10. Antioxidant activity and microorganisms in nest products of *Tetragonisca angustula* from Mérida, Venezuela.

PÉREZ-PÉREZ Elizabeth Mariana[1*], SUAREZ Esther[1], PEÑA-VERA María José[1], GONZÁLEZ Ana Carolina[2], VIT Patricia[3,4]

[1] Laboratorio de Biología Molecular, Facultad de Farmacia y Bioanálisis, Universidad de Los Andes, Mérida 5101, Venezuela.

[2] Laboratorio de Anaerobios "Roberto Gabaldón", Departamento de Microbiología y Parasitología, Facultad de Farmacia y Bioanálisis, Universidad de Los Andes, Mérida 5101, Venezuela.

[3] Apiterapia y Bioactividad, Departamento Ciencia de los Alimentos, Facultad de Farmacia y Bioanálisis, Universidad de Los Andes, Mérida 5101, Venezuela.

[4] Cancer Research Group, Discipline of Biomedical Science, Cumberland Campus C42, The University of Sydney, 75 East Street, Lidcombe NSW 1825, Australia.

* Corresponding author: Elizabeth Mariana Pérez-Pérez, Email elimariana@ula.ve

Received: October, 2012 - Accepted: February, 2013

Abstract

There is abundant information about the antioxidant activity of honey, pollen and propolis, but antioxidant activity of stingless bee nest products is less well known. In this work *in vitro* total antioxidant activity (TAA), flavonoid and polyphenol contents of ethanolic extracts of *Tetragonisca angustula* nest products were assessed. The highest TAA was observed in ethanolic homogenates of cerumen pots, followed by propolis, cerumen and honey. Preliminary data are given on isolated microorganisms (*Bacillus* sp., *Candida* sp., *Staphylocuccus* sp.) in different structures of the nest.

Key words:
Antioxidant activity, flavonoids, microorganisms, nest, polyphenol content, *Tetragonisca angustula*, Venezuela

Introduction

Stingless bees are eusocial insects that live in the tropical and subtropical regions of the world in colonies ranging from a few dozens to 100,000 or more adult workers (Michener, 2000). They are key pollinators of local plants (Slaa et al., 2006) and produce highly appreciated honey (Cortopassi-Laurino et al., 2006). Although the amount of honey produced is relatively small, compared to *Apis mellifera*, there is a recent interest in the quality of stingless bee hive products, due to its great potential presenting antitumoral, antimicrobial and antioxidant activities (Gómez-Caravaca et al., 2006).

It is known that honey, pollen and propolis are rich in phenolic compounds, which act as natural antioxidants, and these stingless bee productcs are

becoming increasingly popular because of their potential uses in contributing to human health. Those compounds can also be used as indicators in studies of the floral and geographical origin of the honey and propolis themselves (Jaganathan et al., 2010). However, little is known about antioxidant activity of honey, propolis and other products inside the stingless bee nest itself. There are some reports about the antioxidant activity of stingless bee honey (Persano Oddo et al., 2008; Rodríguez-Malaver et al. 2010); and several reports on antimicrobial, antiproliferative and cytotoxic activities of stingless bee propolis (Umthong et al., 2009; Liberio et al., 2011; Choudhari et al., 2012).

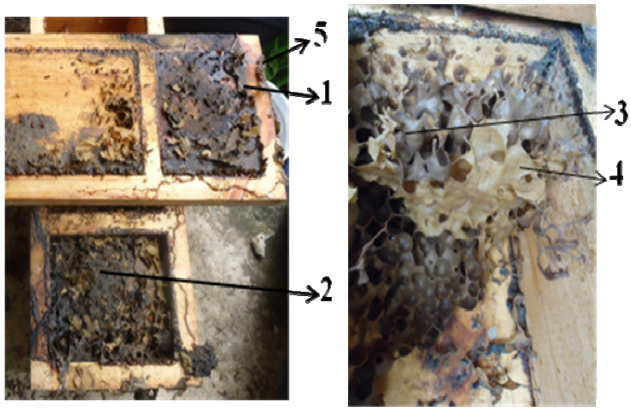
Due to their therapeutic properties, bee products have received renewed interest (Banskota et al., 2001) as an essential natural resource that can be employed in new therapies free from side effects that are often encountered with the use of synthetic chemical medicines (Kelly et al., 2005). However, the market competition on these products imposes extra conditions that can only be ensured by complying with quality assurance and certification protocols (Al-Waili et al., 2012). In this sense, honey can be polluted via different sources of contamination, including pesticides, heavy metals, bacteria, and radioactive materials. In regard to the microorganisms in honey, their presence may influence quality and safety of honey (Al-Waili et al., 2012). The microbes found in honey and honeycomb are bacteria, molds, and yeast; come from the bees, nectar or from external sources, like pollen, honey bee intestines, humans, equipment, containers, wind, and dust (Engel et al., 2012). Pollen may be the original source of microbes in the intestines of honeybees, containing 1% yeast, 27% Gram-positive bacteria (*Bacillus, Bacteridium, Streptococcus* and *Clostridium* spp.) and 70% Gram-negative bacteria (*Achromobacter, Citrobacter, Enterobacter, Erwinia, Escherichia coli, Flavobacterium, Klebsiella, Proteus,* and *Pseudomonas)* (Gilliam and Prest, 1987; El-Leithy and El-Sibael, 1992).

