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PHYSICO-CHEMICAL ANALYSIS AND HEAVY METALS IN SALVADORAN PROPOLIS

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Abstract: Propolis are natural products made by bees from plant resins. They have been traditionally valued for their potential therapeutic benefits, including antibacterial, anti-inflammatory and antioxidant properties. The objective of this study is to evaluate the physicochemical quality and safety of propolis from El Salvador, with a focus on the detection and quantification of heavy metals, specifically lead and cadmium, known to have adverse effects on human health. The methodology used for the determination of physicochemical parameters includes traditional gravimetric techniques and to evaluate the presence of lead and cadmium, atomic absorption spectroscopy was used. As results, all samples analyzed were found to be within the limits allowed by Salvadoran standard NSO 65.19.02:03 for moisture, ash and mechanical impurities, lead and cadmium. However, out-of-specification results were observed in three samples in the ethanol solubility test and in two samples for wax content. In conclusion, this study underlines the importance of good quality propolis from Chilanga, Morazán and Los Naranjos Sonsonate. In addition, it corroborates the compliance of not exceeding the limits of lead and cadmium of all the analyzed propolis; providing relevant data for the regulation and safe use of these products in medicinal and cosmetic applications in El Salvador.

Keywords: Propolis, cadmium, lead, heavy metals, physicochemical analysis.

INTRODUCTION

Propolis is a resinous substance produced by bees from plant products and has been widely recognized for its medicinal and therapeutic properties (Bankova et al., 2000). In El Salvador, a country with rich biodiversity, propolis represents a valuable natural resource whose quality and composition need to be investigated (Castaldo & Capasso, 2002), and the study of the quality of Salvadoran propolis is of great scientific relevance due to its diversity of uses in human health and local industry.

The physicochemical quality of Salvadoran propolis includes parameters such as moisture content, ash, waxes, ethanol-soluble material and organoleptic characteristics, which affect its therapeutic efficacy and industrial processing (Marcucci, 1995). On the other hand, the quantification of heavy metals in propolis, is a critical point to be evaluated, because their accumulation, can be harmful to human health (Castro-Vázquez et al., 2018). Evaluating the concentration of these metals and establishing safe limits for their consumption is essential.

In addition, knowledge of their physicochemical characteristics will allow maximizing their use both in traditional medicine and in the pharmaceutical industry, as well as a better commercial positioning of the products to be developed from propolis. The objective of this study is to characterize the physicochemical quality and evaluate the presence of heavy metals in Salvadoran propolis, contributing to scientific knowledge about this resource and facilitating its safe and sustainable use (Marcucci, 1998).

METHODOLOGY

Six propolis samples were collected in the departments of Sonsonate, La Libertad and Morazán, between May and July 2019, using the hive scraping method. The samples were coded according to their collection site and surrounding flora, which are detailed in *Table 1*. From each sample, 20g were taken for physicochemical analysis, with the remainder stored at -20°C for subsequent grinding.

Code	Propolis Color	Collection Place	Surrounding Flora
M1	Coffee	La Laguna, Morazán	*Secondary forest with timber vegetation: conacaste Enterolobium cyclocarpum, copinol Hymenaea courbaril. In addition to: Mangifera indica, cashew Anacardium occidentale.*
M2	Coffee	El Rosario, Morazán	
M6	Dark Coffee	Jocoaitique, Morazán	
M3	Coffee	Huizúcar, La Libertad	Mature evergreen forest: mango *Mangifera indica*, carreto *Pithecollobium saman*, maquilishuat *Tabebuia pentaphylla*.
M4	Coffee	Los Naranjos, Sonsonate	Coniferous forest: pine *Pinus sylvestris*, cypress *Cupressus lusitanica* Mill, araucaria *Araucaria heterophylla* Salisb.
M5	Green	Chilanga, Morazán	Deciduous forest: laurel *Cordia alliodora*, Mangollano *Pithecellobium saman*, mother cocoa" *Gliricidia sepium* and charcoal tree *Mimosa tenuiflora*. Willd.Poir.

Table 1. General information on the Salvadoran propolis under study.

PHYSICOCHEMICAL DETERMINATIONS

The physicochemical determinations were carried out in triplicate and the results were expressed in percentages (w/w) and compared with the Salvadoran Standard NSO 65.19.02:03 "Quality of raw propolis" (OIRSA Standard).

