Aroma profile and physico-chemical properties of artisanal honey from Tabasco, Mexico

Manuel Viuda-Martos,1 Yolanda Ruiz-Navajas,1 Juan M. Zaldivar-Cruz,2 Victor Kuri,3 Juana Fernández-López,1* Ángel A. Carbonell-Barrachina4 & José Á. Pérez-Álvarez1

1 IPOA Research Group (UMH-1 and REVIV-Generalitat Valenciana), AgroFood Technology Department, Escuela Politecnica Superior de Orihuela, Universidad Miguel Hernández, Ctra. Beniel km. 3,2. E-03312 Orihuela Alicante
2 Ciencia de Alimentos e Ingeniería, Colegio de Postgraduados Campus Tabasco, Carr. Cardenas-Huimanguillo, C.P. 86500, Cardenas, Tabasco, Mexico
3 Food, Health and Nutrition, School of Biomedical and Biological Sciences, PSQA410, University of Plymouth, Drake Circus, Plymouth PL4 8AA, United Kingdom
4 AgroFood Technology Department, Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández, Ctra. Beniel km. 3,2, E-03312 Orihuela, Alicante

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Summary The aim of this work was to characterise, physico-chemically, sugar composition and aroma profile of ten honey samples collected by small producers during two seasons and produced in different agricultural ecosystems from Tabasco State (Mexico). The mean values obtained for the physico-chemical parameters were: pH 3.63; 19.25% moisture; 78.8°Brix sugar; 0.64% ash; 0.25 mS cm⁻¹ electrical conductivity and 0.58 water activity. Based on colour parameters, Tabasco honeys can be placed in the group of dark honeys. In the analysed samples; fructose was the major sugar (39.45%), followed by glucose (35.74%) while sucrose only represented 2.93%. The volatile profiles of the ten honey samples were obtained by gas chromatography-mass spectrometry. All the honeys were characterised by their high contents of benzene and furan-related compounds. As a result, artisanal honeys from Tabasco (Mexico) can be considered to present a good level of quality.

Keywords Honey, physico-chemical parameters, volatile compounds.

Introduction Honey is produced in almost every country of the world and is widely used as a food. It is naturally a sweet substance that bees (Apis mellifera) produce by transforming the nectar of plants, from flowers secretions of plants (nectar honey) or sweet secretions from other part of the plant (honeydew honey) (Silva et al., 2009). Depending on the raw material used by the bees, honey may be classified as nectar, honeydew or mixed nectar-honeydew (Juszczak et al., 2009). Considering the number of possible floral sources, it is understandable that no honey is completely the same as another. The actual composition of honey varies, depending on many factors, such as the pollen source, climate, environmental conditions and the processing it undergoes (Viuda-Martos et al., 2008).

In general, honey is composed of at least 181 components and is basically a solution supersaturated in sugars, of which fructose (38%) and glucose (31%) are the most abundant. The moisture content is about 17.7%, total acidity 0.08% and ashes constitute 0.18% (Gheldof et al., 2002). In addition, honey contains a great variety of minor components, including phenolic acids and flavonoids, such as chrysin, fisetin, pinocembrin etc. (Viuda-Martos et al., 2008), vitamins, such as niacin, pyridoxine and ascorbic acid; enzymes, including invertase, phosphatases, glucose oxidase and catalase; lipids; carotenoids; organic acids, particularly gluconic acid, pyruvic acid, malic acid and citric acid; Maillard reaction products; amino acids; proteins and α-tocopherol (Blasa et al., 2006) all of which may provide an indication of a honey’s origin, variety, colour and flavour.

Honey cannot be considered a complete food by human nutritional standards, but it offers potential as a dietary supplement. In ancient times, honey was the only concentrated form of sugar available and, in addition it forms part of traditional medicine in many cultures (Gómez-Caravaca et al., 2006). Several aspects of its use indicate that it also functions as antibacterial, antioxidant, antitumoral, anti-inflammatory, inhibition of enzymatic browning and antiviral agent

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(Viuda-Martos et al., 2008). There are several reports on the use of physical and chemical parameters as well as data on aroma profile to characterise honey from different countries (Juszczak et al., 2009; Saxena et al., 2010). However, little information is available on Tabasco honeys. Honey production in Mexico has very long traditions going back to ancient times; however, its composition and functional properties have not been studied comprehensively. Mexico is the world’s fifth largest honey producer after China, Argentina, USA and Turkey, with an annual production of 57,000 tonnes, of which approximately 44% is exported. Mexico is the third largest exporter after only China and Argentina. The state of Tabasco in Mexico produces over 200 tonnes, and local artisan producers differentiate their products according to the collection area or type of farm where it is collected such as citrus, cacao, coconut, multifloral, etc. (Ruiz-Navajas et al., 2010).