This study was designed to assess *in vitro* antioxidant activity and phenolic composition of ethanolic and methanolic extracts of *Tetragonisca angustula* nest products and structures (honey, propolis, cerumen pots, cerumen entrance tube) and for preliminary characterization of associated microbiota.

10.1 Stingless bee products and structures

Pot-honey of *T. angustula* was extracted from cerumen pots with a pipette. Propolis and cerumen (entrance, pots, involucrum) were also collected, as shown in Figure 1. A weight of 100 ± 10 mg of each product was placed on a glass homogenizer (Thomas No. A3528, USA), and 1 mL of ethanol 95% (v/v) (Riedel de Haën, Europe) were added, and homogenized on an ice bath. Homogenates were centrifuged in a BHG Optima II (USA) centrifuge at 3,000 rpm for 10 min, and supernatants were used for biochemical analysis.

To isolate microorganisms, sterile hyssops were rubbed on nest surfaces were placed in capped tubes with 3 mL sterile brain heart infusion BHI (Imedia, London, United Kingdom), and incubated at 37ºC during 24-48h, until turbidity was perceived.



Photograph: EM Pérez-Pérez

Figure 1. Beehive products used in this study

1. Propolis, 2. Nest cerumen, 3. Dark cerumen honey pot, 4. Light cerumen honey pot, 5. Entrance cerumen.

10.2 Analytical methods

All experiments were done in triplicate. Data were analyzed by ANOVA, with average comparison through *post hoc* Scheffé test using SPSS 12.0 (SPSS) software. Are indicated statistical differences to $p< 0.05$.

10.2.1 Total antioxidant activity by the $ABTS^{+\bullet}$ method

The assay method of ethanolic decolorization with ABTS (Sigma, Canada) was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{+\bullet}$) was produced by reacting 7 mM stock solution with potassium persulfate (Merck, Darmstadt, Germany) to a final concentration of 2.45 mM (in water), in the dark at room temperature (RT) for 12-16 h before use (Re et al., 1999). For bee product analysis, $ABTS^{+\bullet}$ solution was diluted with 20% ethanol (v/v) until 0.60-0.70 absorbance units at 735 nm, 100 µL of homogenates were diluted in methanol or ethanol, and mixed with 7.5 ml of $ABTS^{+\bullet}$ solution diluted in ethanol 20% (v/v). Absorbance values were measured 6 min after mixture. A solution of 8 mM Trolox (Sigma, Steinheim, Germany) was used as antioxidant standard. Trolox was diluted to obtain 1,

2, 4 and 8 µM in 5 mM PBS buffer (pH 7.4). Decolorization percentages at 734 nm after 6 min was calculated and plotted as a function of different Trolox concentrations, and TAA was reported accordingly. TAA value for a given sample would be equivalent to Trolox concentration that produces the same decolorization percentage.

10.2.3 Biochemical analytical methods

Polyphenol content was analyzed by spectrometry at 765 nm using Folin-Ciocalteu (Sigma-Aldrich, St. Louis, USA) reagent (Singleton et al., 1999). One hundred microliters of a was mixed with 500 µL of Folin-Ciocalteu's reagent diluted 1/10 with water, to which 400 µL of sodium carbonate was added (Sigma, Steinheim, Germany) 7.5% (w/v). 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).

Protein content was determined by spectrometry at 750 nm by the method of Lowry et al. (1951) with bovine serum albumin (Riedel de Haën, Europe) as the standard.

