

## Water content of stingless bee honeys (*Apidae*, *Meliponini*): interspecific variation and comparison with honey of *Apis mellifera*\*

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**Abstract** – Honey samples were taken on both islands of the country of Trinidad and Tobago, from colonies of *Melipona favosa*, *Melipona trinitatis*, *Plebeia tobagoensis*, *Trigona nigra* and *Apis mellifera*. The Moisture Content (MC) of honeys of the various bee species differed significantly. The honey of the smaller species, *P. tobagoensis* and *T. nigra*, had higher MC values than that of the larger *Melipona*. The highest MC value was found in honey from *P. tobagoensis* (42.0%). The other MC values were 36.2% for *T. nigra*, 31.2% for *M. favosa* and 32.2% for *M. trinitatis*. The lowest MC was found in honey of *A. mellifera* (20.2%). There was little variation between colonies of the same species at the same site, but honey of *M. favosa* from Trinidad had a higher MC (35.1%) than that from Tobago (30.2%). The finding that the MC of honeys of stingless bee species varies according to the species and to the area where it is produced, complicates the establishment of a quality standard for these honeys.

**honey composition / *Melipona* / *Trigona* / *Plebeia***

### 1. INTRODUCTION

Social organisation and colony living evolved in several taxa of the family *Apidae* but large colonies that store considerable amounts of food occur only in the stingless bees (*Meliponini*) and in the honeybees (*Apini*). The *Meliponini* (about 400 species) are exclusively tropical whereas the *Apini* contain tropical as well as temperate species. Perennial colonies require a steady flow of food, and food storage makes it possible to cope with short interruptions in food availability.

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The composition of stingless bee honeys appears to differ markedly from that of *Apis* honey. These honeys are often not sweet. Nogueira-Neto (1970) and Cortopassi and Gelli (1991) reported that some stingless bee honeys tasted acidic. A high water content of stingless bee honey has been reported by various authors (Nogueira-Neto, 1970; Cortopassi and Gelli, 1991; Vit et al., 1994, 1998; De Bruijn and Sommeijer, 1997; Torres et al., 2004). In a survey of 27 species, Roubik (1983) found an average moisture content (MC) of 31%. The diversity of the stingless bees in body size and colony population is reflected in the volume of their honey production. Certain species can produce considerable amounts (5–10 dm<sup>3</sup>) of honey per year, while other species only produce a few hundred cm<sup>3</sup>.

Beekeeping with stingless bees is a well-known and wide-spread tradition in tropical countries. This is particularly the case in the neotropics. The honey from stingless bees is highly appreciated locally and traditionally considered to be more powerful as a natural medicine for treating common diseases than honey of the introduced and more productive honey bee (*Apis mellifera*). Only few studies have been conducted to analyse the composition of stingless bee honey. Recently, based on data from earlier papers, Vit et al. (2004) proposed some recommendations for quality standards for stingless bee honeys compared with official standards for *A. mellifera* honey.

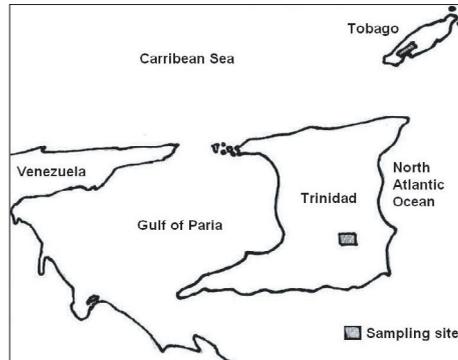
De Bruijn and Sommeijer (1997) presented their first results on the composition, properties and antibiotic activity of honeys of different species of *Melipona* and compared them to those of *Apis*. They found that honey of *M. favosa* and *M. trinitatis* contained on average 23.5% moisture (N = 28). The *Melipona* honeys were further characterized by a significantly lower pH and by having more free acids. In addition, these honeys did not contain diastase, and contained a different microflora, characterized by the (near) absence of yeasts and a dominant presence of *Bacillus*. In a recent study, Torres et al. (2004) found that the honey of the small stingless bee, *Tetragonisca angustula*, had an antimicrobial effect on cultivations of bacteria but not on fungi.

The objective of this paper was to investigate the moisture content (MC) of honey produced by different sympatric species of stingless bees and to compare these honeys with the honey of *A. mellifera* kept in the same tropical environment. This comparison may contribute to the understanding of the difference between honeys of stingless bees and honeybees. The results may also provide a background for the medicinal value that is widely being ascribed to the different honeys of stingless bees in tropical America.

