

Venezuelan stingless bee honeys characterized by multivariate analysis of physicochemical properties

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Abstract – Stingless bee honey samples collected from 27 nests in Venezuela, were analysed for ten compositional factors (acidity, ash, electrical conductivity, diastase activity, hydroxymethylfurfural, invertase activity, nitrogen, reducing sugars, sucrose and water). The entomological origin of the honeys based on these factors was explored using three methods of multivariate analysis. Clustering was adequate to separate the honey samples into two stingless bee tribes with the exception of honeys from one genus of Trigonini. Principal component analysis confirmed these findings and grouped the honeys to species level. Although discriminant analysis of the ten quality factors under consideration positioned all the samples in their respective entomological group, lowering the number of variables to reducing sugars, sucrose and diastase activity was still satisfactory for a correct classification. A fourth variable that could be used to assign correct membership was either acidity or nitrogen content. © Inra/DIB/AGIB/Elsevier, Paris

honey / multivariate analysis / physicochemical properties / stingless bees / Venezuela

1. INTRODUCTION

Stingless bee honeys were widely relied in the tropics before the introduction of *Apis mellifera* L. [22]. Stingless bees belong

to the family Hymenoptera and subfamily Meliponinae, which is divided into two tribes, Meliponini and Trigonini. The genus *Melipona* is endemic to the Neotropics. The honey produced by stingless bees is mark-

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eted as a natural remedy for eye treatment, and this specific market niche makes the value of stingless bee honey 20 times the value of *A. mellifera* honey. However, stingless bee farms are of reduced size, and generally have no more than ten hives, sufficient to satisfy only the local demand. No stingless bee honeys have been registered by sanitary authorities, in part due to the absence of official quality standards. The characterization of the honeys produced by stingless bees has been the subject of only a few studies compared to the extensive documentation available on *A. mellifera* honeys. There are three main reasons for this imbalance: 1) international interest is devoted to commercial *A. mellifera*; 2) the high diversity of stingless bees with more than 500 species increases the complexity of sampling, and limits the collection of data on single species and monofloral honeys; 3) stingless bee hives have lower honey yields than *A. mellifera*. One way to overcome these limitations is to unify information from different sources and to build up a common database of quality factors of stingless bee honeys, with harmonized methods of analysis. Although considerable data on stingless bee honey has been published in proceedings of local meetings, only a few studies have been published in scientific journals.

Stingless bees store honey in pots rather than in combs. Their honey is also nectar or honeydew that has been concentrated and transformed. Previous works [6, 11, 17, 27] have pointed out differences between the composition of stingless bee honeys from Brasil, India and Venezuela, and the composition of other *Apis* honeys. The honey samples studied in the present work have been considered elsewhere in more detail for diastase and invertase activities [28] and the three frequently occurring sugars [30]. The phenolic fraction has also been compared between honeys of *A. mellifera* and *Melipona* spp. to estimate its value as an entomological predictor based on differences in bee diets. However, it was found to be

more related to the geographical origin of the honeys than the entomological origin [29]. A further proposal was to investigate the flavonoid content to assess the putative anticataract activity of stingless bee honeys [31]. In this work some parameters generally used to analyse *A. mellifera* honeys have been used to analyse samples of stingless bee honeys.

The classification of honeys by their botanical and geographical origin has an economical ground of authenticity. The study of unifloral honeys does not only provide trading standards, but also represents a simplified approach to the complex and multifactorial chemical composition of honey. Therefore, any practical model for classifying honeys should be based on observations of the recurrent elements that define different categories [16].

Multivariate analysis techniques have been used to detect the geographical origin of honey based on free amino acid content [10], and to classify honeys of the same botanical origin according to chemical and physical descriptors [12]. Other work has been carried out on the mathematical classification of honey [15, 24, 25].

In the present work we present a comparative approach using three methods of multivariate analysis to predict the entomological origin of stingless bee honeys from the west and south of Venezuela, based on ten compositional factors generally accepted to assess honey quality: acidity, ash, diastase activity, electrical conductivity, hydroxymethylfurfural (HMF), invertase activity, nitrogen, reducing sugars, apparent sucrose and water content.

2. MATERIALS AND METHODS

2.1. Samples

Honey samples were collected in 1993 from 27 stingless bee nests located in the west (Acarigua, Barinas, Barrancas, El Guayabo, Elorza, Guasdalito, Mérida and Punto Fijo) and south

(Guaramajé, Maroa, Santa Elena de Uirén, San Fernando de Atabapo and San Juan de Manapiare) of Venezuela. The entomological origin of the samples is indicated in table 1. Meliponini honey samples in the study were collected from *Melipona* species: two *M. compressipes compressipes*, one *M. crinita*, one *M. eburnea*, six

M. favosa favosa, three *M. lateralis kangarumensis*, four *M. paraensis* and one *M. sp. group fulva*. Honey samples from the Trigonini tribe included one *Nannotrigona sp. aff. chapadana*, one *Trigona (Frieseomelitta) nigra paupera*, two *Trigona (Frieseomelitta) sp. aff. varia* and three *Trigona (Tetragonisca) angustula angustula*.

