Pharmaceutica Analytica Acta

Patricia et al., Pharm Anal Acta 2016, 7:12
DOI: 10.4172/2153-2435.1000525

Research Article

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Chemical Composition of Ecuadorian Commercial Pot-Honeys: *Trigona fuscipennis* "Abeja De Tierra", *Melipona mimetica* "Bermejo" and *Scaptotrigona ederi* "Catiana"

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Received date: November 25, 2016; **Accepted date:** December 14, 2016; **Published date:** December 16, 2016

Abstract

Objective: The quality of fifteen commercial pot-honeys produced by "abeja de tierra" *Trigona fuscipennis*, "bermejo" *Melipona mimetica*, and "catiana" *Scaptotrigona ederi* stingless bees in Ecuador was evaluated for ash, free acidity, hydroxymethylfurfural, reducing sugars, and sucrose and water contents. These pot-honeys were also described for their visual viscosity, color, smell, aroma, dominant taste and other physiological sensations in the mouth.

Method: Fifteen pot-honeys were purchased in El Oro, Loja and Manabí Ecuadorian provinces. Acceptance was done on six honeys with 40 assessors using a 10 cm unstructured line scale anchored with the expressions 'like' expressions. Sensory dominant taste, visual appearance, smell, and aroma (using the odor-aroma table for pot-honey) and other physiological sensations were described. Measurements of ash content were done by gravimetric method, free acidity by potentiometric method, hydroxymethylfurfural by spectrophotometric method, reducing sugars and sucrose by cuprimetric method, and moisture by the refractometric method.

Results: Pot-honey produced by Trigona is the most different from *Apis mellifera* with free acidity some 12-20 times higher than the maximum of 40 meq/kg, double water content of the maximum 20 g/100 g, and a third of the minimum 65 g/100 g of reducing sugars. Pot-honey produced by *Melipona* and *Scaptotrigona* may fulfill *Apis mellifera* standards, with a slightly higher moisture up to 27.88 g/100 g and free acidity up to 76.77 g/100 g, but lower contents of reducing sugars (50.75-63.38) g/100 g. Sucrose content of pot-honey produced by Trigona, *Melipona* and *Scaptotrigona* is lower than 5 g/100 g in the *Apis mellifera* honey standards. Smell and aroma were more "floral" for *Melipona*, "citrusy" for Trigona and "pollen" for *Scaptotrigona* pot-honey.

Conclusion: Compositional and sensory data on pot-honey is a contribution to the database of the revised Ecuadorian honey standards NTE INEN 1572, and will eventually support the inclusion of standards in a new pot-honey norm.

Keywords: Ecuador; Entomological origin; Meliponini; Chemical analysis; Pot-honey; Quality standards; Sensory descriptors

Introduction

Aproximately 500 species of stingless bees belong to the Meliponini tribe [1], and live in tropical and subtropical regions [2]. These bees store honey in cerumen pots, therefore the term "pot-honey" was coined [3] to differentiate them from honey produced in beeswax combs by *Apis mellifera* and other *Apis* spp. In Latin America stingless bee keeping is known as meliponiculture, the origin of the term is uncertain, and could be linked to the *Melipona* genus or to the subfamily Meliponini. The traditional stingless bee keeping or meliponiculture should be protected to prevent its extinction [4], and paradoxically, stingless bees should be protected from stingless bee keepers for a sustainable instead of predatory practice. The decline of forest and plant species diversity, increase competition for food in large *Melipona* [5], and reduce pot-honey yields. Therefore, the traditional practice needs input from current knowledge on stingless bee keeping and environmental protection, to pinpoint an ultimate philosophy "caring gentle bees to protect forests" [6]. As an indicator of the great biodiversity of stingles bees, 89 species of Meliponini are reported in the Southern region of Ecuador [7].

The medicinal use of honey or pollen produced in cerumen pots by eight taxa of Brazilian stingless bees was investigated in the zootherapy study of Costa-Neto [8]. These medicinal properties need to be demonstrated, and one approach is to study bioactive compounds such as flavonoids [9]. Honey alone or combined with conventional therapy was recently reviewed as a novel antioxidant [10]. The antioxidant activity of honey varies according to the entomological source of honey [11]. In the research on Ecuadorian pot-honey, a comprehensive biopharmaceutical approach was done in a *Scaptotrigona* mixture collected by Achuars in Morona Santiago province [12].

Although the oldest fossil of a bee in our planet is a stingless bee [13], and Precolumbian honey was produced only by stingless bees; pot-honey is not included in the honey regulations because they are currently devoted to *Apis mellifera* which was a species introduced after the discovery of America [14].

The first draft for a norm of honey produced by stingless bees was presented by Vit during the 1999 Annual Meeting of the International Honey Commission held in the European Center of Taste Science in Dijon, France, with scientific representatives of 18 countries [15]. Since the standards suggested by Guatemala, Mexico and Venezuela in 2004 [16], and the review done to set quality standards in 2006 [17], the proposal of a norm for pot-honeys of the world is now supported by new data, e.g. from Argentina [18], Australia [19], Bolivia [20], Brazil [21], Colombia [22], Guatemala [23] and Venezuela [24].

