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

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**Abstract**

**Objective:** The quality of fifteen commercial pot-hones produced by “abeja de tierra” *Trigona fusciennis*, “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 Manabi 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.

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

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**Introduction**

Approximately 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 stingless 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 Manabi 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 Universidad 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 Ramirez; 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. Ethnics 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.
Trigona were the most frequent descriptors perceived in the smell and bitter. Four honey samples −two of Trigona and two of Scaptotrigona −caused salivation while tasting. Floral for Melipona and citrusy for Scaptotrigona.

There was acceptance of the honeys varied as follows: Apis mellifera light amber 6.5 ± 2.8, A. 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.

**Table 2**

| 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 | 0.12b (0.02) [0.10-0.14] | 631.7b (108.54) [497.03-810.01] | 41.4b (9.2) [31.40-60.03] | 37.1b (9.92) [34.53-38.74] | 23.97b (1.52) [16.21-33.90] | 2.89b (1.00) [1.42-4.15] |
| Melipona mimetica | n=5 | 0.03a (0.01) [0.01-0.04] | 49.02a (15.05) [38.92-76.77] | 11.8a (12.91) [0.30-28.00] | 22.27a (2.15) [18.77-24.89] | 58.71b (4.56) [50.75-63.38] | 2.01b (1.14) [0.99-4.01] |
| Scaptotrigona ederi | n=5 | 0.08b (0.04) [0.04-0.15] | 40.95b (9.52) [25.23-48.93] | 25.88b (10.88) [5.75-35.05] | 21.97a (3.22) [19.43-27.88] | 42.01b (5.82) [36.33-51.82] | 2.66a (1.08) [1.35-4.34] |
| Apis mellifera | NTE INEN 1572 | Max0.5 | Max40 | Max 50 | Max 20 | Min 65 | Max 5 |

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*.

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. brachythaeta* and 24.1 for *M. grandis* from Bolivia [20], 28.02 M. quinquenfasciata [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. brachythaeta* and 16.0 for *M. grandis* from Bolivia [20], 28.02 for M. quinquenfasciata [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 [45], 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 (meq/kg) | Acidity (mg/kg) | HMF (mg/kg) | Moisture (g/100 g) | Reducing sugars (g/100 g) | Apparent sucrose (g/100 g) |
|---------------------|---------------|--------------|-----------------|-------------|-------------------|--------------------------|---------------------------|
| Trigona             | 0.12 Max. 0.5 | 40.85 [31.40-60.03] | 41.48 Max. 60  | 37.12 [34.53-38.74] | 23.97 [16.21-33.90] | 2.89 [1.42-4.15] | Max. 5                   |
| Melipona            | 0.03 Max. 0.5 | 11.81a [0.30-28.00] | 49.02 Max. 50  | 22.27 [18.77-24.89] | 56.71b [60.60-63.38] | 2.01 [0.99-4.01] | Max. 5                   |
| Scaptotrigona       | 0.08 Max. 0.5 | 25.88 [5.75-35.05] | 40.95 Max. 50  | 21.79 [19.43-27.88] | 42.01 [36.33-51.82] | 2.66 [1.35-4.34] | Max. 5                   |
| *Apis mellifera*    | 0.12 Max. 0.5 | 37.12 [34.53-38.74] | 41.48 Max. 60  | 23.97 [16.21-33.90] | 2.89 [1.42-4.15] | 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 Gonnert and Lavié [47]. A leading research with *Melipona quadridascita* by French and Brazilian scientists made the observations of her delicious thin and sour honeys [48]–always re-discovered by young mellitologists 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 Geotrigna, 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 rain 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 S/125 g, whereas in El Oro province the cost is 20 S/750 g. Ecuadorian Kichwas and Shuars, like Brazilian Enawene-Nawe [58] do not breed bees, but other Brazilian indigenous societies do, like the Guaraní [59] and the Pankararé [60]. Native knowledge about stingless bees connect social groups with nature, in a sort of ecological cosmовision of ancestral awareness.

Government enterprises are needed for producers, possibly to subsidize the quality control procedures, to generate and also to assess the acceptance of pot-honey. Further data is needed to demonstrate putative medicinal properties, and to open the 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 Kayaw, 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] and the Sustainable Development Goals 2015-2030 [57].

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.

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