Loss on drying: The thermogravimetric method (AOAC) was used, weighing 1.0g of raw propolis, which was dried in an oven at 105°C for three hours until constant weight was obtained.

Ash determination: It was carried out by gravimetry following the AOAC method, subjecting 1 g of propolis to calcination in a muffle at 550°C for three hours.

Determination of waxes and ethanol extractable material (MEE): The percentage of waxes and MEE was determined by gravimetry according to the protocols of the technical regulation and the stipulated guidelines of identity and quality of propolis of the Ministry of Agriculture of Brazil (APACAME Standard). The crude propolis was deposited in a cellulose thimble and soxhlet extraction was performed for six hours with 96%v/v ethanol. The ethanolic extract was cooled to -22°C for 24h. Subsequently, the precipitated waxes were eliminated by filtration on Whatman N°1 paper; the waxy precipitate retained on the filter paper was dried in an oven until it reached a constant weight at a temperature no higher than 50ºC. The difference in weight was expressed as percentage of waxes (w/w). Finally, 3g of the solvent from the filtrate was evaporated in a watch glass and dried in an oven at 105°C for 60min until constant weight; the residue obtained was expressed as percentage extractable with ethanol w/w.

Determination of insoluble material. The insoluble residue retained in the cellulose thimble at the end of the soxhlet extraction process was placed in an oven at 37ºC until constant weight. The content of non-extractable material was expressed as a percentage (w/w).

HEAVY METALS

Lead and Cadmium: Lead *and Cadmium* were determined by atomic absorption spectroscopy in a graphite furnace (Shimadzu AA-7000) after treatment of the ashes of the propolis samples, following the procedure for the quantification of heavy metals (Popova et al. 2004).

RESULTS AND DISCUSSION

In propolis, the moisture parameter refers to the loss of mass during the drying process, i.e., water and other volatile compounds pass to the gaseous state due to heat. All propolis samples analyzed are below the limit value established by the Salvadoran standard for crude propolis (OIRSA Standard), which allows a maximum of 8% moisture, as shown in *Table 2*. This parameter is linked to the type of surrounding flora and the time of collection (Salatino et al., 2011). Although all samples comply with the moisture limit, the one with the highest value is the one from the municipality of Chilanga (M5), with 7.53%, close to the allowed limit. It is possible that this is due to the incorporation of chlorophyll from the trees by the honeybees during propolis production, which could increase the moisture content. In addition, it is important to consider that the samples were collected at the beginning of the rainy season, which could also influence this result.

Table 2 shows the values of the ash parameter, where all the samples in this study comply with Salvadoran regulations, which establish a maximum of 5%. This suggests that good beekeeping practices have been followed, avoiding the incorporation of inorganic materials such as clay, small rocks or corroded metal.

Ethanol soluble material (ESM) is a critical quality parameter in propolis, and significant differences were found among the samples analyzed. The propolis with the highest MSE content were those from Huizúcar M3, Los Naranjos M4 and Chilanga M5, with values of (32.3 ± 0.74%), (51.0 ± 1.38%) and (30.5 ± 1.32%) respectively; which comply with Salvadoran regulations that establish a minimum of 30.0%. These results indicate a good proportion of secondary metabolites present in the resins of plant species near these hives.In El Salvador, a high MSE content in propolis is associated with a higher presence of terpenes, flavonoids and chalcones (Galoetti et al. 2018).

In contrast, samples from La Laguna M1, El Rosario M2 and Jocoaitique M6 showed low MSE content. Another relevant parameter for propolis quality is wax content. Most of the samples are within the limits established by Salvadoran regulations (<25%), except samples M1 La Laguna and M4 Los Naranjos, which presented a high wax content. This could be due to the fact that, during propolization, the bees mix resins with a higher proportion of wax to seal the holes of the frames in the hive. Generally, a high wax content is associated with lower quality; the compounds of this fraction are non-polar and usually lack biological activity. However, some studies have reported the presence of triterpenic alcohols and their acetates in this fraction, with antimicrobial properties, (Ristivojević, 2016). The average wax content in this study, is 21.27%, lower than those reported by (Palomino et al. 2010) and (Chaillou et al. 2004), who obtained average values of 72.34% and 30.04% respectively, using n-hexane as solvent for wax extraction.