In this work, Ecuadorian pot-honeys produced by *Trigona fuscipennis* Friese, 1900. named "abeja de tierra", and *Melipona* (Michmelia) mimetica Cockerell, 1914 named "bermejo", and *Scaptotrigona ederi* (Schwarz, n.p.) named "catiana" were studied to contribute for the proposal of its inclusion in the Ecuadorian honey norm [25] either in the same table of standards for *Apis mellifera* –as suggested for the most abundant pot-honey in Venezuela produced by *Melipona favosa* known as "erica" [26], in the annex like in the Colombian regulations [27], or in a new norm for pot-honey.

Materials and Methods

Pot-honeys

Fifteen pot-honeys were purchased from local stingless bee keepers or in markets from El Oro, Loja and Manabí Ecuadorian provinces, and kept frozen until analysis. Stingless bees were collected from the entrances of nests in logs or hives using isopropyl alcohol, dried and kept in plastic boxes before sending them to Dr. Silvia R.M. Pedro for identification at Universidade de Sao Paulo, Ribeirão Preto, Brazil; and using the book on stingless bees from the South of Ecuador [7] for "abeja de tierra" that is referred to few species of *Geotrigona* spp. and *Trigona fuscipennis*. Duplicates of some bees are deposited in the Entomology Laboratory of Universidad Nacional de Loja, Ecuador, with Professor José Ramírez; Pontificia Universidad Católica de Ecuador, Quito, with Professor Clifford Keil; Kansas University, USA, with Professor Charles D. Michener†; and Smithsonian Tropical Research Institute, Panama, with Dr. David W. Roubik. Ethnic and scientific names of stingless bees producing the Ecuadorian pot-honey samples analyzed here are given in Table 1.

Ethnic name of stingless bees	Scientific name of stingless bees
"abeja de tierra"	*Trigona fuscipennis Friese*, 1900
"bermejo"	*Melipona mimetica Cockerell*, 1914
"catiana" or "catana"	*Scaptotrigona ederi* (Schwarz, n.p.)

Table 1: Entomological origin of pot-honeys.

Chemical analyses

Only the honey produced in combs by *Apis mellifera* is considered for the ten quality standards in the Ecuadorian honey norm NTE INEN 1572 [25] and corresponding analytical methods: relative density and moisture, reducing sugars, sucrose, ratio fructose: glucose, free acidity, insoluble solids, ash, hydroxymethylfurfural (HMF), and diastase number. ARCSA (Spanish acronym for Agencia Nacional de Regulación, Control y Vigilancia Sanitaria; in English, National Agency of Regulation, Control and Sanitary Vigilance) complies with five of these parameters for the sanitary registration of honey: Ash, free acidity, hydroxymethylfurfural, moisture, reducing sugars and sucrose, for the consideration to be included in a proposal of standards for pot-honey in the Ecuadorian Honey Norm NTE INEN 1572. The methods used by this laboratory are moisture NTE INEN 1632 [28], reducing sugars, sucrose NTE INEN 1633 [29], acidity NTE INEN 1634 [30].

Sensory analysis

Sensory analysis was done to describe the dominant taste [31] visual appearance, smell, and aroma, using the odor-aroma table for pot-honey [32]. Other physiological sensations were also observed. An acceptance test was done with 40 assessors for honeys of Trigona, *Melipona*, *Scaptotrigona*, two commercial *Apis mellifera* one amber and the other light amber using a 10 cm unstructured line scale anchored with the expressions 'like it a little' and 'like it a lot', in the left (1 cm) and right ends (9 cm), respectively. The acceptance scores were measured.

Statistical analysis

Chemical results and acceptance scores were statistically processed with SPSS [33] to compare means of Trigona, *Melipona* and *Scaptotrigona* pot-honeys with ANOVA, post-hoc Tukey test.

Results

Chemical analysis

Fifteen commercial pot-honeys were collected during field work in El Oro, Loja and Manabí Ecuadorian provinces, and analyzed for six chemical parameters currently done in routine analysis by the Ecuadorian sanitary authority ARCSA, namely ash, free acidity, hydroxymethylfurfural (HMF), moisture, reducing sugars and apparent sucrose. Raw data, averages and *Apis mellifera* Ecuadorian standards are given in Table 2. The following tendencies of pot-honey contrasted with the NTE INEN 1572 *Apis mellifera* honey were observed: 1) Moisture is generally higher in pot-honey [18.77-38.74] g/100 g, compared to the *Apis mellifera* standard, maximum 20 g/100 g. 2) Free acidity is variable; *Melipona* and *Scaptotrigona* pot-honeys contents of 25.23-76.77 meq/kg are more similar to the *Apis mellifera* standard, maximum 40 mg/kg; whereas Trigona has contents 12-20 times higher with a range of 497.03-810.01 meq/kg. 3) Reducing sugars are lower in pot-honey (16.24-63.38 g/100 g) than the *Apis mellifera* standards, minimum 65 g/100 g. 4) Sucrose content of Trigona, *Melipona* and *Scaptotrigona* pot-honeys studied here is lower than the maximum 5 g/100 g permitted for *Apis mellifera* honey.