Table 1. Means \pm standard errors and ranges (in brackets) of physicochemical characteristics of stingless bee honeys from Venezuela. Values followed by different superscripts are significantly different at $\alpha = 0.05$.

| Compositional factors | Tribe | Trigonini | |
|---|---|--|---|
| | Meliponini ¹ | <i>Scaptotrigona</i> spp. ² | Other genera ³ |
| | <i>n</i> | 2 | 7 |
| Acidity (meq/kg honey) | 34.6 \pm 3.4 ^a (9.2 – 69.6) | 52.0 \pm 32.0 ^{ab} (20.0 – 84.0) | 57.8 \pm 3.9 ^b (43.6 – 72.4) |
| Ash (g/100 g honey) | 0.16 \pm 0.03 ^a (0.02 – 0.40) | 0.31 \pm 0.0 ^{ab} (0.29 – 0.32) | 0.41 – 0.04 ^b (0.29 – 0.52) |
| Electrical conductivity (mS/cm) | 2.2 \pm 0.3 ^a (0.7 – 6.5) | 2.9 \pm 0.6 ^a (2.3 – 3.4) | 7.6 \pm 0.2 ^b (7.1 – 8.4) |
| Diastase activity (DN ⁴) | 2.9 \pm 0.1 ^a (2.6 – 3.5) | 2.6 \pm 0.0 ^a (2.6 – 2.6) | 15.6 \pm 3.6 ^b (6.6 – 35.6) |
| Hydroxymethylfurfural (mg·kg ⁻¹ honey) | 11.1 \pm 2.4 ^a (0.4 – 31.6) | 5.7 \pm 0.2 ^a (5.5 – 5.8) | 8.9 \pm 2.8 ^a (4.2 – 20.4) |
| Invertase activity (IU ⁵) | 58.1 \pm 12.0 ^a (9.5 – 169.1) | 26.0 \pm 10.1 ^a (15.9 – 36.1) | 96.5 \pm 30.6 ^a (20.0 – 214.3) |
| Nitrogen (mg/100 g honey) | 49.6 \pm 7.9 ^a (10.6 – 114.6) | 27.1 \pm 1.5 ^a (25.6 – 28.5) | 129.0 \pm 14.5 ^b (47.7 – 162.6) |
| Reducing sugars (g/100 g honey) | 65.3 \pm 1.2 ^a (53.7 – 73.1) | 53.0 \pm 0.7 ^{ab} (52.4 – 53.7) | 61.2 \pm 2.7 ^a (51.2 – 70.4) |
| Sucrose (g/100 g honey) | 1.6 \pm 0.3 ^a (0.6 – 5.6) | 1.3 – 0.1 ^a (1.2 – 1.4) | 2.7 \pm 0.9 ^a (0.3 – 6.1) |
| Water (g/100 g honey) | 26.5 \pm 0.6 ^a (22.9 – 31.5) | 27.4 \pm 2.2 ^{ab} (25.2 – 29.5) | 22.4 \pm 1.3 ^b (17.9 – 27.5) |

¹ Meliponini included here are *Melipona* species: *M. compressipes compressipes* (Mcc), *M. crinita* (Mc), *M. eburnea* (Me), *M. favosa favosa* (Mff), *M. lateralis kangarumensis* (Mlk), *M. paraensis* (Mp) and *M. sp. group fulva* (Mf).

² The two *Scaptotrigona* species are *S. ochrotica* (So) and *S. polystica* (Sp).

³ Other Trigonini species are *Nannotrigona sp. aff. chapadana* (N), *Trigona (Frieseomelitta) nigra paupera* (TFnp), *Trigona (Frieseomelitta) sp. aff. varia* (TFv) and *Trigona (Tetragonisca) angustula angustula* (TTaa).

⁴ The diastase number (DN) expresses g starch hydrolysed/100 g honey/h, at pH 5.2 and 40 °C.

⁵ An invertase unit (IU) indicates μ moles p-nitrophenyl glucopyranoside hydrolysed/kg honey/ min, at pH 6.0 and 40 °C.

Two samples of *Scaptotrigona* species were collected from *S. ochrotica* and *S. polystica*. Following the method of Louveaux et al. [9], 14 of the samples were considered monofloral (*Terminalia catappa*, *Julbernardia* sp., *Myrcia* sp., *Moraceae*, *Spondias mombin*, *Zanthoxylum fagara*, *Weinmannia* sp., *Astronium graveolens*, *Protium* sp., *Angelonia* sp.). However, too few samples were obtained for each monofloral type to embark further comparisons according to their botanical origin. Samples were stored at -20°C until analysis.

2.2. Analytical methods

General parameters such as acidity, ash and water content were analysed following the Codex Alimentarius Commission Recommended European Regional Standard for Honey [5]. Acidity was measured potentiometrically. Ash content was determined gravimetrically after ignition in a muffle furnace. Water content was determined with the refractometric index. Diastase activity was determined with the Phadebas method developed by Bogdanov [3]. Invertase activity was measured following the method described by Siegenthaler [23]. Electrical conductivity was measured following the method described by Louveaux et al. [8]. A photometric determination of hydroxymethylfurfural and the Munson and Walker cuprimetric determination of reducing sugars and apparent sucrose were carried out following the methods described in the Venezuelan Regulations for Honey Quality Control [7]; however, the analysis of sugars by HPLC was not included in these methods. Nitrogen content was determined using the Kjeldahl volumetric technique described by the Association of Official Methods of Analytical Chemists [2].

2.3. Statistical methods

One-way ANOVA followed by Scheffé test was used to compare the means of the quality factors among the three groups of honey. Principal component analysis (PCA) and discriminant analysis are multivariate ordination techniques. PCA projects multidimensional units on to a space of fewer dimensions to derive successive orthogonal axes, which maximizes the variation represented by each component. Discriminant analysis requires a prior grouping of the samples by producing linear combinations of the

descriptors, to maximize variation between the groups [19, 26, 32]. Hierarchical cluster analysis is a classification technique that permits the grouping of experimental units into discrete groups at different levels of association [20]. Hierarchical clustering was applied using the Euclidean distance and Ward's method on data transformed to a normal distribution and standardized to a range of -1 to $+1$. PCA was limited to the plot of the scores along the first two principal components. Canonical variate analyses were performed using all the parameters. Systematic reduction of variables resulted in selection of four of the parameters as the minimum necessary for correct groupings. SPSS was the statistical package used for the analyses.