On the other hand, the results indicate that all the Salvadoran propolis samples comply with the maximum limit of mechanical impurities allowed, which is 40.0%, results that reflect a relatively low average of impurities, indicating a harvesting practice free of foreign inorganic materials and consistent with the quality of the propolis.

As for the determination of heavy metals, the results for lead and cadmium are detailed in *Table 3*. Lead concentrations are well below the limit established by Salvadoran regulations (<5 ppm). However, the regulations do not contemplate a specific parameter for cadmium in propolis, so a study conducted in Turkey under forest conditions similar to those in El Salvador was used as a reference, where a cadmium concentration range between 0.60

Code	% Humidity (Maximum: 8.0 %)	% Ash (Maximum: 5.0 %)	% Ethanol soluble material m/m (Minimum: 30.0 %)	Waxes (Max.: 25.0 %)	Mechanical impurities (Maximum: 40.0 %)
M1	3.80 ± 0.16	2.07 ± 0.10	14.6 ± 1.49	25.1 ± 0.54	33.5 ± 0.32
M2	4.90 ± 0.03	2.80 ± 0.07	21.6 ± 1.67	24.3 ± 0.90	22.6 ± 0.47
M3	5.01 ± 0.16	2.11 ± 0.16	32.3 ± 0.74	18.5 ± 0.52	30.8 ± 0.29
M4	4.77 ± 0.04	2.36 ± 0.01	51.0 ± 1.38	30.4 ± 0.78	28.1 ± 0.54
M5	4.32 ± 0.14	2.24 ± 0.01	30.5 ± 0.72	22.0 ± 0.60	24.7 ± 0.55
M6	7.53 ± 0.16	1.93 ± 0.13	7.3 ± 0.50	7.3 ± 0.50	30.5 ± 0.18

Results of the physicochemical determinations of the propolis under study.

Code	Location	Pb ($\underline{X} \pm S$) Max. 5 ppm	Cd ($\underline{X} \pm S$) 0.8-0.6 ppm
M1	Los Claros Farm. Canton La Laguna, El Rosario.	0.560 ± 0.06	ND**
M2	Quinta Neftaly. Canton La Laguna, El Rosario.	0.332 ± 0.06	ND**
M3	Apiary Leonardo. Municipality of Huizúcar	0.297 ± 0.03	ND**
M4	Apiary Mario Calzadilla. Canton Los Naranjos, Municipality of Juayúa.	0.276 ± 0.02	ND**
M5	Apiary Carlos Vargas. Canton Las Joyas of the Municipality of La Chilanga.	0.149 ± 0.01	ND**
M6	Apiary Mauricio Castro. Municipality of Jocoaitique.	0.617 ± 0.12	0.13 ± 0.0

Results of lead and cadmium heavy metals by atomic absorption method.

* Matin, G., Kargar, N., & Buyukisik, H. B. (2016). Bio-monitoring of cadmium, lead, arsenic and mercury in industrial districts of Izmir, Turkey by using honey bees, propolis and pine tree leaves. Ecological Engineering, 90, 331-335.

** ND: Not Detectable (< 0.01 ppm)

and 1.80ppm was established (Martín et al. 2016). The results obtained in this study are satisfactory, detecting only traces in most of the samples *(see Table 3)*.

The low concentrations of lead and cadmium in Salvadoran propolis suggest that the collection areas are free of heavy metal contamination from agricultural or industrial activities.

CONCLUSIONS

Compliance with all the parameters established in the Salvadoran propolis standards is attributed only to the Huizucar M3 and Chilanga M5 samples. A significant correlation has been observed between the M5 propolis from deciduous forests and the plant species *Mimosa tenuiflora*. Although Los Naranjos M4 propolis, from coniferous forests, does not comply with the ethanol soluble material parameter, it complies satisfactorily with the other parameters evaluated. The results obtained for the levels of heavy metals lead and cadmium evaluated in this study indicate that all the propolis sampled from the different areas of El Salvador are safe for human consumption.

The critical parameter of resin content in propolis is met only by propolis from Huizúcar M3 and Chilanga M5. This determination of physicochemical indicators provides bee producers with valuable information to improve their production processes and suggests its potential application in traditional medicine and its use as a basis for the development of nutraceuticals, functional foods and various products with high commercial value.

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