Bee taxa	Number of pot-honey samples	Ash (g/100 g)	Free acidity (meq/kg)	HMF (mg/kg)	Moisture (g/100 g)	Reducing sugars (g/100 g)	Apparent sucrose (g/100 g)
Trigona fuscipennis	n=5	0.12^c (0,02) [0.10-0.14]	631.77^b (108.54) [497.03-810.01]	41.48^c (9.92) [31.40-60.03]	37.12^b (1.52) [34.53-38.74]	23.97^a (7.70) [16.21-33.90]	2.89^a (1.00) [1.42-4.15]
Melipona mimetica	n=5	0.03^a (0.01) [0.01-0,04]	49.02^a (15.05) [38.92-76.77]	11.81^a (12.91) [0.30-28.00]	22.27^a (2.15) [18.77-24.89]	58.71^b (4.56) [50.75-63.38]	2.01^a (1.14) [0.99-4.01]
Scaptotrigona ederi	n=5	0.08^b (0.04) [0.04-0.15]	40.95^a (9.52) [25.23-48.93]	25.88^b (10.88) [5.75-35.05]	21.97^a (3.22) [19.43-27.88]	42.01^b (5.82) [36.33-51.82]	2.66^a (1.08) [1.35-4.34]
Apis mellifera	NTE INEN 1572	Max0.5	Max40	Max 50	Max 20	Min 65	Max 5

Table 2: Chemical analysis of *Melipona*, *Geotrigona* and *Scaptotrigona* pot-honey, and *Apis mellifera* honey standards.

In the lower rows of each parameter, averages ± (SD) [minimum-maximum] values are given. Different superscripts indicate significant difference in honey composition between the two groups. P<0.05. The NTE INEN 1572 standards have two honey types, Class I (upper value) and Class II (lower value).

Sensory analyses

All the pot-honeys analyzed here were liquid, and few of them developed tiny crystals after freezing, causing a visual milky viscosity in three Trigona honeys. The color varied from light to dark amber. The smell varied in the bee, candy, caramel, menthol, fermented, floral, fruity notes. The aromas were similar with bee, citrusy, floral, lemon zest, fermented, fruity, menthol, pollen, and resinous. *A Trigona* honey had book glue off-odor and stable off-aroma. Dominat flavors are sweet for *Melipona* honeys and sour for Trigona honeys, *Scaptotrigona* honeys are more variable sweet, sour sweet, sour astringent and even bitter. Four honey samples–two of Trigona and two of *Scaptotrigona* –caused salivation while tasting. Floral for *Melipona* and citrusy for Trigona were the most frequent descriptors perceived in the smell and aroma; pollen was frequently perceived in the smell and aroma of *Scaptotrigona*.

The acceptance of the honeys varied as follows: *Apis mellifera* light amber 6.5 ± 2.8, *Apis mellifera* amber 5.1 ± 2.8, Trigona amber 3.9 ± 3.0, *Melipona* light amber 6.7 ± 2.5, *Scaptotrigona* light amber 5.8 ± 2.8. There were no significant differences between the acceptances in the five honey types tested here P<0.05. The highest acceptances were assessed for the light amber honeys, both *Apis mellifera* and *Melipona*, and slightly lower *Scaptotrigona*. For a group of 16 over 40 assessors, the light amber *A. mellifera* was the best honey, while the *Melipona* honey was the best for 10 assessors, both rated with values from 5.9 to 10.0; the *Scaptotrigona* honey was chosen as the best honey by 9/40 assessors who rated it with acceptances from 8.2 to 10.0.

Discussion

Although pot-honeys are major honeys in the forests–as stated by Dr. D.W. Roubik, they are still minor honeys in the market. Therefore, they need promotion, protection and development of their infant industry [34]. Educational initiatives from Australia [35] and pot-honey shows [36] do expand the knowledge of stingless bees, meliponiculture, pot-honey, sensory appeal, composition, and understanding of medicinal uses by the public.

Melipona stingless bee species build bigger cerumen pots to process their honey [37] and have higher honey yields. Therefore their honeys have been studied more frequently. The chemical composition of *Melipona* honeys varies according to the species. Average water contents (g/100 g) are 24.9 for *M. brachychaeta* and 24.1 for *M. grandis* from Bolivia [20], 28.02 *M. quinquefasciata* [38], 28.84 *M. scutellaris* [39] and 24.8 *M. subnitida* [40] from Brazil, 25.8 *M. compressipes*, 27.6 *M. eburnea*, and 24.8 *M. favosa* from Colombia [22], 17.32 for *M. beecheii*, 19.66 for M. solani and 20.37 for *M. aff. yucatanica* from Guatemala [41], and 28.0 *M. favosa* from Venezuela [42]; with a range of 17.32 to 28.84 g water/100 g. Moisture varied from 18.85 to 22.80 g/100 g for the Ecuadorian *Melipona* honeys, within the moisture range of honey from eleven *Melipona* species from Bolivia, Brazil, Colombia, Guatemala, and Venezuela [20,22,38,40,41,42]. Average free acidities (meq/kg) are 10.4 for *M. brachychaeta* and 16.0 for *M. grandis* from Bolivia [20], 28.02 for *M. quinquefasciata* [38], and 32.49 for *M. subnitida* [40] from Brazil, 23.23 for *M. beecheii*, 4.95 for *M. solani* and 10.59 for *M. aff. yucatanica* from Guatemala [41], and 51.7 for *M. favosa* from Venezuela [42]; with a range of 4.95 to 51.7 meq/kg. In our study with five *Melipona* honeys, the variation of free acidity from 38.92 to 76.77 meq/kg is within the free acidity range of honey produced by eight *Melipona* species from Bolivia, Brazil, Guatemala, and Venezuela [20,38,40-42].