Flavonoid content was measured using a modification of the aluminum chloride method (Woisky and Salatino, 1998). The flavonoid concentration was measured with a calibration curve using standard solutions of quercetin (Sigma, Steinheim, Germany) diluted up to 25, 50 and 100 µg/mL in ethanol 80% (v/v). Standard solutions (0.5 mL) were mixed with 1.5 mL 95% (v/v) ethanol, 0.1 mL of aluminum chloride (Fisher Scientific, New Jersey, USA)10% (w/v), 0.1 mL of potassium acetate (Sigma-Aldrich, USA) 1M and 2.8 mL of distilled water. After incubation at ambient temperature for 30 min, absorbance was recorded at 415 nm. In a similar way, 0.5 mL of ethanolic extracts of bee products reacted with aluminum chloride for flavonoid content determinations.

10.2.3 Identification of microorganisms in the nest

Standard microbiological procedures (Velazco et al., 2008) were used to isolate and identify microbial growth. Turbid BHI incubated tubes, were plated to isolate and screen the growth of yeast and *Bacillus* (in blood agar), Enterobacteria (in MacConkey agar), *Staphylococcus* (in mannitol salt agar). Further Gram tincture of isolated colonies for the observation of microscopic morphology were done before the following confirmation tests: *Bacillus* (catalase, blood agar), Enterobacteria (oxidase, Kligler, LIA, MIO, citrate), *Staphylococcus* (catalase, coagulase), yeast (blood agar, bilis agar).

10.3 Relationship of antioxidant activity to total polyphenol content

The highest polyphenol content was observed in dark cerumen honey pots (Table 1). The flavonoid and protein contents were highest in propolis.

Honey from different pots in the same nest was uniform. A positive correlation was found between TAA values and polyphenol content (R^2 = 0.987). Finally, TAA values were similar to, and sometimes higher, tahn those found for other antioxidants extensively studied like lipoic acid, quercetin and melatonin (Table 2).

Table 1. Flavonoid, polyphenol and protein contents of ethanolic extracts of *Tetragonisca angustula* nest products

Tetragonisca angustula products	Flavonoids content (mg of quercetin equivalents/100 g pot-honey, cerumen or propolis)	Polyphenol content (mg equivalents gallic acid/100 g pot-honey, cerumen or propolis)	Protein content (mg proteins/ 100 g pot-honey, cerumen or propolis)
Pot-honey 1	336.5 ± 1.9^{b}	418.4 ± 1.2^{a}	486.0 ± 1.3^{d}
Pot-honey 2	362.1 ± 2.0^{b}	458.5 ± 4.2^{a}	531.4 ± 1.6^{e}
Pot-honey 3	359.1 ± 1.1^{b}	413.4 ± 8.2^{a}	560.1 ± 1.1^{e}
Pot-honey 4	364.2 ± 1.6^{b}	411.6 ± 4.4^{a}	655.5 ± 4.5^{f}
Pot-honey 5	359.6 ± 1.7^{b}	444.1 ± 2.9^{a}	601.8 ± 1.6^{f}
Dark cerumen honey pot	300.1 ± 2.2^{a}	**667.6 ± 1.9^{d}**	179.5 ± 3.2^{b}
Light cerumen honey pot	317.6 ± 2.9^{a}	598.4 ± 1.4^{c}	48.1 ± 3.3^{a}
Nest cerumen	471.5 ± 1.9^{c}	589.7 ± 3.4^{c}	380.5 ± 3.2^{c}
Entrance cerumen	364.7 ± 1.8^{b}	575.1 ± 3.1^{b}	656.4 ± 1.7^{f}
Propolis	**518.1 ± 1.6^{d}**	591.4 ± 3.5^{c}	**676.8 ± 2.6^{f}**

Data are mean ± SE values (n = 3). Columns within a sample sharing the same letter are not significantly different by ANOVA *post hoc* Scheffé test (P <0.05). Cerumen is highlighted.

Table 2. Total antioxidant activity (TAA) of ethanolic extracts of *Tetragonisca angustula* products

Tetragonisca angustula nest products	Total antioxidant activity (TAA) μmoles Trolox equivalents (TEAC/100 g pot-honey, cerumen or propolis)
Pot-honey 1	122.1 ± 5.9[b]
Pot-honey 2	96.9 ± 2.1[a]
Pot-honey 3	128.2 ± 1.3[b]
Pot-honey 4	128.8 ± 5.0[b]
Pot-honey 5	137.9 ± 4.4[b]
Dark cerumen honey pot	**307.2 ± 5.4[f]**
Light cerumen honey pot	225.3 ± 17.1[e]
Nest cerumen	221.6 ± 10.9[e]
Entrance cerumen	161.6 ± 6.1[c]
Propolis	190.6 ± 4.7[d]
Quercetin	100,6 ± 1.7[a]
Melatonin	97.6 ± 5,.1[a]
Lipoic acid	124.7 ± 3.1[b]
R^2	0.987
P	0.021

Data are mean ± SE values (n = 3). The same superscript letter is for not significant differences by *post hoc* Scheffé test (P <0.05). R^2 correlation and P refer to the lineal correlation between polyphenol content and the antioxidant activity. Cerumen is highlighted.

