Research Article

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Production of propolis and geopropolis by stingless bees

Abstract

Propolis is a resinous product produced by honey bees (*Apis mellifera*) with plant exudates or buds, to which they aggregate beeswax. Most stingless bees (meliponines) produce propolis in ways similar to honey bees. Several meliponines include soil in their propolis, with the effect that the final mass and volume of the product is increased. For this reason, meliponine propolis is in general called geopropolis. In the present work propolis of two meliponines native in Brazil, *Melipona marginata* and *Scaptotrigona xanthotricha*, were analyzed for determination of the contents of moisture, ashes, wax, total phenols and sequestering activity. The content of ashes of propolis of *M. marginata* was very high (73.66%), indicating that the product, in fact, contains soil. On the contrary, the content of ashes of *S. xanthotricha* (1.99%) is comparable with the ash content of propolis of honey bees. Thus, the propolis of *S. xanthotricha* does not contain soil, and thus, in this case, the designation geopropolis is not appropriate. The contents of the wax of propolis were 1.44% for *M. marginata* and 28.82% for *Scaptotrigona*. These results suggest opposed strategies adopted by meliponines, with the consequence of enlarging propolis volume and mass. The strategies are an aggregation of either soil (*M. marginata*) or wax (*S. xanthotricha*). The contents of total phenols for both species were low, below the minimum admitted by Brazilian official rules. Coherent with the content of total phenols, the sequestering activity also was lower for propolis of both species.

Keywords: Meliponini, geopropolis, wax, phenolic substances, antioxidant activity, quality control

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Introduction

So far, papers about propolis refer to honey bees (*Apis mellifera*). Propolis is a hive product composed of plant resin, beeswax, pollen, and other minor constituents. Not much has been done about propolis from species of stingless bees. These insects belong to the family Apidae (same as *Apis*), subfamily Apinae, and tribe Meliponini. Most meliponines do not produce honey, although the Brazilian *Melipona scutellaris* ("uruçu") produce honey available commercially. On the other hand, many stingless bees from Tropical America and Australia produce propolis. The composition of the resin of this propolis has been reviewed.[1,2] In general, propolis from *Apis* and meliponine contains phenolic compounds as main constituents. In some cases, both bee groups share the same plant source and the composition of their propolis is almost identical.[3] Antioxidant and antimicrobial activities, among many other biological properties, have been reported for propolis of stingless bees.[2]

The importance of meliponine conservation, particularly in tropical countries, has been emphasized, particularly due to the role these insects have in the pollination of either native[4] or crop plants.[5] lately, meliponiculture has been practiced with both conservation and commercial aims in several tropical and subtropical countries. Presently, the production of meliponine propolis is an emerging market in tropical America[6] and Australia.[7]

Propolis productivity by meliponines is small in comparison with honey bees. Meliponine species add soil material to propolis, a means that probably increases the volume and mass of the product. Meliponine propolis containing soil material is referred to as geopropolis, a designation that is commonly applied to propolis of all meliponine species. However, the unrestricted use of the term may be misleading, because there is no experimental evidence demonstrating that all meliponines include soil in their propolis. We hypothesize that other strategies may be used by some meliponines, which also add mass and volume to propolis. We suggest that accretion of a large quantity of beeswax may be one of these alternatives.

In either case, substantial quantities of biologically inert material are added to the propolis, bringing as a consequence a drop in the relative content of active constituents. The aim of the present investigation is to carry out a preliminary approach to evaluate the content of soil and wax, as well as other parameters, in propolis produced by two common meliponines in Brazil, in order to test the possibility of existence of distinctive strategies toward increasing propolis mass and volume. With this in mind, it was planned to determine the contents of ashes, waxes and other parameters in propolis of both species.

Material and methods

Samples collection

The samples of propolis analyzed were obtained from three colonies of *Melipona marginata* ("manduri") and *Scaptotrigona xanthotricha* ("mandaguari amarela"). Hives of both species are commonly grown in meliponaries in many localities in Brazil because the honey they produce is appreciated and exploited commercially. Propolis samples used in the present work were obtained from the same meliponary, located in the municipality of Guarujá, littoral of the state of São Paulo (southeast Brazil; 23° 59' 41" S, 46° 15' 25" W).

Moisture content

The moisture content plus volatile substances of the propolis samples was determined by placing 3g of the product in a ventilated oven at 80°C until constant weight.

Ash contents

To obtain the values of ash contents, 3g of the dried samples were incinerated inside a crucible, which was placed inside a muffle at 600°C until complete mineralization.

Wax contents

Waxes were extracted by treatment of 3g of samples pulverized with the aid of liquid nitrogen with dichloromethane in a Soxhlet extractor for 6h. The solvent was evaporated at reduced pressure, the residue solubilized with hot ethanol and then placed in a freezer at -20°C. The residue obtained was filtered through a previously weighed filter paper. The paper and flask were transferred to a desiccator until constant weight.

Total phenolic contents and sequestering activity

The content of total phenolic substances was determined using the reagent Folin-Ciocalteau. Details of all procedures are available elsewhere.[8] The EC_{50} values regarding DPPH sequestering activity were determined according to Schmidt et al.[9] using extracts of the propolis samples at 5mg/ml.

Results and discussion

Effect of color of soil

A striking difference was observed comparing the color of the residues remaining in the crucibles after calcination of the propolis of *M. marginata* and *S. xanthotricha* . The ashes of the former have a red-brown color, typical of iron oxides and salts. This result indicates that *M. marginata* propolis contains clay as a relevant constituent, and thus it is a real geopropolis. On the other hand, the residue in crucibles corresponding *S. xanthotricha* had a gray color, similar to ashes derived from calcination of propolis from *Apis* (personal observation). This color suggests that propolis of *S. xanthotricha* is devoid of soil.