Scaptotrigona mexicana honey from Guatemala has a free acidity of 12.68 meq/kg and water content of 18.74 g/100g, 57.22 g reducing sugars/100 g, and 0.06 g apparent sucrose/100 g [41]; the water content for the Ecuadorian *Scaptotrigona* honey in Table 2 is also 18.74 g/100g, but the free acidity 40.1 meq/kg is higher than that found in Guatemala, and consequently the content of 42.25 g/100 g reducing sugars is lower than the minimum 65% of the *Apis mellifera* standard. The low sucrose has no problem because the standard establishes a maximum of 5%, perhaps a more refined limit could be suggested with a lower maximum value for sucrose of pot-honeys.

Dardón and Enríquez [41] reported a free acidity of 85.53 meq/kg and a water content of 32.09 g/100g for the underground honey produced by *Geotrigona acapulconis*; these were the highest acidity and moisture between the honeys produced by nine species of Meliponini in Guatemala. Also in Table 3, the Ecuadorian underground Trigona honey shows the highest free acidity and moisture. The fact that average of free acidity in the Ecuadorian Trigona is 609.33 meq/kg should be explained by the species and the interactions of the underground nest with the soil. Behavioral observations of the underground bees and their nests are needed to understand such a different pot-honey [43], and hypothesize the origin of such chemical array. This is a very thin honey with 37.06 g/100 g water content, almost double of the 20% maximum permitted in the Ecuadorian honey norm [25]. These elevated compositional values of water and acids, explain a decrease on reducing sugars to a 24.53 g/100 g average which is lower than the minimum of 65% established in the norm [25].

The statistical analysis from Table 2 show that honey produced by *Melipona* and *Scaptotrigona* are more similar between each other in free acidity, moisture, and reducing sugar contents than honey produced by Trigona in Ecuador. However apparent sucrose is not different in the three honey types. Similarly, these observations was done with Venezuelan *Melipona*, *Scaptotrigona* and Trigona honeys from Venezuela in 1998 [44], although pot-honey produced by Trigona are different. Quality standards needed by pot-honey were substantiated in 1999 [15] during the Annual Meeting of the International Honey Commission, and later proposed for Guatemala, Mexico, and Venezuela in 2004 [45], and later also for Brazil in 2006 [46] and 2013 [39]. The first official insert in the Annex of the Colombian honey norm ICONTEC [27] derived from the comprehensive review done in 2006 [16]. The proposal to expand the Ecuadorian honey norm NTE INEN 1572 [25] to other honey bee species, namely stingless honey bees was one reason for the current revision; during the third meeting the *M. favosa* model [26] was presented as an option for the crucial decisions to be taken, and the best option for further development of meliponiculture.

A provisional idea for pot-honey standards in Ecuador is given in Table 3, with concerns that 30-40 honey samples of each group should be analyzed for a sensible and solid database. Averages and [minimum-maximum] values from Table 2 are retained in Table 3 to visualize the proposal of quality standard for each parameter (free acidity, moisture, reducing sugars and apparent sucrose) for the three pot-honey groups based on their entomological origin Trigona, *Melipona* and *Scaptotrigona*. The column of apparent sucrose is highlighted in grey because the standard of a permitted maximum of 5 g/100 g remains the same for the honey produced by the three genera of Meliponini. Conservative proposals are made when the *Apis mellifera* honey standard is met by pot-honey. Compared to the free acidity maximum value 40 meq/kg in the *Apis mellifera* honey standards, reference values should be increased up to 800 for Trigona and 50 for *Melipona* and *Scaptotrigona* honey. The maximum moisture of 20% also needs to be increased up to 25% for *Melipona*, 30% for *Scaptotrigona* and 40% for the Trigona. On the other hand, the 65% minimum of reducing sugars requires a reduction to 16% for Trigona, 50% for *Melipona* and 35% for *Scaptotrigona*. This proposal is a trend that needs to be validated with more pot-honeys from Ecuador. This is not the first proposal, and therefore it is supported by research done in Brazil, Guatemala, Mexico, and Venezuela [45]. Ecuador is about to start a contribution in regulatory promotion of pot-honey. The final outcome would have consensual value by the Technical Committee TC-NTE INEN 1572 revising and updating the honey norm, with the agreement to create the pot-honey norm in 2016.

