

Physicochemical and microbiological quality of honeys produced by stingless bees *Scaptotrigona polysticta*, *Melipona illota* and *Tetragonisca angustula* (Apidae: Meliponini) in San Martín, Peru

Calidad físico - química y microbiológica de las mieles producidas por las abejas sin aguijón *Scaptotrigona polysticta*, *Melipona illota* y *Tetragonisca angustula* (Apidae: Meliponini) en San Martín, Perú

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Abstract

The honeys from stingless bees (Hymenoptera: Apidae: Meliponini) are recognized for their medicinal properties. They are commonly used by many indigenous groups around the world. However, in Peru stingless beekeeping is practiced in an artisanal way and the honeys remain products whose qualities are still little studied. The objective of this work was to analyze physicochemical characteristics and microbiological quality of honeys produced by *Scaptotrigona polysticta* Moure, *Melipona illota* Cockerell and *Tetragonisca angustula* Latreille in apiaries of department of San Martín, Peru. In June and November 2019, 30 honey samples were collected from 24 colonies housed in two apiaries in the towns of Tarapoto and Chasuta. Significant differences ($p < 0,05$) were found for pH ($3,8 \pm 0,6$; $3,5 \pm 0,6$; $4,7 \pm 0,6$) and the percentage of total solutes ($70,4 \pm 2,5\%$; $61,2 \pm 3,2\%$; $73,0 \pm 2,7\%$), among the honey samples of *S. polysticta*, *M. illota*, and *T. angustula*, respectively. The color of *S. polysticta* honey was variable, from light amber to dark yellow (115 ± 23 mm Pfund), while *T. angustula* honey was darker yellow (178 ± 33 mm Pfund). Values for mesophilic and anaerobic sulfite-reducing microorganisms were under the level of 1 CFU / mL for all species. Fungi and yeasts exceeded the allowed value (> 10 CFU / mL) according Peruvian sanitary norm N° 071-Minsa/Digesa-V.01, in 30 and 50% of the samples of *S. polysticta* and *T. angustula*, respectively. These results contribute to the knowledge of the stingless bee honeys of Peru providing important references for the quality standard setting process and the advancement of stingless beekeeping.

Keywords: Meliponini, honeys, Peruvian Amazon, Tarapoto, meliponiculture.

Resumen

Las mieles de abejas sin aguijón (Hymenoptera: Apidae: Meliponini) son reconocidas por sus propiedades medicinales y comúnmente utilizadas por muchos grupos indígenas de todo el mundo. Sin embargo, la crianza de abejas sin aguijón en el Perú se practica de forma artesanal y las mieles son productos de cualidades aún poco estudiadas. El objetivo de este trabajo fue analizar las características fisicoquímicas y la calidad microbiológica de las mieles producidas por *Scaptotrigona polysticta* Moure, *Melipona illota* Cockerell y *Tetragonisca angustula* Latreille en apiarios del departamento de San Martín, Perú. En junio y noviembre de 2019 se recolectaron 30 muestras de miel de 24 colonias alojadas en dos colmenares en las localidades de Tarapoto y Chasuta. Se encontraron diferencias significativas ($p < 0,05$) para el pH ($3,8 \pm 0,6$; $3,5 \pm 0,6$; $4,7 \pm 0,6$) y el porcentaje de solutos totales ($70,4 \pm 2,5\%$; $61,2 \pm 3,2\%$; $73,0 \pm 2,7\%$), entre las muestras de miel de *S. polysticta*, *M. illota*, y *T. angustula*, respectivamente. El color de la miel de *S. polysticta* varió de ámbar claro a amarillo oscuro (115 ± 23 mm Pfund), mientras que lo de la miel de *T. angustula* se presentó amarillo

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más oscuro (178 ± 33 mm Pfund). Los valores de microorganismos mesófilos y anaerobios sulfitos reductores estuvieron por debajo del nivel de 1 UFC / mL para todas las mieles. Los hongos y levaduras excedieron el valor permitido (> 10 UFC / mL) según la norma sanitaria peruana N° 071-Minsa/Digesa-V.01, en 30 y 50% de las muestras de *S. polysticta* y *T. angustula*, respectivamente. Estos resultados contribuyen al conocimiento de las mieles de abejas sin aguijón del Perú, al proporcionar referencias para establecer estándares de calidad que promuevan el avance de la meliponicultura.