Effect of soil and wax

Table 1 contains data about several parameters determined for propolis from both meliponine species. The content of ashes of *M. marginata* propolis is six-fold over the content of *S. xanthotricha* propolis. Conversely, the content of wax of *S. xanthotricha* propolis is 20 times higher the content of *M. marginata* propolis. The results indicate that each meliponine species aggregate either soil (*M. marginata*) or wax (*S. xanthotricha*) while producing propolis. While the designation "geopropolis" is appropriate referring to propolis of *M. marginata*, it is not adequate regarding *S. xanthotricha*. Data of Table 1 about the contents of ashes and wax suggest that meliponines adopt distinct strategies that compensate their relatively low productivity of propolis. The content of moisture was higher in propolis of *S. xanthotricha* (Table 1). The aggregation of material other than plant resins to propolis, be it moisture, soil or wax, decreases the relative content of biologically active substances, such as phenols. The higher content of total phenols in propolis of *S. xanthotricha* (mean: 1.08%) than in propolis of *M. marginata* (mean: 0.62; Table 1) is coherent with the amount of material not derived from plants, e.g. soil and wax. In fact, the sums of the means of these parameters regarding *M. marginata* and *S. xanthotricha* are 75.1% and 30.81%, respectively (Table 1). In consequence, the propolis of the latter bee has higher antioxidant activity (lower value EC_{50}) than the propolis of the former (Table 1). A correlation between total phenols and antioxidant activity often has been reported.[10,11]

In several countries, rules have been put forward aiming to guarantee standards of quality of propolis. In this way, minimum or maximum contents of moisture, wax, ashes, total phenols, total flavonoids, among other parameters, were established. Argentine, Brazil, Bulgaria, and Russia are examples of countries that have adopted rules about propolis identity and quality. In Brazil, standards of propolis quality are defined by the Technical Regulation of Propolis Identity and Quality (TRPIQ), of the Brazilian Ministry of Agriculture.[12]

Quality of propolis

The parameters of both propolis analyzed are not in full conformity with the requisites of STRQP (Table 1). Regarding *S. xanthotricha*, the moisture and wax contents (13.84% and 28.82%, respectively) lie above the maximum admitted (8% and 25%, respectively). On the other hand, the content of total phenols (1.08%) is below the minimum admitted (5%). On its turn, the propolis of *M. marginata* has the content of ashes (73.66%) far above the maximum (5%), as well as content of total phenols below the minimum (5%) admitted by STRQP (Table 1). The low contents of phenols of both propolis account for the high values of EC_{50} of sequestering activity (578.49µg/ml) and (91.92µg/ml) regarding *M. marginata* and *S. xanthotricha*, respectively). Values of EC_{50} regarding the sequestering activity of samples of green propolis of Africanized honey bees from the State of Minas Gerais (southeast Brazil), derived from resins of *Baccharis dracunculifolia* (Asteraceae), lay in the range 10-40µg/ml.[13] The EC_{50} of two samples of Propolis of *Apis mellifera* from northeast Brazil, derived from *Mimosa tenuiflora* (Leguminosae), were 56.2 and 72.9 µg/ml.[14] The higher activity of honey bee propolis, in comparison with meliponine propolis, is accounted for the lower content of phenolic substances in the latter, due to aggregation of material not derived from plant resins.

Future requirements

The present work raises evidence that at least some meliponine species produce propolis without soil, i.e. they do not produce geopropolis. Considering the possibility that meliponine propolis becomes a commercial product for use by humans or domestic animals, it is crucial to find out if the product contains soil or not. The presence of soil in propolis may turn out an obstacle for commercial exploitation, due to the possibility of soil contamination by fungi and bacteria, among other inconveniences. In this case, microbiological analysis is going to be an additional step among the parameters considered for quality control. This inconvenience does not apply to propolis devoid of soil material, such as *S. xanthotricha*. This is a relevant topic to consider, because of the current trend of use of meliponiculture products including propolis.[6]

Further investigations are needed to get a broad picture of chemical profiles of propolis of stingless bees, in particular, those involving parameters included in TRPIQ and similar international rules. If products of some meliponine species become appreciated in the propolis market, more relaxed, or specific parameters for chemical quality control will have to be established.

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Table 1 Parameters determined for propolis of *Melipona marginata* ("manduri") and *Scaptotrigona xanthotricha* ("mandaguari amarela") and requisites established by the Technical Regulation of Propolis Identity and Quality (TRPIQ).[12] Means with the same letter between columns are not significantly different (α=0.05)

Parameters	Melipona	Scaptotrigona	TRPIQ
Moisture (%, m/m)	5.63±0.07a	13.84±0.62b	8% maximum
Ashes (%, m/m)	73.66±0.16a	1.99±0.21b	5% maximum
Wax (%, m/m)	1.44±0.12a	28.82±2.95b	25% maximum
Total phenols (%, m/m)	0.62±0.02a	1.08±0.14b	5% minimum
EC50 (DPPH; µg/ml)	578.49	91.62	-

Conclusion

a. There seem to be distinct ways stingless bees adopt to add volume and mass to their propolis.
b. Evidence raised in the present work suggests that some species aggregate soil to propolis.
c. In these cases, the content of propolis wax is similar to propolis of honey bees. Other stingless bees, however, add a high quantity of wax, which also increases propolis volume and mass.
d. In this case, the parameter that is similar to honey bee propolis is the ash content, indicating that no soil is included. Thus, the unrestricted designation "geopropolis" regarding all species of stingless bees should be avoided.

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Conflicts of interest

The authors declare that there was no conflict of interest.

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