## 2. MATERIALS AND METHODS

### 2.1. Bees and colonies

*Melipona favosa* is a small *Melipona* species with a body length (worker) of 7–8 mm. Colonies



**Figure 1.** Map of sampling sites. Tobago (11° 12' N and 60° 44' W, 0–100 m above sea level), Trinidad (10° 18' N and 61° 10' W, 0–100 m above sea level).

are generally small with about 200–500 bees in Trinidad and Tobago. (Thriving colonies of this species in the Surinam mangrove area were observed to contain as many as 800 bees (observation Sommeijer)).

*Melipona trinitatis* is a larger species of this genus, with a body length of 10–11 mm. Colonies can contain up to 1000–2000 individuals and the nests can be several times larger than those of *M. favosa*. *M. trinitatis* only occurs in Trinidad, W.I.

*Plebeia tobagoensis* is a small stingless bee with a body size of about 4 mm, and a colony population ranging from 40–1500 bees. This species is described from Tobago, but may have a wider distribution.

*Trigona nigra* is a widely distributed species with a slender body of about 5–6 mm. The colony population in Trinidad and Tobago is 500–1200 bees.

The general biology of stingless bees is reviewed in e.g. Michener (1974), Sakagami (1982), Roubik (1989) and Sommeijer and de Bruijn (1994).

### 2.2. Honey samples

Samples were taken during two field trips to Trinidad and Tobago (Fig. 1), in August 1998 and in October 2002. Our sampling site in Trinidad was in the south eastern humid forested area of the island, near Rio Claro and the colonies in Tobago were all situated in the southern part of this island.

A total of 717 honey samples were taken from open and closed storage pots in colonies, managed by beekeepers, of *M. favosa*, *M. trinitatis*, *P. tobagoensis*, *T. nigra* and from sealed honey storage in

*A. mellifera*. In August 1998, honeys of *M. favosa* (N = 408 from 18 different colonies in Tobago) were collected. In October 2002 honeys were collected from *M. favosa* (N = 86 from two colonies in Tobago, and N = 22 from one colony in Trinidad), *M. trinitatis* (N = 62 from two colonies in Trinidad), *P. tobagoensis* (N = 71 from two colonies in Tobago), *T. nigra* (N = 57 from two colonies in Tobago), *A. mellifera* (N = 2 from two colonies in Trinidad, and N = 9 from nine colonies in Tobago).

### 2.3. Measuring honey storage

In 2002, the total number of honey pots per colony was counted in two average-sized colonies of each species except *M. trinitatis*. The volume of open and closed pots was measured by sampling from at least 10 pots per hive. Honey was extracted from the pots by the use of a syringe and the volume of the individual pots was measured in a small, calibrated cylinder. The sum of these measurements was averaged. Based on these results, the total volume of honey per hive could be estimated. Only in the case of *M. trinitatis* in Trinidad, where we could not use small calibrated cylinders, the pot content was estimated by collecting honey from pots in a part of the hive, in one big calibrated cylinder and dividing the volume by the estimated number of honey pots.

### 2.4. Measure of moisture content

Moisture content (MC) of the samples was measured directly in the field using a handheld refractometer (Atago). MC is expressed as percentage (g moisture per 100 g fresh weight). Values obtained for the determination of moisture by refractometry are not absolutely correct (Bogdanov et al., 2000). However, it is a very simple and reproducible method, with an acceptable error. For the MC measurements, all closed honey pots of a colony were opened at the top and samples were taken by the use of Pasteur pipettes. A separate pipette was used for each pot and MC in all samples from one colony were measured directly after collecting. A comparison of the MC of honeys from closed pots with honeys from open pots could only be made for samples taken in August 1998, because of low numbers of open pots in October 2002.

### 2.5. Statistical analysis

We performed ANOVA analyses with a hierarchical model (samples nested within colonies and colonies nested within species). Since the design was unbalanced, Satterthwaite's correction was used (Satterthwaite, 1946; Zar, 1999). Arcsin transformation was used, but these results did not differ from the results done on proportions. For each island, we did a nested ANOVA followed by Tukey HSD for multiple comparison of means on the MC of honeys from closed pots of different bee species in 2002. A comparison of closed honey pots of *M. favosa* and *A. mellifera* was made between the two islands in 2002 (intraspecific difference), and between the two years (1998 and 2002) on the island of Tobago.

## 3. RESULTS AND DISCUSSION

### 3.1. Honey storage per hive

The average total number of honey pots, volume of honey per pot, and total amount of honey are shown in Table I. In addition to the honey pots, the number of pollen pots are also shown in this table.