3. RESULTS

The ten compositional factors of honey measured in the current study are presented in *table I*. Mean values, standard errors of the means (s.e.m.) and ranges are compared between honeys produced by Meliponini, *Scaptotrigona* spp. and other genera of Trigonini. Since the number of samples per stingless bee was small, we grouped them according to the two tribes. The two *Scaptotrigona* spp. samples are listed in a separate column because the results of the multivariate analysis (below) indicated they were more similar to Meliponini than Trigonini tribe to which they belong. HMF, invertase activity and sucrose were not significantly different among the three groups of honey. Acidity, ash, reducing sugars and water content were different between Meliponini and Trigonini, but not between *Scaptotrigona* spp. and the two groups of honeys. Conductivity, diastase activity and nitrogen were not significantly different between *Melipona* and *Scaptotrigona* spp. honeys but were significantly lower in those groups than in the Trigonini.

Table II is a condensed table with the ten quality factors of honey produced by the four species from which at least three samples were collected. In this table, mean values for acidity vary from 30.40 to 48.27 meq·kg⁻¹ honey. The highest value was pre-

Table II. Average of ten physicochemical characteristics of stingless bee honeys. Values are mean \pm (s.e.m.).

| Bee species ¹ | <i>n</i> | Quality factors | | | | | | | | | |
|--------------------------|----------|------------------------|---------------------|---------------------------------|-----------------------------------|---------------------|------------------------------------|---------------------------|--------------------------------|-------------------------|-----------------------|
| | | Acidity (meq/kg honey) | Ash (g/100 g honey) | Electrical conductivity (mS/cm) | Diastase activity DN ² | HMF (mg/100g honey) | Invertase activity IU ³ | Nitrogen (mg/100 g honey) | Reducing sugar (g/100 g honey) | Sucrose (g/100 g honey) | Water (g/100 g honey) |
| Mff | 6 | 36.80 (3.56) | 0.15 (0.03) | 2.06 (0.39) | 2.86 (0.15) | 17.06 (3.72) | 90.08 (19.85) | 70.87 (8.64) | 70.27 (0.68) | 2.00 (0.44) | 24.17 (0.42) |
| Milk | 3 | 40.67 (2.08) | 0.11 (0.04) | 1.65 (0.29) | 2.76 (0.12) | 3.85 (0.62) | 58.90 (16.10) | 23.42 (3.73) | 64.79 (2.78) | 28.83 (0.24) | 1.08 (1.84) |
| Mp | 4 | 30.40 (13.31) | 0.14 (0.05) | 1.37 (0.45) | 2.90 (0.16) | 3.36 (1.26) | 19.77 (3.76) | 14.34 (2.23) | 60.80 (2.44) | 1.17 (0.29) | 26.40 (1.34) |
| TTaa | 3 | 48.27 (4.08) | 0.38 (0.04) | 7.32 (0.23) | 23.00 (6.30) | 9.83 (5.28) | 50.13 (11.53) | 142.27 (3.87) | 23.17 (2.61) | 2.05 (0.86) | 65.90 (0.37) |
| all | 27 | 41.90 (3.63) | 0.23 (0.03) | 3.62 (0.52) | 6.15 (1.41) | 10.12 (1.75) | 65.67 (11.58) | 68.52 (9.48) | 63.34 (1.23) | 1.86 (0.32) | 25.49 (0.64) |

¹ For species abbreviations, see *table 1*.² The diastase number (DN) expresses g starch hydrolysed/100 g honey/h, at pH 5.2 and 40 °C.³ An invertase unit (IU) indicate (μoles p-nitrophenyl glucopyranoside hydrolysed/kg honey/min, at pH 6.0 and 40 °C).

sented by *T. (Tetragonisca) angustula angustula* and the lowest by *M. paraensis* with the highest intraspecific variations. The ash content varied from 0.11 to 0.38 g/100 g honey. The electrical conductivity varied from 1.37 to 7.32 mS/cm. Acidity, ash content, electrical conductivity and diastase activity were highest in *T. (Tetragonisca) angustula angustula* honeys; acidity and electrical conductivity were lowest in *M. paraensis*, whereas ash and diastase were lowest in *M. lateralis kangarumensis* honey samples. The highest values of HMF (mg/100 g honey) were found in *M. favosa favosa* (17.06), followed by *T. (Tetragonisca) angustula angustula* (9.83). The other two *Melipona* spp. presented similar low values of HMF (3.36–3.85). The highest invertase activity was also a characteristic of

M. favosa favosa, and the lowest of *M. paraensis* honeys, varying from 90.08 to 19.77 IU, respectively. Average nitrogen content varied from 14.34 to 142.27 mg/100 g honey, respectively, in *M. paraensis* and *T. (Tetragonisca) angustula angustula*. Reducing sugars (g/100 g honey) were higher in honeys produced by *M. favosa favosa* (70.27) and varied from 60.80 to 65.90 in the remaining three species, while sucrose presented a very narrow range (1.08–2.05 g/100 g honey) in all the species. The highest average water content was found in *M. lateralis kangarumensis* (28.83), followed by *M. paraensis* (26.40), *M. favosa favosa* (24.17) and the lowest in *T. (Tetragonisca) angustula angustula* (23.17).

