Chapter 34
Antioxidant Activity of Pot-Honey

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34.1 Introduction

Stingless bee honey has been used in traditional medicine for centuries. In countries including Peru, Guatemala, Mexico, and Venezuela, this honey is used widely and sold at local markets, often as a sweetener, but more often as an ingredient of folk medicine (Vit et al. 2004). This honey is a complex mixture that contains different botanical and entomological compounds. Such compounds contribute to honey's bioactive properties and are important in apitherapy.

Although there is a vast Neotropical biodiversity of 391 stingless bee species (Camargo and Pedro 2007), only the honey produced by a few species has been studied. In general, the main differences between stingless bee honey and *Apis mellifera* (honey bee) honey are a higher water content and acidity, lower diastase, and a different sugar content in the stingless bee honey compared to *Apis mellifera* honey (Vit et al. 2004; Souza et al. 2006).

It has been demonstrated that fermentation increased the antioxidant bioactivity of *Tetragonisca angustula* honey. This observation, signaling the importance of antioxidants, could partly explain the reputed medicinal properties of stingless bee honey (Pérez-Pérez et al. 2007).

Rodríguez-Malaver et al. (2007) measured the antioxidant capacity of *Apis*, *Melipona*, and *Trigona* honey from Venezuela with three oxidative systems, to test the effectiveness of honey at scavenging (i.e., removing) superoxide anions, hydroxyl radicals, and benzoate degradation. All the honey samples showed higher antioxidant capacity indicators than those of artificial honey and lipoic acid. The authors suggested that the antioxidant capacity could serve as a test to detect and then control adulterated honey on the commercial market.

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P. Vit et al. (eds.), *Pot-Honey: A legacy of stingless bees*,
DOI 10.1007/978-1-4614-4960-7_34,

In this chapter, the antioxidant capacity of pot-honey is reviewed, and further scrutinized using information available for stingless bee pollen and propolis.

34.2 Bioactivity of Stingless Bee Products (Honey, Propolis, Pollen)

Among natural products, honey bee-derived apicultural products such as pollen and propolis have been applied for centuries in traditional medicine, as well as in food diets and supplementary nutrition (Kroyer and Hegedus 2002). Propolis has been used as a folk medicine and has been reported to possess therapeutic or preventive effects against inflammation, heart disease, diabetes mellitus, microbes hepatotoxity, and cancer (Burdock 1998).

Kujumgiev et al. (1999) report no differences in the antibacterial, antifungal, and antiviral activities of propolis from different geographic origins, including four samples from Brazilian *A. mellifera* and two stingless bees. The flavonoids in propolis (mainly pinocembrin) are considered responsible for its inhibitory effect on bacteria and fungi, but only traces of these compounds have been found in propolis of South American origin (Tomás-Barberán et al. 1993); thus, propolis from that region may possess other active compounds.

Farnesi et al. (2009) demonstrated that the antibacterial activity of green propolis from honey bee nests against *Micrococcus luteus* and *Staphylococcus aureus* was superior to that taken from nests of stingless bee, *Melipona quadrifasciata* and *Scaptotrigona*, propolis. Two samples of propolis (green propolis and *Scaptotrigona* propolis) were effective against *Escherichia coli*. *Melipona quadrifasciata* propolis was more active than green propolis and *Scaptotrigona* propolis against *Pseudomonas aeruginosa*, suggesting a potential importance for human and veterinary medicine.

It was found that Fenton reagent causes a decrease in salivary total antioxidant activity (TAA) and *Apis mellifera* propolis protects and even increases salivary TAA. On the other hand, *Melipona favosa* propolis only protects salivary TAA against oxidative stress (Sánchez et al. 2010).

Silva et al. (2009) show that the extracts of pollen from *Melipona rufiventris* are good scavengers of active oxygen species. Those authors suggest this property of pollen is important in prevention of diseases such as cancer, cardiovascular disease, and diabetes, among others.

34.3 Comparison of Pot-Honey and *Apis mellifera* Honey

Pot-honey shows differences in antioxidant activity, in comparison to *Apis mellifera* honey. In a study on Peruvian stingless bee honey from ten species, the Trolox equivalent antioxidant capacity (TEAC) ranged from 93.84 to 569.65 μmol Trolox equivalents (TE)/100 g (Rodríguez-Malaver et al. 2009). Some species (*Nannotrigona melanocera*) showed higher TEAC than both Czech *A. mellifera* honey (from 43.55 to 290.35 μmol TE/100 g) (Vit et al. 2008) and Venezuelan *A. mellifera* (from 34.90 to 203.21 μmol TE/100 g) (Vit et al. 2009a). In this work, flavonoid and polyphenol contents of stingless bee honey were measured; they ranged from 2.6 to 31.0 mg quercetine equivalents (QE)/100 g, and 99.7–464.9 mg gallic acid equivalents (GAE)/100 g, respectively. Those values were higher than Czech *A. mellifera* honey (from 1.90 to 15.74 mg QE/100 g and from 47.39 to 265.49 mg GAE/100 g) and Venezuelan *A. mellifera* honey (from 2.32 to 14.41 mg QE/100 g and 38.15 and 182.10 mg GAE/100 g).