### 3.2. Interspecific differences in moisture content of honey

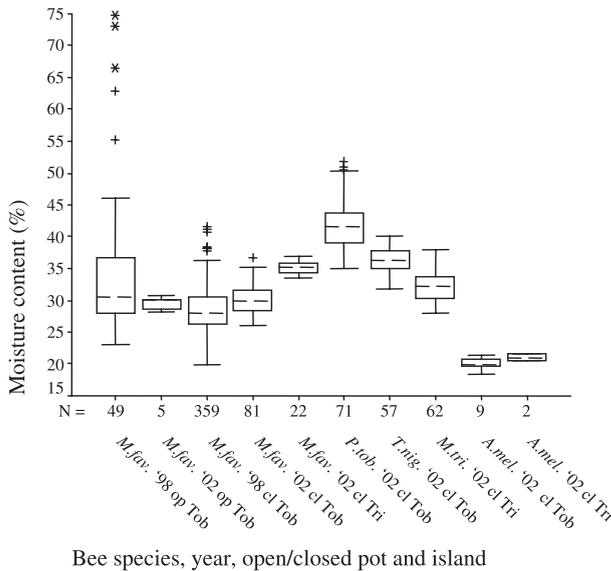
The average MC values are plotted in Figure 2. The highest mean value for MC in closed pots taken in October 2002 was found in *P. tobagoensis* 42.0% (SD 4.0; N = 71) and the lowest mean value was found for *A. mellifera* on Tobago 20.0% (SD 1.0; N = 9).

Nested ANOVA, on closed pots of different stingless bee species on the island of Tobago in 2002, revealed significant differences (F-value = 69.6; df = 3, 5.68;  $P < 0.001$ ) and Tukey's HSD showed that all pairwise comparisons, between the water content of honeys from different species, were significant ( $P < 0.001$ ).

Nested ANOVA, on closed pots of *M. favosa* and *M. trinitatis* on the island of Trinidad in 2002, revealed no significant differences (F-value = 3.5; df = 2, 1.05;  $P = 0.343$ ).

**Table I.** Food storage per hive. Average number of pots for honey (open and closed) and pots for pollen for two colonies and average honey volume per pot (at least 10 pots taken from each of the two hives).

Bee species	closed honey pots/hive	open honey pots/hive	pollen pots/hive	Amount of honey/pot in mL (N)	SD	Total amount of honey/ hive in mL
<i>M. favosa</i> (1998)	25	3	8	6.2 (39)	1.6	174
<i>M. favosa</i> (2002)	32	1	5	6.6 (37)	2.3	218
<i>P. tobagoensis</i>	125	0	43	0.8 (22)	0.5	100
<i>T. nigra</i>	62	2	15	1.2 (23)	1.1	77
<i>M. trinitatis</i>	58	0	25	25.0		1450



**Figure 2.** Boxplots of moisture content of honey for the different species. N = number of samples; *M.fav.* = *Melipona favosa*, *M.tri.* = *Melipona trinitatis*, *P.tob.* = *Plebeia tobagoensis*, *T.nig.* = *Trigona nigra*, *A.mel.* = *Apis mellifera*; '98, '02 = year; op = open pots, cl = closed pots; Tob = from Tobago, Tri = from Trinidad.

Honeys of different species of stingless bees did have significant differences in MC, with exception of honey of *M. favosa* and *M. trinitatis*. In the study of De Bruijn and Sommeijer (1997) the honeys of *M. trinitatis* and *M. favosa* were also found to have the same MC. However in that study, the MC value was considerably lower (23.5%) than the values in this study. This may be explained by the fact that the sampling periods in 2002 (October) and in 1998 (August) were at the end of the rainy season, whereas the honey samples from the 1997 report were collected in the dry season.

It should be noted that the honey of smallest species of our study, *P. tobagoensis* and *T. nigra* have much higher mean MC values than that of the larger *Melipona*'s. The finding by Torres and collaborators of 20.5% MC for the honey of the small *Tetragonisca angustula*, does not confirm the hypothesis that smaller species in general produce honeys with higher MC than larger stingless bees (Torres et al., 2004). For *P. tobagoensis*, a high MC may be related to the fact that nests of this very small species were usually located low to the ground and in rather humid conditions.