The output of the hierarchical cluster analysis is presented in figure 1. The honeys

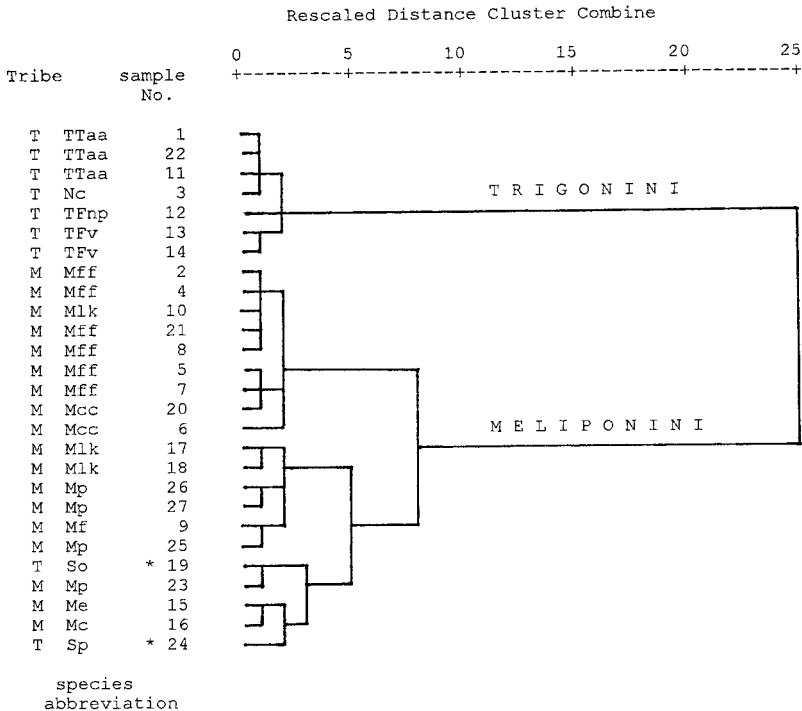


Figure 1. Dendrogram using Ward’s method to cluster stingless bee honeys by their acidity, ash, diastase activity, electrical conductivity, hydroxymethylfurfural, invertase activity, nitrogen, reducing sugars, sucrose and water content, standardized within the range -1 to +1. M= Meliponini, T= Trigonini. See table 1 for species abbreviations. * Trigonini honey included in the Meliponini cluster.

produced by the two stingless bee tribes were successfully positioned in two different clusters except for the two samples of *Scaptotrigona* spp.

In figure 2 the scores plotted along the first two principal components accounted for the 59.6 % of the total variation among all the variables measured in the study. In this two dimensional representation, the principal component 1 (PC1) separated Trigonini from Meliponini, except *Scaptotrigona* spp., as previously observed in the dendrogram. The principal component 2 (PC2) generated further divisions, grouping honeys at different levels. *M. compressipes* compressipes and *M. fava fava* formed one group, and *M. lateralis kangarumensis* and *M. paraensis* were closely positioned. *S. polystica* was also close to these species. No major comments can be made on single samples of honey from *M. crinita*, *M. eburnea*, *S. ochrotica* and *M. aff. group fulva*

which did not overlap with previous taxa. One of the four samples collected from *M. paraensis* did not locate in the vicinity of the other three honeys produced by the same bee species. It is worth mentioning that a field observation on the structure of the honey pots from which this sample was collected was very different from the pots in the other three nests.

PC1 accounted for 38.9 % of the variability and was strongly associated with positive weights of electrical conductivity, ash and nitrogen, and negative diastase. PC2 presented high loadings for reducing sugars, HMF, invertase and sucrose and explained 20.7 % of the total variance. Acidity and HMF were moderately weighted in PC3, contributing with an additional 11.9 %, to complete a 71.4 % of cumulative percentage of the total variation.

The results of discriminant analysis are presented in figure 3. Further reductions

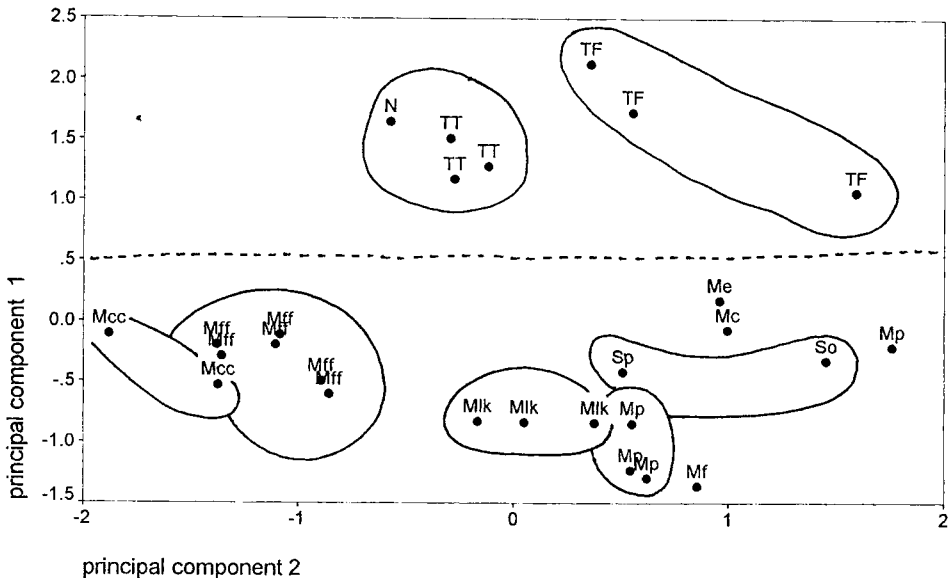


Figure 2. Plot of the scores along the two first principal components produced by the ten honey quality factors under consideration, without rotation. See table 1 for species abbreviations. Two major groups at the tribe level were separated by the first component (---), Trigonini on the upper band and Meliponini with *Scaptotrigona* spp. in the lower band, similar to the output of cluster analysis (figure 1). The second component (---) permitted additional separation within the tribe level. Boundaries were drawn only for illustrative purposes.