Bee taxa	Ash (mg/100 g)	Free acidity (meq/kg)	HMF (mg/kg)	Moisture (g/100 g)	Reducing sugars (g/100 g)	Apparent sucrose (g/100 g)
Trigona	0.12 [0.10-0.14] Max. 0.5	631.77 [497.03-810.01] Max. 800	41.48 [31.40-60.03] Max. 60	37.12 [34.53-38.74] Max. 40	23.97 [16.21-33.90] Min. 16	2.89 [1.42-4.15] Max. 5
Melipona	0.03 [0.01-0.04] Max. 0.5	49.02 [38.92-76.77] Max. 50	11.81[a] [0.30-28.00] Max. 30	22.27 [18.77-24.89] Max. 25	56.71[b] [50.60-63.38] Min. 50	2.01 [0.99-4.01] Max. 5
Scaptotrigona	0.08 [0.04-0.15] Max. 0.5	40.95 [25.23-48.93] Max. 50	25.88b [5.75-35.05] Max. 40	21.79 [19.43-27.88] Max. 30	42.01 [36.33-51.82] Min. 35	2.66 [1.35-4.34] Max. 5
Apis mellifera Honey Type 1 INEN 1572	Max. 0.5	Max. 40	Max. 50	Max. 20	Min. 65	Max. 5

Table 3: Suggested pot-honey standards for *Geotrigona*, *Melipona*, and *Scaptotrigona*. Averages and [minimum-maximum] values are given.

Sensory characteristics and defects of honey from *A. mellifera* were based on the wine sensory experience adapted to the perception of honey bee keepers and consumers by Gonnet and Lavie [47]. A leading research with *Melipona quadrifasciata* by French and Brazilian scientists made the observations of her delicious thin and sour honeys [48]–always re-discovered by young melittologists and endlessly communicated in seminars, workshops and papers of pot-honey produced by stingless bees–as already stated by Schwarz since 1948 [49] for the widely relished honeys in tropical America before Columbus. An ayurvedic observation on dominant flavor of food to prepare balanced meals [50] was incorporated in early sensory approach of commercial honeys from Venezuela [32], where the sugary matrix does not always convey to the sweet perception collectively assigned to honey, even in botanical types with more than 65% reducing sugars, such as the bitter Castanea and Arbutus unedo European honeys. Comparing honey extracted from beeswax combs and cerumen pots, needs adaptations to keep the similarities and to insert differences. For instance, the fermentive off-odor is a defect for a matured *Apis mellifera* honey [47], but it is a feature in some types of pot-honeys [51], and therefore this descriptor was included as a family in the odor-aroma table [33] because Meliponini process their honey diversely.

Bee products have healing properties because they improve circulation, decrease inflammation and boost immune protection [52,53]. Pot-honey has been studied for the antibacterial [54], antioxidant [10,17] and anticancer activity [55]. A vast research is needed to demonstrate putative medicinal properties and the active components derived from stingless bees, their diet or perhaps originated with their interactive microbiota [56]. Discoveries such as the C-glycosides in honeys [9] are possible due to the great biodiversity of Meliponini bees [57].

In the Ecuadorian coast Geotrigona, Trigona and *Scaptotrigona* honeys are more abundant. According to meliponicultors the two *Melipona* species known as "bermejo" and "cananambo" were easily kept in the past, some 10-20 years ago, but now feral nests of *Melipona* are scarce and these bees abandon the hive more easily than *Scaptotrigona* named "catiana", as if the bees are more sensitive to management and climate change. In the Ecuadorian Amazon rainy forest, *Melipona* honey is harvested by pot-honey hunters and sold by Kichwa and Shuar nationalities in native Indian fairs from Puyo, Pastaza province, Tena, Napo province and El Coca, Orellana province with a top price of almost 10 $/125 g, whereas in El Oro province the cost is 20 $/750 g. Ecuadorian Kichwas and Shuars, like Brazilian Enawene-Nawe [58] do not breed bees, but other Brazilian indigenous societies do, like the Guarani [59] and the Pankararé [60]. Native knowledge about stingless bees connect social groups with nature, in a sort of ecological cosmovision of ancestral awareness.

Government enterprises are needed for producers, possibly to subsidize the quality control procedures, to generate scientific research needed to demonstrate putative medicinal properties, and to open marketing channels. Ministries of Health, and of Agriculture and Livestock could support a strategic planning for ancestral knowledge embracing traditional stingless beekeeping and pot-honey-hunting in Ecuador, among other agricultural practices to participate in the Change of Productive Matrix as envisaged by the Knowledge Sharing Program (KSP) from South Korea [61] and the Good Living (originally from Kichwa language Sumak Kawsay, translated into Spanish as Buen Vivir). Ecuador will join the seminal initiative Route of Living Museums of Meliponini in the World, launched by Costa Rica and Venezuela in 2013 [37], with a leading role for the regulatory concepts, with the current systematic Prometeo Project location of stations for *melipona*ries and stingless bee nests (Vit, unpublished data). Besides their great biodiversity, the identified stingless bees keep changing their names [62], therefore a specialized entomologist is mandatory in any scientific team investigating pot-honey. The unifloral Italian honeys were carefully filed in a book [63] that could be imitated to illustrate the tropical meliponine honeys for a better knowledge of the public and the scientists. However, this year the melissopalynological analysis were excluded from the first revision of the Ecuadorian honey norm [64], as well as the Annex with reference values for pot-honey standards as in the Colombian honey norm reviewed in 2007 [27], only the scientific references were left to inform on pot-honey produced by stingless bees from the tribe Meliponini.