Introduction

Meliponini (Hymenoptera: Apidae), commonly called stingless bees, are important pollinators that inhabit the tropical and subtropical areas of the world (Yáñez-Ordóñez *et al.*, 2008). In the Amazon region, these insects would pollinate 38% of all plant species (Kerr *et al.*, 2001).

Since the time of the Maya, the Meliponini were managed for production of honey, whose antioxidative (Cauch *et al.*, 2015), antiseptic, antimicrobial, anticancer and anti-inflammatory properties have been proven in several studies (Alvarez-Suárez *et al.*, 2012; Silva *et al.*, 2013). Nowadays, stingless beekeeping or “meliponiculture” remains a widespread practice in many Latin American countries (Nates-Parra & Rosso-Londoño, 2013; Paris *et al.*, 2018). Despite the importance attached to their honeys, little has been done to value the resource (de Oliveira, 2013). The introduction of stingless bee honey to the market is limited by the low knowledge of the characteristics that define the quality, as well as the absence of regulations and technical procedures that define the standards.

In Peru, about 170 species of stingless bees have been reported (Rasmussen & Gonzalez, 2009) and meliponiculture is practiced in an artisanal way by native peoples. *Melipona illota* Cockerell, *Tetragonisca angustula* Latreille and *Scaptotrigona spp.* Moure are commonly raised (Rasmussen & Castillo, 2003). The quality standards for honey of exotic honeybee (*Apis mellifera* Linnaeus 1758) have been normed in Peru (NTP 209.168) (Instituto Nacional de Defensa de la Competencia y de la Protección de la Propiedad Intelectual [Indecopi], 2014), while stingless bee honey remains an unknown product. To date, there is hardly a single scientific work to characterize it (Rodríguez-Malaver *et al.*, 2009; Red de Seguridad Alimentaria - Consejo Nacional de Investigaciones Científicas y Técnicas [RSA-Conicet], 2018). The purpose of this study was to determine the physicochemical characteristics and microbiological quality of the honeys produced by *Scaptotrigona polysticta*, *Melipona illota* and *Tetragonisca angustula* in apiaries of San Martín, Peruvian Amazon.

Materials and Methods

Procedure in the apiaries. The study was carried out in two apiaries in the localities of Tarapoto and Chasuta, a transitional area of the tropical dry-humid forest in Huallaga river basin of San Martín, Peru (Figures 1-2). In June and November 2019, 30 honey samples of *S. polysticta*

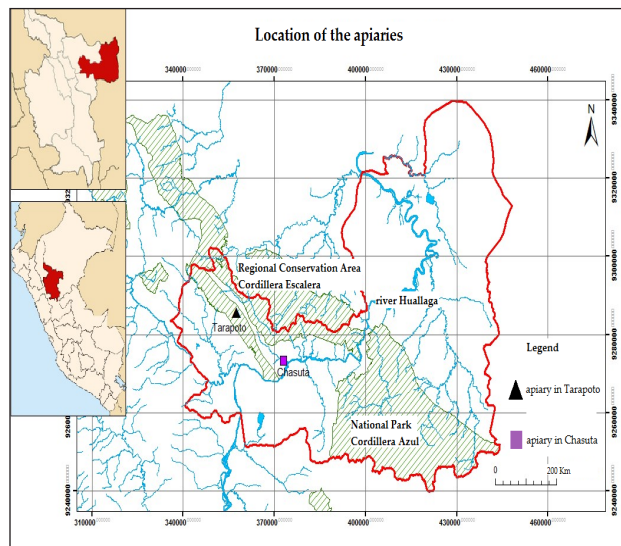


Figure 1. Location of the apiaries.



Figure 2. 2.A Meliponario Chasuta. 2.B Meliponario Tarapoto.

($n_1 = 15$), *M. illota* ($n_2 = 4$) and *T. angustula* ($n_3 = 11$) were collected from 24 colonies (Table 1) reared in modern hives. Sterile syringes and a basic glove, lab coat and mask were used to extract the honey. Each sample consisted of the total amount of honey extracted from ten ripe pots randomly chosen within the colonies. The samples were

stored at a temperature of 4 °C. In the apiary of Chasuta were present only beehives of the species *Tetragonisca angustula*.

Table 1. Number of honey samples collected by apiary.