10.4 Pot-honey and propolis antioxidant activity

The TAA activity of ethanol extracts of several nest products of *T. angustula* from Mérida-Venezuela. was decreasing from was dark cerumen pot, light cerumen honey pot, light cerumen honey pot, propolis, laminate involucrum and entrance cerumen, honey (Table 2). In the same way, the highest polyphenol content values were observed in propolis, dark cerumen honey ethanolic homogenates; and the same trend is observed in flavonoids content (Table 1). There is a positive relation between polyphenol content and antioxidant activity (TAA values).

The evidence about the antioxidant activity of honey and propolis and its relationship with total polyphenol content, and especially flavonoid concentration, is numerours. 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, among them amino acids and proteins, carotenes, phenolic compounds and flavonoids, ascorbic acid, organic acids, and Maillard reaction products (Al-Mamary et al., 2002; Gheldof et al., 2002).

According to Aljadi and Kamaruddin (2004), the antioxidant capacity of honey and propolis is due to the content of phenolic compounds and flavonoids, and there is a high correlation between them and the antioxidant capacity of honey, although a synergism between several compounds is present (Johnston et al., 2005; Kücük et al., 2007). Propolis also contains amino acids, phenolic acids, flavonoids, terpenes, steroids, aldehydes, and ketones which account for its antioxidant activity (Borrelli et al., 2002).

However, the majority of studies are done with honeys and other *Apis mellifera* products, and very little is known about the composition and biological activity of stingless bee products. In this sense, the results presented in this study are in concordance with the report of Persano Oddo et al. (2008), who studied several physicochemical parameters of honey produced by *Tetragonula carbonaria* from Australia. In this work, the antioxidant activity, was 233.96 ± 50.95 μM Trolox equivalents/100 g of honey, while in the present study is ranged between 96.9 to 307.2 μM Trolox equivalents/100 g of honey, being the highest for dark and light cerumen pots.

The results also resemble those reported by Rodríguez-Malaver et al. (2009), on properties of honey produced by 10 stingless bee species (*Melipona crinita*, *M. eburnea*, *M. grandis*, *M. illota*, *Nannotrigona melanocera*, *Partamona epiphytophila*, *Ptilotrigona lurida*, *Scaptotrigona polystica*, *Scaura latitarsis*, and *Tetragonisca angustula)* from Peru, ranging in polyphenol contents from 99.7 to 464.9 mg gallic acid equivalents/100g, and the antioxidant capacity from 93.8 to 569.6 micromoles Trolox equivalents/100 g. The entomological differences between the samples reported in this study and those reported in literature can be due to several factors like nectar source, climate, soil type, genetic factors, among other possible factors (Yao et al., 2003).

As mentioned previously, the antioxidant activity is basically due to the presence of phenolic compounds and flavonoids, although the mechanism of action is unknown. The free radical sequestration, hydrogen donation, metallic ion chelation, or their action as substrate for radicals such as superoxide and hydroxyl, are proposed mechanisms (Al-Mamary et al., 2002). The biophenols may also interfere with propagation reactions, or inhibit the enzymatic

systems involved in initiation reactions (Maruyama et al., 2010).

According to the above, the antioxidant activity can represent an important parameter to determine if a honey or propolis is genuine, and increase added value of honey and propolis of *T. angustula* for nutritional and pharmaceutical applications.

10.5 Antioxidant activity of honey pots, nest and entrance cerumen

In the results presented in this study we find that the highest antioxidant activities belong to dark and light cerumen pots, batumen and cerumen. These TAA values are even greater that those founded for purified and known antioxidants like lipoic acid, quercetin and melatonin (Table 2). These TAA values are higher than those found for honey and propolis of the same beehive, which is an interesting finding because it is the first time that the antioxidant capacity of these products from the hive of stingless bees has been studied. A positive correlation between TAA values and total polyphenol content was found (Table 1 and 2).

The nests of stingless bees have been studied and described from different points of view by different authors. The stingless bee nests in almost any cavities that are available, from tree holes up space in homes of towns (Dos Santos et al., 2010). Conditioning of the bee nests serves to house young and old, to ward off enemies and weather (rain, wind, temperature), and also to store the food collected from plants (nectar).