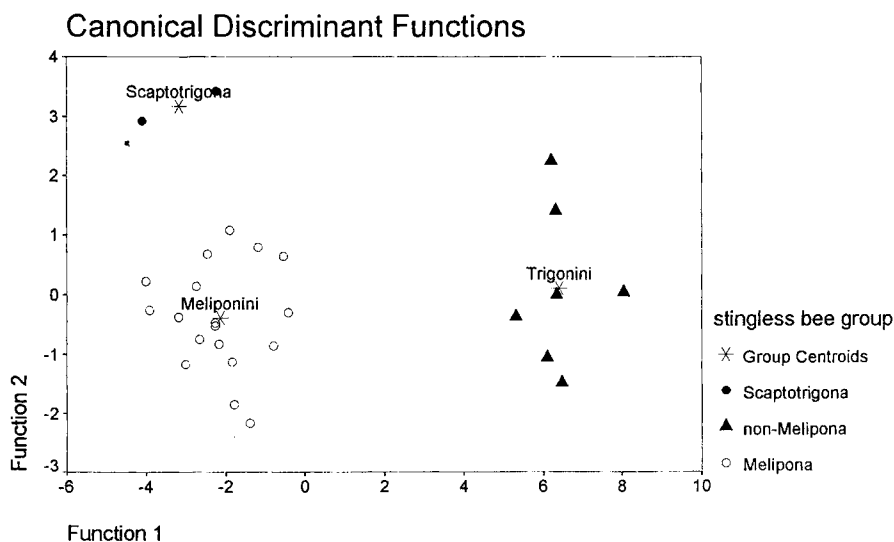


Figure 3. Plot of stingless bee honeys relative to the first two canonical discriminant functions obtained from the ten compositional factors of this study. Honeys from three stingless bee groups were readily observable. Function 1 separated Meliponini and *Scaptotrigona* spp. from Trigonini honeys, as in the dendrogram (figure 1). Function 2 provided an additional discrimination between Meliponini and *Scaptotrigona* spp. honeys.

were made by evaluating all possible combinations between groups of nine to two of the studied variables. The minimal number of variables required to position correctly all the samples was four. The reduction from ten to four variables (figure 4) produced 100 % correctly grouped samples; however, it increased their scatter.

4. DISCUSSION

Stingless bee honeys may be divided into two obvious groups based on the two tribes. However, after studying the enzyme activity [28] and the sugar content by HPLC [30] of the same group of stingless bee honeys from Venezuela, the membership of *Scaptotrigona* spp. was controversial because although the bees belong to the Trigonini tribe, their honeys were consistently positioned in the Meliponini cluster. These findings were confirmed in the present study by multivariate analysis of ten factors asso-

ciated with honey (figures 1 and 2). For that reason, in the present work, the Trigonini data are divided into two subgroups, *Scaptotrigona* spp. and other Trigonini genera (table 1).

The average acidity of stingless bee honeys was high compared to the standards for *A. mellifera* [5]. The Trigonini, excluding the samples from *Scaptotrigona* spp., produced a more acidic honey than Meliponini. However, we did not find the exceptionally high values, up to 160 meq·kg⁻¹ honey, reported by Cortopassi-Laurino and Gelli [6].

Ash and electrical conductivity are two parameters bound to honey mineral content. Ash represents a direct measure of the inorganic residue after honey carbonization, while electrical conductivity measures all ionizable organic and inorganic substances. The relationship between the two parameters has been shown by several authors [1, 18, 21]. When compared to values of conducti-

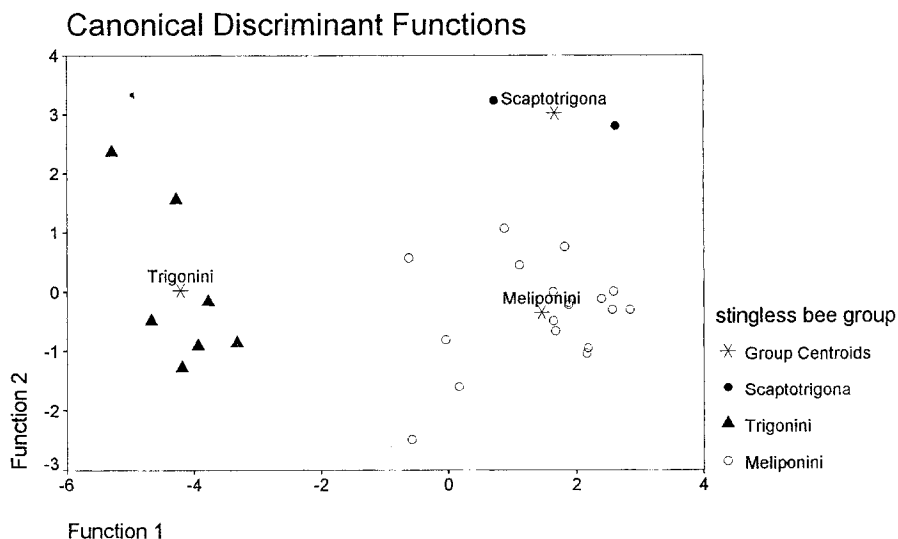


Figure 4. Plot of stingless bee honeys relative to the first two canonical discriminant functions obtained from four compositional factors: reducing sugars, sucrose, diastase activity and nitrogen. Honeys from three stingless bee groups observed in *figure 3* are also observed here but with higher scatter.

vity in *A. mellifera* honey [16] stingless bee honeys had very high values, and were exceptionally high in Trigonini samples. On the contrary, ash values fall in the same range of *A. mellifera* honey. This fact is probably related to the organic ionizable fraction and should be investigated more deeply. The ash content of stingless bee honeys was similar to *A. mellifera* honeys, and was higher in Trigonini (excluding *Scaptotrigona* spp.) than in Meliponini. Conductivity of Venezuelan stingless bee honeys collected in 1987–1988 was found to be significantly higher in non-*Melipona* honeys [4]. However, compared with the present work, conductivity values were approximately tenfold lower. This difference should be addressed in the future, especially considering that ash contents were similar in both works, and that both Liebefeld and Rome involved labs are reference labs for honey and have ring trial experience.