The antioxidant activity, flavonoid and polyphenol contents are compared in pot-honey produced by several stingless bee genera. The highest values are found in *Nannotrigona* honey, followed by *Scaura* and *Ptilotrigona*. The lowest values are found in *Melipona* and *Partamona*, followed by *Tetragonisca* and *Scaptotrigona*. However, such comparisons are only preliminary, because more honey samples are needed. Only one honey was available for most of the genera, whereas 28 *Melipona* honeys and 18 *Tetragonisca* honeys were analyzed (Gutiérrez 2008).

34.4 Factors that Explain the Antioxidant Capacity and Possible Role for Authentication

Persano Oddo et al. (2008) report that the TEAC of *Tetragonula carbonaria* (formerly named *Trigona carbonaria*) honey from Australia is higher (233.96 ± 50.95 μmol/100 g) than that reported for Czech floral honey of *Apis mellifera*, while the radical scavenging activity (RSA) (48.03 ± 12.58% ascorbic acid equivalents) is similar to that of floral and honeydew blends of Spanish honey (Pérez et al. 2007). The flavonoid content of *T. carbonaria* honey (10.02 ± 1.59 mg QE/100 g) is higher than those of Czech floral and honeydew honey (6.59 and 7.25 mg QE/100 g, respectively). In contrast, the polyphenol content is higher in the floral (115.03 mg GAE/100 g) and honeydew (129.03 mg GAE/100 g) Czech honeys than in *T. carbonaria* honey (55.74 ± 6.11 mg GAE/100 g) (Vit et al. 2008). The authors suggest that organic acids might explain its high antioxidant activity. The antioxidant capacity of *T. carbonaria* and other stingless bee honey represents an important added value, to encourage further research on medicinal attributes with both nutritional and pharmaceutical application. In a recent study, a high level of antibiotic activity was found in honey from *T. carbonaria* (Irish et al. 2008).

In another study with pot-honey from Guatemala, *M. beecheii* "abeja criolla" and *M. solani* "chac chow" were compared. The antioxidant activity, flavonoid and polyphenol contents are given in Table 34.1. The TEAC values, flavonoid and polyphenol contents were significantly higher in *M. beecheii* than in *M. solani* honey (Gutiérrez et al. 2008). Such a difference could be explained by the floral species visited. Asteraceae and Melastomataceae were the most abundant plant families in the *Melipona* honey pollen spectrum in Guatemala (Dardón and Enríquez 2008).

Table 34.1 Bioactivity of *Melipona* honey from Guatemala (permission granted by Revista de la Facultad de Farmacia)

	Stingless bee species	
Bioactive parameter	*M. beecheii*, $N=4$	*M. solani*, $N=2$
Flavonoids* (mg QE/100 ghoney)	3.60 ± 0.61	1.88 ± 1.64
Polyphenols* (mg GAE/100 g honey)	107.35 ± 17.79	68.66 ± 15.11
TEAC* (μmol TE/100 ghoney)	87.38 ± 12.92	39.07 ± 10.52

Averages ± SD values
*Significant differences between *M. beecheii* and *M. solani* ($P<0.05$), *t*-test

Tetragonisca fiebrigi Schwarz, 1938 is a stingless bee named "yateí" in Argentina and Paraguay. Vit et al. (2009b) compared a honey sample from both countries and found that TEAC was higher in honey from Argentina (160.15 ± 60.50 μmol TE/100 g) compared to Paraguay (120.91 ± 38.67 μmol TE/100 g). However, they did not find a difference in flavonoid (14.37 ± 11.11 and 12.66 ± 4.82 mg QE/100 g) and polyphenol (240.74 ± 94.05 and 148.29 ± 17.75 GAE/100 g) content.

High nitrite content was found in Peruvian pot-honey (Rodríguez-Malaver et al. 2009). It was hypothesized that nitric oxide and/or nitrite might be responsible, in part, for the biological and therapeutic effects of honey (Al-Waili 2003). In addition, this metabolite could be used for authentication of honey. Also in this research, there were positive Pearson correlations ($P<0.01$) between flavonoids-TEAC (0.879), polyphenols-TEAC (0.942), proteins-TEAC (0.911), color-TEAC (0.771), and nitrites-TEAC (0.422). Those correlations indicated compounds that could be involved in the antioxidant action of stingless bee honey. Similar results have been reported for polyphenols, flavonoids, and color in *A. mellifera* honey (Bertonceij et al. 2007; Frankel et al. 1998; Taormina et al. 2001; Vela et al. 2007, 2008). It has also been reported that the antioxidant activity of stingless bee honey increases with free acidity ($r^2=0.97$, $P<0.01$) (Vit et al. 2006). Due to a controversy about which compounds signify honey antioxidant activity, Gheldof et al. (2002) suggested that total antioxidant content of honey may be better explained by interactions of a wide range of compounds, including phenolics, peptides, organic acids, enzymes, and Maillard reaction products.

34.5 Conclusions

Diversity of stingless bees in America is very high. Thus, bioactivities of stingless bee products are diverse because they depend on bee species, their habits, and also on external factors such as geography, climate, season, harvesting method, etc. Comparisons of bioactivities from bee products of native stingless bee species has been widely studied and reported. It was found that both internal and external factors affect classes, types, and contents of active compounds and their derivatives, which mainly belong to phenolic compounds and flavonoids.

The correlation between chemical compounds such as water, sugars and free acidity and the bioactivities has been widely studied. Standard control of stingless bee products in traditional medicine would require identifying new bioactive agents of interest in order to demonstrate their bee origin, and to avoid or reduce the side-effects of using present modern medicine.

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