The primary use of resins by honey bees is for caulking cracks and crevices in the walls of nest cavities, for adding it to brood combs, for embalming intruders and for disinfection (Winston, 1987; Roubik 1989; Michener 2000). In stingless bees, cerumen is also important for colony defense, and it is kept in deposits as a sticky material. In colonies of *Tetragonisca angustula* (Latreille) and species of *Plebeia*. Nogueira-Neto (1997) isolated viscous propolis clusters throughout the interior of the colonies. The bees use these substances in threatening situations, for incapacitating small enemies, as observed in *Plebeia saiqui* (Pick and Blochtein, 2002). When mixed with the product of wax glands, propolis will form the cerumen, which is incorporated mainly in the involucrum, on the food pot walls and on the nest entrances (Roubik, 2006).

According to what we mention above, several resins are present in a stingless bee hive. In first place the honey and pollen pots, used for honey and pollen deposits and beekeeping, respectively. These pots are constructed from cerumen, which is a mixture of wax and resins. Stingless bees have secretory wax glands located between the segments of the upper abdomen. This wax is mixed with resins collected from some trees required for the formation of cerumen (Roubik, 2006). In the second place the egg cells and nest layers. Sometimes adding stones, leaves, fibers or animal waste improves the cerumen strength, especially in exposed nests. In third place the bitumen, a hardened material resulting from the mixture of cerumen with mud or sand, or even plant fibers. This layer serves to seal cracks or delimit the nest in tree cavities (Teixeira et al., 2005).

With respect to the antioxidant activity of honey pots, nest cerumen and entrance cerumen, this is the first report known about TAA values of these beehive products. The only report about some bioactivity of cerumen is the report of Massaro et al. (2011) who studied the potential anti-inflammatory properties of cerumen produced by *Tetragonula carbonaria* from Australia, finding that cerumen extracts had similar potency to Trolox to inhibit the *in vitro* of 5-lipoxygenase (5-LOX) in a cell-free assays, but were less potent than honeybee propolis or gallic acid. The results presented in this study, warrant further investigation of the ecological and medicinal properties of stingless bee cerumen, honey pots and nest cerumen, which may herald a commercial potential for the Venezuelan beekeeping industry.

10.6 Microorganisms isolated from the nest

Microorganisms isolated from different structures of the *T. angustula* nest are indicated in Table 3. Plant and insect vectors may explain their presence until functional relationships will be revealed. *Bacillus* sp. was the most frequent microorganism in the studied nest products. This spore-forming bacteria was found in the inner involucrum, entrance tube, propolis, pot-pollen, column. Gilliam et al. (1990) previously reported the presence of three species of *Bacillus* (*B. alvei*, *B. circulans*, *B. megaterium*) in *Melipona fasciata*, currently renamed as *M. panamica*.

The presence of unidentified yeast species in the tip of the entrance tube, suggests its environmental origin outside the nest –as explained by Rosa et al. (2003) and Menezes et al. (2013), or associated with adult bees, since they walk on the entrance tube. Indeed, some yeast species found in adult bees are distinctive in the flowers they may visit (Rosa et al., 2003).

Table 3. Isolated microorganisms in the *T. angustula* nest

Tetragonisca angustula nest products	Isolated microorganism
Foam of pot-honey	-
Pollen	*Bacillus* sp.
Tip of entrance tube	unidentified yeast species
Base of entrance tube	*Bacillus* sp.
External cerumen pot	-
Inner cerumen pot	*Staphylococcus* sp.
External involucrum	-
Inner involucrum	*Bacillus* sp.
Cerumen column	*Bacillus* sp.
Propolis	*Bacillus* sp.

Cerumen is highlighted.

In general, the action of molds and yeasts on sweetened foods is merely infective (*Candida albicans*, *Cryptoccus neoformans*), but mold level leading to the formation of mycotoxins (*Aspergillus* spp., *Fusarium* spp.) should be prevented. Certain molds can cause poisoning in children or adults with digestive problems, and probably that the presence of *C. albicans* is occasional or accidental, introduced into the entrance tube by chance or unhygienic handling during harvesting or processing of honey (Martins et al., 2003). Rosa et al. (2003) studied yeast communities associated with stingless bees, and found a strong association of *T. angustula* with the yeast *Starmerella meliponinorum*.