Water content of stingless bee honey is known to be higher than in *A. mellifera*

honeys [1, 6, 11, 13, 27]. In this work Meliponini honeys had a higher water content than Trigonini honeys excluding those from *Scaptotrigona* spp. According to the previous three parameters, *Scaptotrigona* spp. honeys were not different from Meliponini and the other Trigonini honeys. Mean values of electrical conductivity, diastase activity and nitrogen content were not statistically different between Meliponini and *Scaptotrigona* spp. honeys, but were lower than honey samples from the other genera of Trigonini. Reducing sugars were generally lower or in the limit of the *A. mellifera* honey standards. They were very low in *Scaptotrigona* spp. honeys compared to honey obtained from Meliponini. The other Trigonini presented intermediate values for this parameter. HMF, invertase activity and sucrose were not different in the three groups of stingless bee honeys, but sucrose was considerably lower than the maximum suggested by the honey standards for *A. mellifera* [5].

The honeys were separated into two major groups and four subgroups with further subdivisions after plotting the scores of PC1 and PC2 (figure 2). A major division separates Trigonini from Meliponini honeys, except the two *Scaptotrigona* spp. Secondary divisions were drawn to illustrate the five subgroups obtained for: *T. (Tetragonisca) angustula angustula* and *Nannotrigona* sp. (1), *T. (Frieseomelitta)* spp. (2), *M. favosa favosa* and *M. compressipes compressipes* (3), *M. lateralis kangarumensis*, *M. paraensis*, *Melipona* sp. group *fulva*, *S. polystica*, *M. eburnea*, *M. crinita* and *S. ochrotical* (4). The first component accounted for 38.9 % of total data variability and was strongly associated with the electrical conductivity, diastase activity, nitrogen and ash content. The second component explained 20.7 % of the variation and was moderately associated with reducing sugars and HMF. Zalewski [32] compared unifloral and honeydew honeys from Poland and, as in our study, found the electrical conductivity, diastase activity and ash content to be strongly associated with the PC1, although he expressed difficulty in interpreting all components. In both cases, the extraction of the first component was based on three common quality factors, to produce separations based on entomological differences in stingless bee honeys from Venezuela and botanical differences in Polish honeys. Screening of significant parameters to distinguish honeys by eliminating the ash content from the data matrix, was suggested by Krauze and Zalewski [12] because of its high correlation with electrical conductivity. Hierarchical cluster analysis also confirmed the PCA results, with one exception for sample 10 (figure 2).

The possibility of eliminating redundant variables by the classification and ordination methods of multivariate analysis offers the advantage of reducing the analysis required to assign membership of honeys according to their origin. After discriminant analysis, four quality factors were sufficient to position 100 % of the stingless bee honeys

either at the tribe level or at the intermediate *Scaptotrigona* spp. group. Ninety-six percent of the honeys were correctly classified by reducing sugars, sucrose and diastase activity, increased to 100 % with either acidity or nitrogen. Between these two variables, nitrogen is more adequate for the reduced set of variables, because acidity presented a considerably high variation in the *Scaptotrigona* spp. group (table 1). However reducing the number of variables decreased group separation (figure 3). Test stingless bee honey samples from each group were treated with the obtained discriminant function and were correctly positioned.

These preliminary findings need to be confirmed through continued sampling prior to their statistical validation as species-specific characteristics of stingless bee honeys. The botanical origin may play a role in determining some physico-chemical differences, as it does in *A. mellifera* honey. However, due to the small number of honey samples and the scant knowledge of Venezuelan bee flora no particular conclusion can be made. It should also be mentioned that the effect of the floral source or the effect of both floral and entomological components of the honey cannot be measured in the present work because of the lack of replications of monofloral honeys to allow statistical testing. Even based on a small number of samples, our data illustrate the main features of stingless bee honeys. This very general picture could be obviously made more precise by continuing the research at a species level. A slow but solid growth of the database on stingless bee honey compositional factors should provide cumulative information and bridge gaps so that comparisons may be made at the more rigorous species level.

The long-term objective in studying the compositional factors of honey produced by stingless bees is to develop adequate standards for honeys produced by specific groups of bees that are important because

of their productivity or due to their attributed medicinal properties. This information may be used as a technical support for the rescue of traditional meliponiculture and its expansion out of the rural market. The development of standards for stingless bee honey can benefit from the enormous information available for *A. mellifera* honeys, with appropriate adaptations. The suggestion to expand the official definition of honey to a wider entomological conception, accepting that stingless bees and other *Apis* spp. also produce honey, has been systematically rejected for the last 10 years. Perhaps it is time to coin a different name for the sweet product stored in pots by tropical bees. A new term such as divine elixir – exclusive for stingless bee honey – will not be more poetic than royal jelly.

ACKNOWLEDGEMENTS

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Résumé – Caractérisation des miels d'abeilles sans aiguillon du Vénézuéla par l'analyse multivariée des propriétés physicochimiques.