The honey market currently shows a tendency to establish geographical limits of production with the aim of protecting a production zone that has developed and marketed a particular standard of quality (Baroni et al., 2009). Honey products from one region may attain a higher value than similar products from another area. However, the labelling of regional honey must be supported by analysis that confirms its provenance (Woodcock et al., 2007). Therefore, the aim of this work was to characterise, physico-chemically, the sugar composition and aroma profile of ten honey samples collected by small producers during two seasons from different agricultural ecosystems in Tabasco State (Mexico).

Materials and methods

Honey samples

For this study, ten honey samples from Tabasco (Mexico) were analysed. Samples of artisanal honey were collected in locations where the local agrifood production ecosystems and the season determine the flora available near the collection point. All the samples were collected in either the high season (February to April) or low season (December) a classification that coincides with the expected flowering patterns of plants. Most samples are considered to be multifloral, but some now are labelled. Collection location, description and collection times are indicated on Table 1.

Physico-chemical properties

The moisture and ash contents were determined by AOAC (1995) methods. The total soluble solids of the honey samples were measured by refractometry using a DR-101 refractometer (Cosecta S.A. Barcelona, Spain) and the results were expressed in °Brix. All the measurements were made at ambient temperature (25 °C).

The CIE LAB colour space was studied. The following colour coordinates were determined: lightness (L*), redness (a*, ± red-green) and yellowness (b*, ± yellow-blue). Colour determinations were made, means a Minolta CR-300 Colorimeter (Minolta Camera Co. Osaka, Japan), with illuminant D65 and 10° observer angle, equipped with an adapter for liquid samples CR-A70 (Minolta Camera Co. Osaka, Japan). For this, the samples were poured into low refractant glass vials. The water activity (aw) was measured at 25 °C using a Novasina TH200 electric hygrometer (Novasina; Axair Ltd, Pfäffikon, Switzerland). The pH values were measured by blending a 5-g sample with 50 mL deionised water for 2 min. Measuring the pH of the resultant suspension with a Crison pH meter (Model 507, Crison, Barcelona, Spain) equipped with a Crison combination electrode (Cat. nr 52, Crison, Barcelona, Spain). Electrical conductivity was measured in triplicate, after checking the cell constant, at 20 °C in 20% (w/w) aqueous solutions of honey, according to the method for honey reported by Bogdanov et al., (2000) using a Hi 8633 conductivity meter (Hanna Instruments, Eibar, Spain) equipped with a Hi 76301W conductivity probe (Hanna Instruments, Eibar, Spain).

Sugar composition

Five grams of honey was homogenised in 50 mL of distilled water and shaken vigorously for 5 min and then centrifuged at 14,463 g for 10 min at 4 °C. Two millilitres of the supernatant were filtered through a 0.45-μm Millipore filter (Millipore Corporation, Bedford, USA) and then 10 μL were injected into a Hewlett-Packard series 1100 HPLC according to the method of Doughtry (1995). The elution system consisted of 0.1% phosphoric acid running isocratically with a flow rate of 0.5 mL min⁻¹. The sugars were eluted through a Supelco column (Supelcogel C-610H, 30 cm 7.8 mm, Supelco