Antimicrobial properties of honey prevent the growth of many microorganisms. However, a few pathogens are found in honey, especially spore forming microorganisms such as *Bacillus cereus, Clostridium perfringens,* and *Clostridium botulinum*. The *Clostridium botulinum* population did not change over a year at 4ºC (Snowdon, 1999). In Nigerian honeys several species of the genus *Bacillus* (*B. cereus, B. megaterium, B. polymyxa, B. licheniformis, B. firmus*, and *B. pumilis*) were found, but yeasts and moulds were not detected (Omafuvbe and Akanbi, 2009). On the other hand, members of *Staphylococcus* have been found in honey and bees from Latvia, besides *Enterococcus* spp., *Corynebacterium* spp., *Corynebacterium* spp. and *Bacillus* spp.) (Lace et al., 2008); particularly *Staphylococcus albus* in honey marketed in Saudi Arabia, (Al-Hindi, 2003).

Most of these studies on microorganisms in bee nests identify isolated bacterial and fungal communities; data on symbiotic relations between bees and microbiota relationships is scarce (Morais et al., 2013). Some microorganisms are widely distributed, and their presence in bees as 'cosmopolitan gut bacteria' is a temporary term used until a function is demonstrated. Lactic acid bacteria isolated from honey and pollen of *T. angustula* may suppress spoilage bacteria (CA Rosa, personal communication), like the antagonist effect against chalkbrood pathogen (Gilliam, 1997; Yoshihama and Kimura, 2009). Considering a plant origin of *Staphylococcus* present in the gut of *Apis cerana japonica* (Yoshiyama and Kimura, 2009) is not sufficient to explain the presence of this bacterium in the inner cerumen pot of the nest of *T. angustula*. Composition of the substrate and interaction with inhibitory compounds may explain the presence of the yeast in the tip of the entrance tube –lighter color than the rest of the cerumen tube, possibly containing less resin, as tested burning these materials with a match. A higher yeast concentration in unripe pot-honey than the final product may indicate their role during honey ripening by *Melipona quinquefasciata* (Calaça, 2011, cited in Morais et al. 2013). Our preliminary data still need to confirm the species of microorganisms possibly with ancient based association with this bee, as assumed by Gilliam (1997).

Bacillus species are used by bees to convert, ferment, enhance, and/or preserve food, this process may occur during collection of nectar and pollen, as storage pots are filled, before brood cells are sealed, and during the 5-10 d delay before eggs hatch. The *Bacillus* species are metabolically active and produce numerous enzymes. Thus, these bacteria could produce chemicals such as fatty acids and antibiotics that inhibit competing organisms (fungi and other bacteria) as well as enzymes that convert food into more digestible products for storage (Gilliam et al., 1990).

These preliminary results confirm the presence of *Bacillus* spp. in nest products as previously reported by Gilliam et al. (1990), related to storage time of the processed materials in cerumen pots after pollen processing and brood-cell provisioning.

Acknowledgements

The nest we used was kindly detected by Mrs. Leandra Rial inside the cavity of an inverted Büchner funnel, in the garden of the Food Science Department, and kindly transferred to the wooden hive by Professor João MF Camargo during his visit to Universidad de Los Andes, March 2008. The authors are grateful to Professor Antonio Rodríguez-Malaver, Laboratory of Adaptive Biochemistry, Faculty of Medicine, Universidad de Los Andes, for collaborative support and equipment used. This work was financed by CDCHT-ULA Grant FA-1312-05-03-A and Apitherapy and Biocativity Group CVI-ADG-FA-04-97. We value comments kindly received from Prof. Carlos Augusto Rosa, Departamento de Microbiologia,

Universidade Federal de Minas Gerais. Final editorial comments of Dr. DW Roubik were greatly appreciated.

References

Al-Hindi RR. 2003. Microbiological qualities of imported and locally produced honey in Saudi Arabia. Arab Gulf Journal of Scientific Research 21: 204-209.

Aljadi AM, Kamaruddin MY. 2004. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. Food Chemistry 85: 513-518.

Al-Mamary M, Al-Meeri A, Al-Habori M. 2002. Antioxidant activities and total phenolics of different types of honey. Nutrition Research 22: 1041-1047.

Al-Waili N, Salom K, Al-Ghamdi A, Ansari MJ. 2012. Antibiotic, pesticide, and microbial contaminants of honey: Human health hazards. The Scientific World Journal 2012: 1-9.

Banskota AH, Tezuka Y, Kadota S. 2001. Recent progress in pharmacological research of propolis. Phytotherapy Research 15: 561-571.

Borrelli F, Maffia P, Pinto L, Ianaro A, Russo A, Capasso F, Ialenti A. 2002. Phytochemical compounds involved in the anti-inflammatory effect of propolis extract. Fitoterapia 73(1): 53-63.