Les caractéristiques physicochimiques suivantes d'échantillons de miels prélevés dans 27 nids d'abeilles sans aiguillon ont été analysées : acidité, teneur en cendres, conductibilité électrique, activité diastasique, HMF, activité de l'invertase, azote, sucres réducteurs, saccharose et teneur en eau. Les valeurs moyennes, les erreurs standard et les valeurs extrêmes sont

présentées dans le *tableau I* pour les miels de Meliponini, les miels des espèces de *Scaptotrigona* et les miels de Trigonini. Il existe des différences dans l'acidité, la teneur en cendres, les sucres réducteurs et le teneur en eau entre les miels de Meliponini et de Trigonini mais pas entre ceux-ci et les miels de *Scaptotrigona* spp. La conductibilité électrique, l'activité diastasique et la teneur en azote sont significativement plus bas dans les miels de Meliponini et de *Scaptotrigona* que dans ceux de Trigonini. Le *tableau II* donne la composition chimique des miels pour chaque espèce (avec au moins trois échantillons/espèce) et pour l'ensemble des échantillons. La possibilité de discriminer l'origine entomologique des miels par les caractères physicochimiques a été étudiée par trois méthodes d'analyse multivariée. L'analyse de groupe (*figure 1*) convient pour séparer les échantillons de miels des deux tribus Meliponini-Trigonini, un genre de Trigonini faisant exception. Ceci a été confirmé par l'analyse en composantes principales (*figure 2*), où d'autres regroupements au niveau de l'espèce ont été faits. L'analyse discriminante des caractères physicochimiques a permis de positionner tous les échantillons dans leur groupe entomologique respectif (*figure 3*). Un nombre restreint de variables, comprenant les sucres réducteurs, le saccharose et l'activité diastasique et la teneur en azote, suffit à classer correctement les échantillons (*figure 4*). © Inra/DIB/AGIB/Elsevier, Paris

miel / propriété physicochimique / Meliponinae/ analyse multivariée / Vénézuéla

Zusammenfassung – Charakterisierung von Honigen stachelloser Bienen durch die multivariate Analyse der physiko-chemischen Merkmale. Aus siebenundzwanzig Nestern Stachelloser Bienen in Venezuela wurden Honigproben entnommen. Zur Einschätzung der Honigqualität wurden zehn Merkmale der Honigzusammenset-

zung untersucht: PH-Wert, Aschegehalt, Leitfähigkeit, Diastaseaktivität, Hydroxymethylfurfural (HMF-Wert), Invertaseaktivität, Stickstoffgehalt, reduzierende Zucker, Rohrzucker – und Wassergehalt. In *Tabelle I* sind Mittelwerte, mittlere Fehler und Wertbereiche dieser Qualitätsmerkmale für Meliponini, *Scaptotrigona* spp und für andere Trigonini aufgeführt. PH-Wert, Aschegehalt, reduzierende Zucker und Wassergehalt unterschieden sich zwischen den Meliponini und den Trigonini, aber nicht zwischen *Scaptotrigona* spp. und den anderen beiden Gruppen. Leitfähigkeit, Diastaseaktivität und Stickstoffgehalt lagen bei den Meliponini und *Scaptotrigona* spp. signifikant niedriger als bei den anderen Trigonini. In *Tabelle II* werden die Qualitätsmerkmale für die Arten angegeben, für die mindestens drei Probenwerte vorlagen, sowie die Mittelwerte aller Proben zusammen. Die mögliche Eignung der untersuchten Merkmale zur Bestimmung der Herkunftsart wurde mit drei Methoden multivariater Analysen untersucht. Mit einer Clusteranalyse (*Abb. 1*) konnten die Honige nach dem Tribus aufgetrennt werden, wobei nur eine Art der Trigonini eine Ausnahme darstellte. Diese Tatsache wurde durch eine Hauptfaktorenanalyse bestätigt (*Abb. 2*), die zudem weitere Gruppierungen auf dem Gattungsniveau aufzeigte. Die zehn Qualitätsmerkmale ordneten in einer Diskriminanzanalyse alle Proben ihrer jeweiligen entomologischen Gruppe zu (*Abb. 3*). Auch eine geringere Anzahl von Merkmalen (reduzierende Zucker, Rohrzuckergehalt, Diastaseaktivität und Stickstoffgehalt) war ausreichend, um eine befriedigend korrekte Zuordnung zu erreichen (*Abb. 4*). Zuletzt gehen wir auf die Entwicklung von Qualitätsstandards für Honige von Stachellosen Bienen und auf die Notwendigkeit einer angemessenen und offiziellen Kenntnisnahme dieses Produkts ein. © Inra/DIB/AGIB/Elsevier, Paris

Honig / multivariate Analyse / zusammengesetzte Faktoren / Stachellose Bienen / Venezuela