Conclusion

Pot-honeys produced by Ecuadorian *Trigona fuscipennis* "abeja de tierra", *Melipona mimetica* "bermejo" and *Scaptotrigona ederi* "catiana" where characterized, and suggested chemical quality standards were compared with those of *Apis mellifera* honey. Sensory analysis was useful to describe the diversity of entomological origin and also to assess the acceptance of pot-honey. Further data is needed to reduce the HMF standard, as is the case for the *Melipona* honey standard of the State of Bahia, Brazil, with a lower HMF limit, up to 10 mg/kg.

Acknowledgement

To Prometeo, Senescyt, Ecuador for the grant to P. Vit at Universidad Técnica de Machala (UTMACH), Provincia El Oro, for the Project "Valorization of pot-honey produced by Ecuadorian Meliponini", and her personal financial support. To Mrs. Elizabeth Brito Administrative Assistant from the Planning Department at UTMACH, Mrs. Esperanza Poma Bustos, Director of Public Relations at UTMACH, and Mrs. Gabriela Yerovi, Secretar of the Principal at Universidad Estatal Amazónica (UEA) for the efficient logistics. To Mrs. Hugarita Cobo, Financial Director at UEA for her administrative support for the analytical service, the UEA driver Mr. Marco Pico, and the UEA security officer Mr. Carlos Muñoz who donated the Amazonian *Melipona* honey for the sensory tests. To Dr. Hugo Cerda, Prometeo Researcher at UEA and his Assistant Mrs. Carolina Ledezma, for their timely support during sensory trials. The Principal of UTMACH Eng. César Abad Quezada provided facilities for field work with the drivers Mr. Francisco Saavedra, Mr. Jairo Riofrio and Mr. Wilson Ríos. To Ecuadorian meliponicultors from El Oro, Loja and Manabí provinces to nurture the tradition of stingless bee keeping and pot-honey extraction that made possible this research. Preliminary data were included in few slides of the plenary session in the 23rd SILAE congress held in Marsala, Sicily, Italy, 7th–12th September 2014. Financial support was received from Schullo Products from Ecuador for half airflight ticket. All honeys were purchased by P. Vit, except two *Melipona* honeys that were received from the meliponicultor Ubertino Matamoro from Piedras Blancas, El Oro province, and from the Professor José Ramírez from Universidad Nacional de Loja, harvested by Mr. Emilio Sarmiento, Loja province. Dr. Hugo Rosero and Eng. Maritza Farinango kindly invited P. Vit to the revision of the Ecuadorian honey norm NTE INEN 1572, as President and Secretar of the Technical Committee, respectively.

References

1. Michener C (2007) The bees of the world (2nd edn.) The Johns Hopkins University Press, Baltimore, USA, p: 992.
2. Crane E (1992) The past and present status of beekeeping with stingless bees. Bee World 73: 29-42.
3. Vit P, Pedro SRM, Roubik DW (2013) Pot-honey. A legacy of stingless bees. Springer; New York, USA, pp: 654.
4. Villanueva R, Roubik D, Colli-Ucan W (2005) Extinction of *Melipona* beecheii and traditional beekeeping in the Yucatan Peninsula. Bee World 86: 35-41.
5. Villanueva-Gutiérrez R, Roubik DW, Colli-Ucán W, Güemez-Ricalde FJ, Buchmann SL (2013) A critical view of colony losses in managed Mayan honey-making bees (Apidae: Meliponini) in the heart of Zona Maya. J Kansas Entomol Soc 86: 352-362.
6. Vit P (2000) Una idea para valorizar la meliponicultura latinoamericana. Tacaya 10: 3-6.
7. Ramírez JA, Ureña J, Camacho A (2013) Las abejas sin aguijón de la Región Sur del Ecuador. Consejo Nacional de Educación Superior; Loja, Ecuador, p: 119.
8. Costa-Neto EM (2004) Implications and applications of folk zootherapy in the state of Bahia, northeastern Brazil. Sust Dev 12: 161-174.
9. Truchado P, Vit P, Ferreres F, Tomás-Barberán F (2011) Liquid chromatography-tandem mass spectrometry analysis allows the simultaneous characterization of C-glycosyl and O-glycosyl flavonoids in stingless bee honeys. J Chromatogr A 1218: 7601-7607.
10. Rodríguez-Malaver AJ, Pérez-Pérez EM, Vit P (2007) Capacidad antioxidante de mieles venezolanas de los géneros Apis, *Melipona* y Tetragonisca, evaluada por tres métodos. Rev Inst Nac Hig Rafael Rangel 38: 13-18.
11. Aguilera G, Gil F, González AC, Nieves B, Rojas Y, et al. (2009) Evaluación de actividad antibacteriana de mieles de Apis mellifera, contra cepas de *Escherichia coli* y *Staphylococcus aureus*. Rev Inst Nac Hig Rafael Rangel 40: 21-25.