### 3.3. Intraspecific differences in moisture content of honey

There was little variation in MC of honey measured in colonies of the same species at the same sampling site. The highest variation was found for honeys collected from *P. tobagoensis* nests. However, samples from the same stingless bee species from different islands presented significant differences: samples from closed pots of *M. favosa* from Trinidad had a higher MC compared to Tobago samples (F-value = 52.2; df = 1, 1.46;  $P = 0.04$ ). The average Trinidad value was 35.1% (SD 1.0; N = 22) and the average for Tobago was 30.2% (SD 2.2; N = 81). The higher MC for *M. favosa* honey from Trinidad may result from a higher humidity on this island. The reported average rainfall value for Trinidad is 2200 mm annually and for Tobago 1900 mm ([www.rlc.fao.org/paises/h2o/trinidad.htm](http://www.rlc.fao.org/paises/h2o/trinidad.htm)).

For *A. mellifera* there was no difference in the MC of honeys originating from the two islands (F-value = 1.1; df = 1, 3.74;  $P = 0.353$ ).

### 3.4. Comparing the moisture content of honey from two years

Honey of closed pots of *M. favosa*, on the island of Tobago, of the year 1998 and 2002 were compared to determine the year-to-year variability. Nested ANOVA revealed no significant differences (F-value = 0.46; df = 1, 17.57;  $P = 0.506$ ).

### 3.5. Comparing the moisture content of honey in open and closed pots

For the large series of pots sampled in colonies of *M. favosa* in August 1998, the MC of honey in open pots was in general much higher than in closed pots. The average MC value for closed pots was 28.4% (SD 3.4; N = 359) and for open pots 35.0% (SD 12.1; N = 49). The samples from open pots varied considerably since some of these had very high MC values. Maximum MC value was 74.7% (Fig. 2). Seasonal weather conditions as well as the typical behaviour of bees

in ripening and storing honey may explain the large standard deviation in MC in open pots. During a nectar flow, collected food is discharged in open pots by foragers or by receiving nest bees (De Bruijn et al., 1989). This nectar is subsequently further dehydrated which leads to lower MC. It is therefore expected that open pots will usually have higher MC's than closed pots. However, open pots also may include pots that were opened again for use after storage. In a period of low nectar flow, open honey pots will then contain honey of lower MC than in periods of heavy flow.

## 4. CONCLUSIONS

### 4.1. Interspecific differences in moisture content of honey

Our results indicate that there are differences in the MC of honey of the different species. First, it was confirmed that, in general, honey of stingless bees has a higher water content than honey of *A. mellifera*. Torres et al. (2004) suggested that this is mainly because stingless bees collect small amounts of honey and use it rather than ripening and storing it. This cannot be generally true, since some *Meliponini* species collect considerable amounts of honey and store it for a prolonged period of time e.g. *M. trinitatis* (Sommeijer and Bootsma, 1988) and *M. beecheii* (Van Veen and Arce, 1999).

It is important to further study the seasonal impact on MC of stingless bee honey. It is a well-known fact (and a commercial problem) that *A. mellifera* honey from the humid tropics has a considerably higher MC than honey from a dryer tropical origin.

There may also be an influence of the type of sources visited (botanical origin) and of specific ways in which the honey is transformed and stored by the bees. In future studies it will be important to include data on the environmental context of the colonies. Observational studies on honey storage could provide further explanations for the high water content in sealed pots, e.g. combined with information on longevity of these pots.

#### 4.2. High water content of stingless bee honey associated with antibiotic activity

The occurrence of watery honey in stingless bees may be related to the humid tropical environment, in which it is difficult to extract water to low concentrations from nectar. When it is difficult to produce highly dehydrated honey there is the problem of easy spoilage of the honey. Strikingly, honeys of *M. favosa* and of *T. nigra* do not spoil more readily than well dehydrated honey bee honeys: more than a year after harvesting, these honeys remained clear and of the same taste (pers. comm. de Bruijn). Possibly, in processing nectar into honey, the bees add enzymes or other substances that lead to antibiotic or preservative activity. This hypothesis would also support the claims of the medicinal activity of these honeys.

#### 4.3. Stingless bee honey as a variable bee product

It is clear from these results that it is difficult to produce general quality standards for all stingless bee honey. In our study which focussed solely on the MC of these honeys, we see that in natural colonies the MC varies between 28.4% (Tobago samples of *M. favosa*, August 1998) and 42.0% (Tobago samples of *P. tobagoensis*, October 2002). The finding that the MC of honey of the same stingless bee species also seems to vary according to the area where it is produced, further complicates the establishment of quality standards. In the recommendations by Vit et al. (2004) it is proposed that the water content for honey of *Melipona*, *Scaptotrigona* and *Trigona* honey should be maximally 30%. It is remarkable that nearly none of the honeys collected during our study reached such low levels.