REFERENCES

- [1] Accorti M., Piazza M.G., Persano Oddo L., La conductivité électrique et le contenu en cendre du miel, *Apiacta XXII* (1983) 19–20.
- [2] AOAC, Official Methods of Analysis, 15th ed., Association of Official Analytical Chemists, Washington, DC, 1984.
- [3] Bogdanov S., Honigdiastase, Gegenüberstellung verschiedener Bestimmungsmethoden, *Mitt. Geb. Lebensm. Hyg.* 75 (1984) 214–220.
- [4] Bogdanov S., Vit P., Kilchenmann V., Sugar profiles and conductivity of stingless bee honeys from Venezuela, *Apidologie* 27 (1996) 445–450.
- [5] CAC, Recommended European Regional Standard for Honey, Codex Alimentarius Commission, FAO/OMS. CAC/RS 12-1969, Joint FAO/WHO Food Standards Programme, Rome, 1969.
- [6] Cortopassi-Laurino M., Gelli D.S., Analyse pollinique, propriétés physico-chimiques et action antibactérienne des miels d'abeilles africanisées *Apis mellifera* et de Méliponines du Brésil, *Apidologie* 22 (1991) 61–73.
- [7] COVENIN, Miel de Abejas. Métodos de Ensayo, Comisión Venezolana de Normas Industriales, COVENIN 2191–36, CT10 s/14, Caracas, 1984.
- [8] Louveaux J., Pourtaillier M.P., Vorwohl G., Méthodes d'analyses des miels, mesure de la conductivité du miel, *Bull. Tech. Apic.* XVI (1973) 1–3.
- [9] Louveaux J., Maurizio A., Vorwohl G., Methods of mellissopalynology, *Bee World* 59 (1978) 139–157.
- [10] Gilbert J., Shepherd M.J., Wallwork M.A., Harris R.G., Determination of the geographical origin of honeys by multivariate analysis of gas chromatographic data on their free aminoacid content, *J. Apic. Res.* 20 (1981) 125–135.
- [11] Gonnet M., Lavie P., Nogueira-Neto P., Étude de quelques caractéristiques des miels récoltés par certains Méliponinés brésiliens, *C.R. Acad. Sci. Paris* 258 (1964) 3107–3109.
- [12] Krauze A., Zalewski R.I., Classification of honeys by principal component analysis on the basis of chemical and physical parameters, *Z. Lebensm. Unters. Forsch.* 192 (1991) 19–23.
- [13] Nogueira Neto P., A Criação de Abelhas Indígenas sem Ferão (Meliponinae), Editora Chacaras e Quintais, São Paulo, 1953.
- [14] Persano Oddo L., Valutazione del miele. I mieli uniflorali, in: Persano Oddo L., Piana L., Sabatini A.G. (Eds.), *Conoscere il Miele. Guida all'Analisi Sensoriale*, Istituto Nazionale di Apicoltura, Edizioni Avenue Media, Bologna, 1995, pp. 133–139.
- [15] Persano Oddo L., Stefanini R., Piazza M.G., Accorti M., Diagnosis of unifloral honey. III. Application of a statistical approach to honey classification, *Apicoltura* 4 (1988) 27–38.

- [16] Persano Oddo L., Piazza M.G., Sabatini A.G., Accorti, M., Characterization of unifloral honeys, *Apidologie* 26 (1995) 453–465.
- [17] Phadke R. P., Studies on Indian honeys. 3. Proximate composition and physicochemical characterization of honeys from the wild honeybees *Apis dorsata*, *Apis florea* and *Trigona*, *Indian Bee J.* 30 (1968) 3–8.
- [18] Piazza M.G., Accorti M., Persano Oddo L., Electrical conductivity, ash, colour and specific rotatory power in Italian unifloral honeys, *Apicoltura* 7 (1991) 51–63.
- [19] Randerson P.F., Ordination, in: Fry J. (Ed.), *Biological Data Analysis, A Practical Approach*, Oxford University Press, Oxford, 1993, pp. 173–217.
- [20] Randerson P. F., Classification, in: Fry J. (Ed.), *Biological Data Analysis, A Practical Approach*, Oxford University Press, Oxford, 1993, pp. 173–217.
- [21] Sancho M.T., Muniategui S., Sánchez M.P., Huidobro J.F., Simal J., Relationships between electrical conductivity and total and sulphated ash contents in Basque honeys, *Apidologie* 22 (1991) 487–494.
- [22] Schwarz H., Stingless Bees (Meliponidae) from the Western Hemisphere, *Bull. Am. Mus. Nat. Hist.* 90 (1948) 1–546.
- [23] Siegenthaler U., Eine einfache und rasche Methode zur Bestimmung der (-Glucosidase (Saccharase) im Honig, *Mitt. Geb. Lebensm. Hyg.* 68 (1977) 251–258.
- [24] Stefanini R., Variability and cluster analysis of Italian honeys, *Apiacta* XIX (1984) 109–114.
- [25] Stefanini R., Diagnosis of unifloral honeys. II. Numerical taxonomy of honey and statistical diagnostic test of its botanical origin, *Apicoltura* 4 (1988) 13–26.
- [26] Tabachnick B.G., Fidell L.S., *Using Multivariate Statistics*, 2nd ed., Harper Collins Publishers, New York, 1989.
- [27] Vit P., Bogdanov S., Kilchenmann V., Composition of Venezuelan honeys from stingless bees (Apidae: Meliponinae) and *Apis mellifera* L., *Apidologie* 25 (1994) 278–288.
- [28] Vit P., Pulcini P., Diastase and invertase activities in Meliponini and Trigonini honeys from Venezuela, *J. Apic. Res.* 35 (1996) 57–62.
- [29] Vit P., Soler C., Tomás-Barberán F.A., Phenolic profiles of *Apis mellifera* and *Melipona* spp. honeys from Venezuela, *Z. Lebensm. Unters. Forsch.* 204 (1997) 43–47.
- [30] Vit P., Fernández-Maeso M.C., Ortiz-Valbuena A., Potential use of the three frequently occurring sugars in stingless bee honey, to predict the entomological origin, *J. Appl. Entomol.* 122 (1998) 5–8.
- [31] Vit P., Tomás-Barberán F.A., Flavonoids in Meliponinae honey from Venezuela, related to their botanical, geographical and entomological origin to assess their putative anticataract properties, *Z. Lebensm. Unters. Forsch.* 206 (1998) 288–293.
- [32] Zalewski R.I., Principal component analysis as a tool in organic chemistry and food chemistry, in: Zalewski R.I., Krygowski T.M., Shorter J. (Eds.), *Similarity Models in Organic Chemistry, Biochemistry and Related Fields*, Elsevier Science Publishers, Amsterdam, 1991.