12. Guerrini A, Bruni R, Maietti S, Poli F, Rossi D, et al. (2009) Ecuadorian stingless bee (*Meliponinae*) honey: A chemical and functional profile of an ancient health product. Food Chem 114: 1413-1420.
13. Vit P (2010) Estudio incesante de Meliponini al estilo de JMF Camargo. Rev Inst Nac Hig Rafael Rangel 41: 50-60.
14. Vit P (2008) La miel precolombina de abejas sin aguijón (*Meliponini*) aún no tiene normas de calidad. Rev Bol Centro Investigaciones Biológicas 42: 415-423.
15. Vit P (1999) A draft for stingless bee honey regulations. Annual Meeting of the International Honey Commission, Dijon, France.
16. Souza B, Roubik D, Barth O, Heard T, Enríquez E, et al. (2006) Composition of stingless bee honey: Setting quality standards. Interciencia 31: 867-875.
17. Vit P, Gutiérrez MG, Rodríguez-Malaver AJ, Aguilera G, Fernández-Díaz C, et al. (2009) Comparación de mieles producidas por la abeja yateí (Tetragonisca fiebrigi) en Argentina y Paraguay. ABCL 43: 219-226.
18. Sgariglia MA, Vattuone MA, Sampietro Vattuone MM, Soberón JR, Sampietro DA (2010) Properties of honey from Tetragonisca angustula fiebrigi and Plebeia wittmanni of Argentina. Apidologie 41: 667-675.
19. Persano Oddo L, Heard TA, Rodríguez-Malaver A, Pérez RA, Fernández-Muiño M, et al. (2008) Composition and antioxidant activity of Trigona carbonaria honey from Australia. J Med Food 11: 789-794.
20. Ferrufino U, Vit P (2013) Pot-honey of six species of Meliponini from Amboró National Park in Bolivia, pp: 409-416.
21. Almeida-Muradian LB, Stramm KM, Horita A, Barth OM, Silva de Freitas A, et al. (2013) Comparative study of the physicochemical and palynological characteristics of honey from *Melipona* subnitida and *Apis mellifera*. Int J Food Sci Technol 48: 1698-1706.
22. Fuenmayor CA, Díaz-Moreno AC, Zuluaga-Domínguez CM, Quicazán MC (2013) Honey of Colombian stingless bees: Nutritional characteristics and physicochemical quality indicators, Springer, New York, USA, p: 654.
23. Gutiérrez MG, Enríquez E, Lusco L, Rodríguez-Malaver A, Persano Oddo L, et al. (2008) Caracterización de mieles de *Melipona* beecheii y *Melipona* solani de Guatemala. Rev Fac Farm 50: 2-6.
24. Vit P, Mejías A, Rial L, Ruíz J, Peña S, et al. (2012) Conociendo la miel de *Melipona* favosa en la Península de Paraguaná, Estado Falcón, Venezuela. Rev Inst Nac Hig Rafael Rangel 43: 15-19.
25. INEN(1988) Norma Técnica Ecuatoriana Obligatoria. Miel de Abejas, Requisitos, Instituto Ecuatoriano de Normalización, Quito, Ecuador, pp: 1-4.
26. Vit P (2013) Modificaciones comentadas de la norma Miel de Abejas, hacia la norma Miel de Venezuela: Inclusión de miel de pote y exclusión de mieles falsas, Modificaciones norma miel de Venezuela, pp: 1-8.
27. ICONTEC (2007) Norma Técnica Colombiana. Miel de Abejas, NTC 1273, Bogotá, Colombia, pp: 1-6.
28. INEN (1989) Determinación de la densidad relativa a 27°C y de la humedad. NTE INEN 1632. INEN; Quito, Ecuador, pp: 1- 6.
29. INEN (1989) Miel de abejas. Determinación de azúcares reductores totales, sacarosa, y la relación fructosa-glucosa. NTE INEN 1633. INEN; Quito, Ecuador, pp: 1-8.
30. INEN (1989) Miel de abejas. Determinación de la acidez total. NTE INEN 1634. INEN; Quito, Ecuador, pp: 1- 4.
31. Vit P (1993) Miel de Abejas. Cuaderno Ciencia de los Alimentos No. 1. Consejo de Publicaciones, ULA; Mérida, Venezuela, p: 97.
32. Vit P, González I, Carvalho CAL, Enríquez E, Moreno E, Roubik DW, et al. (2007) Tabla olor-aroma. Taller evaluación sensorial de mieles de abejas sin aguijón; Mérida, Venezuela.
33. https://www.jou.ufl.edu/assets/researchlab/SPSS-Statistcs-Base-Users-Guide-17.0.pdf
34. Heard TA, Dollin AE (2000) Stingless beekeeping in Australia: snapshot of an infant industry. Bee World 81: 116-125.
35. Maginnity S (2013) Using stingless bees as an educational tool in Australian schools. Stingless bees process honey and pollen in cerumen pots, pp: 1-13.
36. Vit P, Rodríguez-Malaver A, Almeida D, Souza B, Marchini L, et al. (2006) A scientific event to promote knowledge regarding honey from stingless bees: 1. Physical-chemical composition. Magistra 18: 270-276.
37. Aguilar I, Herrera E, Vit P (2013) Stingless bees process honey and pollen in cerumen pots. Facultad de Farmacia y Bioanálisis. Universidad de Los Andes; Mérida, Venezuela.
38. São Thiago Calaça PS (2011) Aspectos da biologia de *Melipona* quinquefasciata Lepeletier (Mandaçaia do chão), características físico-químicas do mel, recursos alimentares e leveduras associadas, Ouro Preto, MG, Brazil, p: 109.
39. Carvalho CAL, Alves RMO, Souza BA, Véras SO, Alves EM, et al. (2013) Proposta de regulamento técnico de qualidade fisico-química do mel floral processado produzido por abelhas do gênero *Melipona*, Universidad de Los Andes; Mérida, Venezuela, pp: 1-9.
40. Almeida-Muradian LB, Stramm KM, Horita A, Barth OM, Freitas AS, et al. (2013) Comparative study of the physicochemical and palynological characteristics of honey from *Melipona* subnitida and Apis mellifera. Int J Food Sci Tech 48: 1698-1706.
41. Dardón MJ, Enríquez E (2008) Caracterización físicoquímica y antimicrobiana de la miel de nueve especies de abejas sin aguijón (Meliponini) de Guatemala. Interciencia 33: 916-922.
42. Vit P (2013) *Melipona* favosa pot-honey from Venezuela.Springer; New York, USA, pp: 363-373.
43. Barbosa FM, Alves RMO, Souza BA, Carvalho CAL (2013) Nest architecture of the stingless bee Geotrigona subterranea (Friese,1901) (Hymenoptera: Apidae: Meliponini). Biota Neotrop 13: 377-389.
44. Vit P, Medina M, Enriquez ME (2004) Quality standards for medicinal uses of Meliponinae honey in Guatemala, Mexico and Venezuela. Bee World 85: 2-5.
45. Villas-Bôas J, Malaspina O (2005) Parâmetros físico-químicos propostos para o controle de qualidade do mel de abelhas sem ferrão no Brasil. Mensagem Doce 82: 6-16.
46. Gonnet M, Vacche G (1984) L'Analisi Sensoriale dei Mieli. Come degustare e individuare le qualitá organolettiche di un miele. Federazione Apicoltori Italiani; Rome, Italy, pp: 114.
47. Gonnet M, Lavie P, Nogueira-Neto P (1964) Étude de quelques characteristiques des miels récoltés para certains Méliponines brésiliens. Compt Rend Acad Sci Paris 258: 3107-3109.
48. Schwarz H (1948) Stingless bees (Meliponidae) of the Western Hemisphere. Bull Am Mus Nat Hist 90: 1-546.
49. Bhat K (1983) Qué, Cómo y Cuándo Comer Industria Gráfica Oriental CA; Cumaná, Venezuela, p: 85.
50. Vit P, Rodríguez-Malaver AJ, Pérez-Pérez E, Enríquez E, Pérez A (2009) Fermented Meliponini honey is not spoiled honey (8th edn.) Pangborn Sensory Science Symposium, Florence, Italy.
51. Molan P (2005) Manuka honey as a medicine. Public Service Rev 5: 52-54.
52. Gupta RK, Stangaciu S (2014) Apitherapy: Holistic healing through the honeybee and bee products in countries with poor health system. Beekeeping for Poverty Alleviation and Livelihood Security, pp: 413-446.
53. Zamora G, Arias ML, Aguilar I, Umaña E (2013) Costa Rican pot-honey: its medicinal use and antibacterial effect. Pot-honey, pp: 507-512.
54. Vit P, Yu J, Huq F (2013) Use of honey in cancer prevention and therapy. Pot-honey, pp: 481-493.
55. Morais PB, Sao Thiago CPS, Rosa CA (2013) Microorganisms associated with stingless bees. Pot-honey, pp: 173-186.
56. Camargo JMF, Pedro SRM (2008) Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical Region. Apidologie 39: 387-387.
57. Santos GM, Antonini Y (2008) The traditional knowledge on stingless bees (Apidae: Meliponina) used by the Enawene-Nawe tribe in western Brazil. J Ethnobiol Ethnomed 4: 19.
58. Rodrigues AS (2006) Até quando o etnoconhecimento sobre as abelhas sem ferrão (Hymenoptera, Apidae, Meliponinae) será transmitido entre gerações pelos índios guarani m'byá da aldeia morro da saudade,

