998) attributes this capacity to the presence of aromatic acids and esters, while Takaisi and Schilcher (1994) suggest that it is due to the action of the flavonone pinocembrin and the flavonol galangin, and caffeic acid phenethyl ester, whose action mechanism is based on the inhibition of bacterial RNA polymerase. Cushnie and Lamb (2005) reported that other flavonoids such as galangin also presents antibacterial action. The action mechanism involves degrading the cytoplasm membrane of the bacteria, which leads to a loss of potassium ions and the damage caused provoking cell autolysis. Quercetin, which is also found in honey, increases membrane permeability, and dissipates its potential, leading the bacteria to lose their capacity to synthesis ATP, their membrane transport, and motility (Mirzoeva and others 1997).

While the antibacterial capacity of honey is clear, there seems to be no one clear-cut cause, suggesting that there is a combined or synergistic effect at work.

### Conclusions

Honey, propolis, and royal jelly are food products obtained from bees. All of them are important not only for their nutritional properties but also for their functional and biological properties. Antioxidant, anti-inflammatory, antibacterial, antiviral, and anti-ulcerous activities and also the capacity for the inhibition of enzymatic browning are some of these important properties. These activities are mainly attributed to the phenolic compounds such as flavonoids. Due to the large number of beneficial effects that honey, propolis, and royal jelly presented on the body, these products could be considered as potential ingredients for different foods. In any case, some precautions must be taken for their use in foods to avoid some problems in persons who suffer from allergy by bee-related allergens.

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