	Tarapoto	Chasuta
<i>S. polysticta</i>	15	0
<i>M. illota</i>	4	0
<i>T. angustula</i>	6	5

Chemical-physical analysis. The analyzes were carried out in the Takiwasi Laboratory, in Tarapoto (<http://www.laboratorio.takiwasi.com/>). Hydrogen potential (pH) was measured with a pH meter (Hanna brand, model HI 8424); the total polyphenol content with a spectrophotometer (UNICO brand), according to the procedure described by Meda *et al.* (2005); and the color, determined according to Instituto Argentino de Normalización y Certificación (Iram, 2007), by means of a Pfund Kohler colorimeter and a spectrophotometer (Unico ®). The standards of the United States Department of Agriculture (Usda, 1985) were used to establish the correspondence among mm Pfund values and color. A field Brixometer (0 - 90% Brix) (Texim ®, model TR-099) was used to measure the total solutes. Color and total polyphenols of *M. illota* honey have not been determined due to insufficient sample volume for this analysis.

Microbiological analysis. The colony-forming units (CFU/mL) were counted in plates for: a) aerobic mesophilic organisms, b) anaerobic sulfite-reducing agents (cf. *Clostridium perfringens*), c) molds and yeasts, according to the following standards from International Organization for Standardization (ISO): 4833-1 (ISO, 2019), 15213 (ISO, 2003) and 7937 (ISO, 2004), respectively. The results were compared with the maximum values allowed by the Peruvian sanitary technical standard NTS N° 071-Minsa/Digesa-V.01 (Ministerio de Salud, [Minsa], 2008).

Data analysis

The data are expressed as mean ± standard deviation. A t-Student test was applied for comparison of means. The statistical analyses were performed using Analysis ToolPak for Microsoft Excel 2019 (Microsoft Corporation, Redmond, USA). A p-value <0,05 was considered significant.

Results

Chemical-physical quality. The honeys showed differences in the parameters pH, percentage of total solutes, color, and total polyphenols (Table 2). *T. angustula* honey presented the highest pH value ($4,7 \pm 0,6$) and a high total solute content ($73,0 \pm 2,7\%$), compared to the other species. The *S. polysticta* honeys showed a strong variation of color: from light amber to dark yellow, while

the samples of *T. angustula* were all dark yellow (Table 3). Total polyphenols were higher in *T. angustula* honeys compared to that observed for *S. polysticta* samples (Table 4).

Table 2. Main chemical parameters of samples (n) of honey from *S. polysticta*, *M. illota*. and *T. angustula* (one day after harvest; conservation at 4 ° C).

	n	pH	% Total solutes
<i>S. polysticta</i>	15	$3,8 \pm 0,6^b$	$70,4 \pm 2,5^b$
<i>M. illota</i>	4	$3,5 \pm 0,6^c$	$61,2 \pm 3,2^c$
<i>T. angustula</i>	11	$4,7 \pm 0,6^a$	$73,0 \pm 2,7^a$

Note: Different letters indicate significance for p <0,05.

Table 3. Values of mm Pfund and color of samples (n) of honeys of *S. polysticta* and *T. angustula* (seven days after harvest; conservation at 4 ° C).

Species	n	mm Pfund	Range	Color (spectrum)
<i>S. polysticta</i>	6	115 ± 23^a	77 - 141	Light amber - dark yellow
<i>T. angustula</i>	5	178 ± 33^b	147 - 216	Dark yellow

Note: Different letters indicate significance for p <0,05.

Table 4. Total polyphenol content (TPC) in mg / 100 g for samples (n) of *S. polysticta* and *T. angustula* honeys (seven days after harvest; storage at 4 ° C).

Species	n	TPC (mg/100g)
<i>S. polysticta</i>	6	$8,9 \pm 0,8^a$
<i>T. angustula</i>	5	$12,2 \pm 2,8^b$

Note: Different letters indicate significance for p <0,05.

Microbiological quality. Honey samples from *S. polysticta*, *M. illota* and *T. angustula* were free of bacterial contamination; but 30% and 50% of the samples of *S. polysticta* and *T. angustula*, respectively, exceeded the maximum threshold allowed by the Peruvian sanitary standard for molds and yeasts (Table 5).