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**Résumé – Teneur en eau des miels d'abeilles sans aiguillon (Apidae, Meliponini) : variation interspécifique et comparaison avec le miel d'*Apis mellifera*.** Nous avons étudié la teneur en eau (MC) du miel de diverses espèces sympatriques d'abeilles sans aiguillon et l'avons comparé à celle du miel d'*Apis mellifera* produit dans le même environnement tropical à Trinidad et Tobago. Sept cent dix sept échantillons de miel ont été prélevés dans des réserves operculées et non operculées dans des colonies de *Melipona favosa*, *Melipona trinitatis*, *Plebeia tobagoensis*, *Trigona nigra* et *A. mellifera*. La valeur moyenne la plus forte de MC dans les pots operculés (42,0 %) a été trouvée chez *P. tobagoensis* et la plus faible (20,2 %) chez *A. mellifera*. Les autres valeurs de la MC étaient de 36,2 % pour *T. nigra*, de 31,2 % pour *M. favosa* et de 32,2 % pour *M. trinitatis*. La variation entre colonies de la même espèce sur le même site d'échantillonnage a été faible, mais le miel de *M. favosa* de Trinidad avait une MC plus forte (35,1 %) que celui de Tobago (30,2 %). Pour les grandes séries provenant des colonies de *M. favosa* en août 1998, la teneur en eau du miel dans les pots non operculés était beaucoup plus forte (35,0 %) que dans les pots operculés (28,4 %). Nos résultats confirment que le miel des abeilles sans aiguillon possède une teneur en eau généralement beaucoup plus forte que celui d'*A. mellifera*. Le phénomène du miel aqueux chez les abeilles sans aiguillon peut être lié à l'environnement tropical humide, où il est difficile de produire un miel fortement déshydraté. Le problème de la détérioration facile du miel aqueux pourrait être surmonté si les abeilles ajoutaient des enzymes ou d'autres substances ayant une activité antibiotique, ce qui corroborerait les allégations d'activité médicinale de ces miels.

***Melipona* / *Trigona* / *Plebeia* / miel / teneur en eau / *Meliponini***

**Zusammenfassung – Wassergehalte in Honigen von Stachellosen Bienen (Apidae, Meliponini): interspezifische Variation und Vergleich mit *Apis mellifera* Honig.** In der vorliegenden Studie untersuchten wir den Wassergehalt (WG) in Honigen verschiedener in Trinidad und Tobago sympatrisch vorkommender Stachelloser Bienen und verglichen diese mit *Apis mellifera* Honigen aus der gleichen Tropenregion. Aus Kolonien von *Melipona favosa*, *Melipona trinitatis*, *Plebeia tobagoensis*, *Trigona nigra* und *Apis mellifera* wurden insgesamt 717 Honigproben aus offenen und geschlossenen Honigvorräten entnommen.

Den höchsten WG-Mittelwert für geschlossene Honigtöpfe fanden wir bei *P. tobagoensis*. Er lag bei 42 %. Den niedrigsten Wert (20,2 %) fanden wir bei *A. mellifera*. Die anderen Arten zeigten folgende Werte: 36,2 % bei *T. nigra*, 31,2 % bei *M. favosa* und 32,2 % bei *M. trinitatis*. Wir fanden nur geringe Variationen für diese WG-Werte in Kolonien der gleichen Art und am gleichen Standort. Lediglich *M. favosa* Honig aus Trinidad hatte einen höheren WG (35,1 %) als der aus Tobago (30,2 %). Bei der großen Probenserie aus *M. favosa* Kolonien, die im August 1998 gesammelt wurden, lag der WG für Honige aus offenen Honigtöpfen bei 35,0 % und damit etwas höher als der für geschlossene Töpfe. Unsere Ergebnisse bestätigen frühere Befunde, dass Honige von Stachellosen Bienen einen höheren Wassergehalt aufweisen als *A. mellifera* Honige. Das Phänomen der wasserreichen Honige Stachelloser Bienen könnte mit den feuchten tropischen Klimabedingungen zusammenhängen, die die Produktion wasserarmer Honige erschweren. Das Problem der leichten Verderblichkeit dieser wässrigen Honige können die Bienen vermutlich durch Zusatz von Enzymen oder anderer Substanzen mit antibiotischer Aktivität umgehen.

**Honigzusammensetzung / *Melipona* / *Trigona* / *Plebeia***

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