Choudhari MK, Punekar SA, Ranade RV, Paknikar KM. 2012. Antimicrobial activity of stingless bee (*Trigona* sp.) propolis used in the folk medicine of Western Maharashtra, India. Journal of Ethnopharmacology 141: 363-367.

Cortopassi-Laurino M, Imperatriz-Fonseca VL, Roubik DW, Dollin A. 2006. Global meliponiculture: challenges and opportunities. Apidologie 37: 275-292.

Dos Santos C, Blochtein B, Megiolaro FL, Imperatriz-Fonseca V. 2010. Age Polyethism in *Plebeia emerina* (Friese) (Hymenoptera: Apidae) Colonies Related to Propolis Handling. Neotropical Entomology 39: 691-696.

El-Leithy M, El-Sibael K. 1992. Role of microorganisms isolated from bees, its ripening and fermentation of honey. Egyptian Journal of Microbiology 75: 679-681.

Engel P, Martinson VG, Moran NA. 2012. Functional diversity within the simple gut microbiota of the honey bee. Proceedings of the National Academy of Sciences 109: 11002-11007.

Gheldof N, Wang XH, Engeseth NJ. 2002. Identification and quantification of antioxidant components of honeys from various floral sources. Journal of Agricol and Food Chemetry 50: 5870-5877.

Gilliam M. 1997. Identification and roles of non-pathogenic micro-flora associated with honey bees. FEMS Microbiology Letters 155: 1-10.

Gilliam M, Roubik DW, Lorenz BJ. 1990. Microorganisms associated with pollen, honey, and brood provisions in the nest of a stingless bee, *Melipona fasciata*. Apidologie 21: 89-97.Gilliam M, Prest B, 1987. Microbiology of feces of the larval honey bee, *Apis mellifera*, Journal of Invertebrate Pathology 49; 70–75.

Gómez-Caravaca AM, Gómez-Romero M, Arráez-Román D, Segura-Carretero A, Fernández-Gutiérrez AJ. 2006. Advances in the analysis of phenolic compounds in products derived from bees. Pharmacology and Biomedical Anals 41(4):1220-1234.

Jaganathan SK, Mandal SM, Jana SK, Das S, Mandal M. 2010. Studies on the phenolic profiling, anti-oxidant and cytotoxic activity of Indian honey: in vitro evaluation. Natural Product Research 24: 1295-1306.

Johnston JE, Sepe HA,Miano CL, Brannan RG, Alderton AL. 2005. Honey inhibits lipid oxidation in ready-to-eat ground beef patties. Meat Science 70: 627-631.

Kücük M, Kolayli S, Karaoglu S, Ulusoy E, Baltaci C, Candan F. 2007. Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chemistry 100: 526-534.

Lace J, Valdovska A, Konosonoka IH. 2008. Microbial contamination in honey. International Scientific Conference: Implication of Different Production Technologies on Animal Health and Food Products Quality Indices. Sigulda, Latvia, 4-5 December.

Liberio SA, Pereira AL, Dutra RP, Reis AS, Araújo MJ, Mattar NS, Silva LA, Ribeiro MN, Nascimento FR, Guerra RN, Monteiro-Neto V. 2011. Antimicrobial activity against oral pathogens and immunomodulatory effects and toxicity of geopropolis produced by the stingless bee *Melipona fasciculata* Smith. BMC Complementary and Alternative Medicine 11:1-10.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193: 265-275.

Martins H, Martins L, Bernardo F. 2003. Bacillaceae spores, fungi and aflatoxins determination in honey. Revista Portuguesa de Ciencias Veterinarias 989: 85-88.

Maruyama H, Sakamoto T, Araki Y, Hara H. 2010. Anti-inflammatory effect of bee pollen ethanol extract from Cistus sp. of Spanish on carrageenan-induced rat hind paw edema. BMC Complementary and Alternative Medicine 23: 10-30.

Massaro FC, Brooks PR, Wallace HM, Russell FD. 2010. Cerumen of Australian stingless bees (*Tetragonula carbonaria*): gas chromatography-mass spectrometry fingerprints and potential anti-inflammatory properties. Naturwissenschaften 98: 329-337.

Menezes C, Vollet-Neto A, León Contrera FA, Venturieri GC, Imperatriz-Fonseca VL. 2013. The role of useful microorganisms to stingless bees and stingless bee keeping. pp. 153-171. In Vit P, Pedro S, Roubik DW, eds. Pot-honey: a legacy of stingless bees. Springer; New York, USA. 654 pp.

Michener CD. 2000. The bees of the world. The Johns Hopkins University Press, Baltimore MD, USA. 912.