Discussion

The results of this study are consistent with those obtained by other authors (Souza *et al.*, 2006; Fuenmayor *et al.*, 2012; Vit *et al.*, 2016; Lemos *et al.*, 2017; RSA-Conicet, 2018; Grajales-Conesa *et al.*, 2018) in terms of acidity and total solute content. However, the methods of analysis used in the different scientific papers could be distinct, so it would be difficult to make a comparison among our results and another one. In general, the honeys of stingless bees have been characterized by low pH, because the presence of different acids (gluconic, acetic, lactic, citric, succinic, formic, malic, maleic and oxalic), and a reduced total solute content, if compared with *Apis mellifera* honey (Vit *et al.*, 1998).

Table 5. Frequency of samples (F) that meet the legal standards of microbiological quality of honey from three species of stingless bees: *S. polysticta*, *M. illota* and *T. angustula*.

Species	n	RTAM (UFC/mL)		RTASR (UFC/mL)		RTML (UFC/mL)		
		Value ref.	F	Value ref.	F	Value ref.	Average value	F
<i>S. polysticta</i> .	10	10 ³	100%	10 ²	100%	10	23	30%
<i>M. illota</i>	3	10 ³	100%	10 ²	100%	10	5	100%
<i>T. angustula</i>	6	10 ³	100%	10 ²	100%	10	53	50%

Note: RTAM: Total aerobic mesophilic count. RTASR: Total anaerobic reduction sulfite count (maximum limit allowed = 102). RTML: Total mold and yeast count (maximum allowed limit = 10). Valor ref.: Maximum threshold allowed by NTS N071-Minsa/ Dígesa-V01.

The color of honey, which can usually vary from practically transparent to almost black, reflects the amount of carotenoid, chlorophyll, and xanthophyll pigments (Suescún & Vit, 2008). The darkest hue of the honeys of *T. angustula* could be related to the major presence of minerals and phenolic compounds (Instituto Nacional de Tecnología Agropecuaria [Inta], 2019), as well as calcium and iron phosphate; while the light colored honeys of *S. polysticta* would prove to be richer in vitamin A (Zandamela, 2008).

Phenolic compounds are not only a marker of the floral origin of the honey, but also potential indicators of their biological activity (Quiñones *et al.*, 2012). In this study, the total polyphenols were much lower than those recorded for the same bee species by Rodríguez-Malaver *et al.* (2009). However, due to the lack of information about the area of origin and the season of extraction of the samples in this investigation, it was not possible to speculate on the causes of these differences.

Microorganisms in the honey can originate by primary sources, when they come from the nectar or for influence of the bees themselves, and inoculate by secondary sources, when operating conditions influence the collection, transport and storage of honey (Pucciarelli *et al.*, 2014). Absence of mesophiles and sulfite-reducing anaerobic bacteria in all the analyzed samples would indicate the optimal microbiological quality of the honeys. Although the high water content of stingless bees honeys could favor the growth of molds and yeasts (Gil, 2010), the causes of this presence in the samples of *S. polysticta* and *T. angustula* deserve to be studied in depth, without ignoring presence of yeasts in the “bees bread” (Portillo, 2016), the conservation and sanitation conditions of inert laboratory surfaces (Sarmiento *et al.*, 2014) in storage (Ascencio, 2014) and harvest protocols (RSA-Conicet, 2018).

It assumes that several causes could have concurred to observe significant differences among physicochemical parameters of honeys from *S. polysticta*, *M. illota* and *T. angustula*: changes in floral origin of the honey, the kinds of materials used by different species, the processes of production and storage used for each species, would be considered as the main factors to define the quality of the honey (de Oliveira & Santos, 2011). Anyhow, these aspects were not part of our study, being a preliminary analysis

on the quality of the honeys of some stingless bee species commonly reared in the San Martín region. We believe it should be the following step to deepen the knowledge on the nature of honeys from Peruvian stingless bees.

Conclusions

The results of this research agree with those obtained in other countries and the absence of bacteria in all the samples analyzed would indicate an optimal microbiological quality. However, the presence of mold and yeast in the *S. polysticta* and *T. angustula* samples deserves to be investigated. The characterization of physicochemical parameters and the microbiological quality are essential to define for the process of setting quality standards of the honeys, providing the reference values. Due to the scant knowledge about the stingless bee honeys in Peru, this study represents a starting point for new research aimed at enhancing stingless bee honeys and boosting the meliponiculture in the country.

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