localizada na cidade de São Paulo, estado de São Paulo, Brasil? Sitientibus Ser Ci Biol 6: 343-350.

59. Costa-Neto EM (1998) Folk Taxonomy and cultural significance of "Abeia" (Insecta:Hymenoptera) to the Pankararé, Northeastern Bahia State. J Ethnobiol 18: 1-13.

60. Folleto I (2013) SENPLADES, Secretaría Nacional de Planificación y Desarrollo.

61. Roubik DW (2013) Why they keep changing the names of our stingless bees (Hymenoptera: Apidae; Meliponini): a little history and guide to taxonomy. Universidad de Los Andes; Mérida, Venezuela, pp: 1-7.

62. Persano Oddo L, Sabatini A, Accorti M, Colombo R, Marcazzan G, et al. (2000) I mieli uniflorali italiani. Nuove schede di caratterizzazione. Ministero delle Politiche Agricole e Forestali, Gradoli, Italia, p: 105.

63. NTE INEN (2016) Norma Técnica Ecuatoriana Obligatoria. Instituto Ecuatoriano de Normalización. Miel de Abejas, Requisitos. Primera Revisión, pp: 1-12.

64. Alve RMDO (2015) Regulamento Técnico de Identidade e Qualidade do Mel de Abelha social sem ferrão gênero *Melipona*. Agência Estadual de Defesa Agropecuária da Bahia.