Morais PB, Calaça PSST, Rosa CA. 2013. Microorganisms associated with stingless bees. pp. 173-186. In Vit P, Pedro S, Roubik DW, eds. Pot-honey: a legacy of stingless bees. Springer; New York, USA. 654 pp.

Nogueira-Neto P. 1997. Vida e criação de abelhas indígenas sem ferrão. Editora Nogueirapis; São Paulo, Brasil. 446 pp.

Omafuvbe BO, Akanbi OO. 2009. Microbiological and physico-chemical properties of some commercial Nigerian honey. African Journal of Microbiology Research 3: 891-896.

Persano Oddo LP, Heard TA, Rodríguez-Malaver A, Pérez RA, Fernández-Muiño M, Sancho MT, Sesta G, Lusco L, Vit P. 2008. Composition and antioxidant activity of *Trigona carbonaria* honey from Australia. Journal of Medicinal Food 11: 789-794.

Pick RA, Blochtein B. 2002. Atividades de vôo de *Plebeia saiqui* (Holmberg, 1903) (Hymenoptera; Apidae; Meliponinae) durante o período de postura da rainha e em diapausa. Revista Brasileira de Zoologia 19: 827-839.

Re R, Pellegrini N, Protoggnte A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity in improved ABTS radical cation decolorization assay. Free Radical in Biology and Medicine 26: 1231-1237.

Rodríguez-Malaver AJ, Rasmussen C, Gutiérrez MG, Gil F, Nieves B, Vit P. 2009. Properties of honey from ten species of Peruvian stingless bees. Natural Products Community 4: 1221-1226.

Rosa CA, Lachance MA, Silva JO, Teixeira AC, Marini MM, Antonini Y, Martins RP. 2003. Yeast communities associated with stingless bees. FEMS Yeast Reasearch 4: 271-275.

Roubik DW. 1989 Ecology and natural history of tropical bees. Cambridge University Press; Cambridge, UK. 514 pp.

Roubik DW. 2006. Stingless bee nesting biology. Apidologie 37: 124-143.

Singleton VL, Orthofer R, Lamuela-Raventos RM. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 299: 152–178.

Slaa EJ, Chaves LAS, Malagodi-Braga KS and Hofstde FE. 2006. Stingless bees in applied pollination: practice and perspectives. Apidologie 37: 293-315.

Snowdon J. 1999. The microbiology of honey—meeting your buyers' specifications. American Bee Journal 139: 51-60.

Teixeira EW, Negri G, Meira RM, Message D, Salatino A. 2005. Plant origin of green propolis: bee behavior, plant anatomy and chemistry. Evidence based Complementary and Alternative Medicine 2: 85-92.

Umthong S, Puthong S, Chanchao C. Trigona laeviceps propolis from Thailand: antimicrobial, antiproliferative and cytotoxic activities. 2009. American Journal of Clinical Medicine 37: 855-865.

Velazco J, Araque MC, Araujo E, Longa A, Nieves B, Ramírez AC, Sánchez K, Velazco E. 2008. Manual Práctico de Bacteriología Clínica. Publicaciones Vicerrectorado Académico, Univerisdad de Los Andes; Mérida, Venezuela. 206 pp.

Winston ML. 1987. The biology of the honey bee. Harvard University; London, UK. 281 pp.

Woisky RG, Salatino A. 1998. Analysis of propolis: some parameters and procedures for chemical quality control. Journal of Apiculture Research 37: 99-105.

Yao L, Datta N, Tomas-Barberan FA, Ferreres F, Martos I, Singanusong R. 2003. Flavonoids, phenolic acids and abscissic acid in Australian and New Zealand *Leptospermum* honeys. Food Chemistry 81: 159–168.

Yoshihama M, Kimura K. 2009. Bacteria in the gur of Jpanese honeybee *Apis cerana japonica*, and their antagonistic effect against *Paenibacillus larvae*, the causal agent of American foulbrood. Journal of Invertebrate Pathology 102: 91-96.

how to cite this chapter?

Pérez-Pérez EM, Suárez E, Peña-Vera MJ, González AC, Vit P. 2013. Antioxidant activity and microorganisms in nest products of *Tetragonisca angustula* Latreille, 1811 from Mérida, Venezuela. pp. 1-8. In Vit P & Roubik DW, eds. Stingless bees process honey and pollen in cerumen pots. Facultad de Farmacia y Bioanálisis, Universidad de Los Andes; Mérida, Venezuela. http://www.saber.ula.ve/handle/123456789/35292