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Description</th>
<th>Collection season</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Cacao honey</td>
<td>HS, 2005</td>
<td>Finca Cholula, Comalcalco</td>
</tr>
<tr>
<td>B</td>
<td>Cacao honey</td>
<td>LS, 2005</td>
<td>Finca Cholula, Comalcalco</td>
</tr>
<tr>
<td>C</td>
<td>Coconut honey</td>
<td>LS, 2006</td>
<td>Comalcalco</td>
</tr>
<tr>
<td>D</td>
<td>Multifloral</td>
<td>LS, 2005</td>
<td>Ejido Victoria, Tenosique</td>
</tr>
<tr>
<td>E</td>
<td>Citrus</td>
<td>HS, 2006</td>
<td>Estacion Chontalpa, Huijumangullo</td>
</tr>
<tr>
<td>F</td>
<td>Multifloral</td>
<td>LS, 2005</td>
<td>Ejido Victoria, Tenosique</td>
</tr>
<tr>
<td>G</td>
<td>Multifloral</td>
<td>HS, 2005</td>
<td>Ejido Estapilla, Tenosique</td>
</tr>
<tr>
<td>H</td>
<td>Multifloral</td>
<td>HS, 2005</td>
<td>Ejido Esperanza, Tenosique</td>
</tr>
<tr>
<td>I</td>
<td>Cacao, Coconut</td>
<td>HS, 2006</td>
<td>Colpos, Cardenas</td>
</tr>
<tr>
<td>J</td>
<td>Multifloral</td>
<td>LS, 2005</td>
<td>Ejido Yajon Rio Seco, Tacotalpa</td>
</tr>
</tbody>
</table>
Honey is a very complex matrix endowed with very specific physico-chemical properties that make it unique from other viscous solutions. This complex matrix makes the analysis of honey difficult in terms of its different properties. Table 2 shows the physico-chemical properties of the ten Tabasco honey samples. Moisture is a physico-chemical parameter that is related to the climatic conditions and degree of maturity of honey.
Physico-chemical properties of Mexican honey M. Viuda-Martos et al. (Baroni et al., 2009). The honey samples analysed showed values for this parameter ranging between 18.90 and 24.30%. Except for samples E, G and H, all other tested Tabasco honeys had moisture contents higher that 20%, which is the maximum prescribed limit for the moisture content proposed by the Codex Alimentarius Commision standard for honey (Codex Alimentarius, 2001). The high moisture values obtained were probably because of the high rainfall levels recorded in the Tabasco region. The moisture content of honey is an important factor contributing to its stability. A higher moisture content could lead to undesirable honey fermentation during storage caused by the action of osmotolerant yeasts and resulting in the formation of ethyl alcohol and carbon dioxide (Saxena et al., 2010). The alcohol may break down to acetic acid and water, giving the honey a distinctly sour or ‘off’ taste and a runny texture with small bubbles, surface heaving or foaming (Sanford, 2009).

The ash content of samples analysed varied from 0.06 to 0.08% with no statistically significant differences \( P > 0.05 \) between the samples. The low ash content obtained was probably because of a variety of factors, such as differences in soil and atmospheric conditions as well as to the type and physiology of each plant. The values recorded fell within the range typical of natural nectar honeys but not of honeydew honeys, which have a higher ash content (Juszczak et al., 2009). The Codex Alimentarius Commision standard for honey (Codex Alimentarius Commission Standards, 2001) proposed an ash content of not more than 0.6% for normal honey. Certain nitrogen compounds, minerals, vitamins, pigments and aromatic substances contribute to the ash content of honey (Mairaj et al., 2008) which is considered a quality criterion indicating the possible botanical origin of honey.

The analysed samples presented Brix degrees ranging from 77.0 to 80.67, which are similar to those others honey samples of different origins (Silva et al., 2009; Saxena et al., 2010). Anomalous values of Brix degrees (directly related with sugar content) may be a reliable index of adulteration (Terrab et al., 2004).

Most colour values reported in the literature generally correspond to measurements taken on the Pfund scale (mm) (Al et al., 2009), while very few studies have used CIE LAB \( \text{L}^*, \text{a}^*, \text{b}^* \) to measure colour in nectar and honeydew honey (Terrab et al., 2004; Bertoncelj et al., 2007). Honey samples A and B had the highest values (33.00 and 37.68, respectively) for parameter \( \text{L}^* \) which indicates lightness. No statistically significant differences \( P > 0.05 \) were found between samples C, D, E, G, H, and J with values ranging from 26.90 to 27.67. Samples F and I showed the lowest values for lightness. González-Miret et al., (2005) classified honey samples into two groups from the point of view of lightness: light honeys with \( \text{L}^* > 50 \) and dark honeys with \( \text{L}^* < 50 \). Considering this classification, Tabasco honeys can be placed in the group of dark honeys. For the red-green coordinate, redness, \( \text{a}^* \) values varied from 0.42 to 3.32 (samples G and D, respectively). This coordinate is affected by pigment content and disposition. For the yellow-green coordinate, yellowness, \( \text{b}^* \) values ranged between 2.21 and 11.09 (samples F and B, respectively). All Tabasco honey samples showed positive values for this parameter \( \text{b}^* \).

The colour of honey is one of the factors determining its price in the world market, and also its acceptability by consumers. Honey can undergo darkening during shipping or storage, and parallel changes in its organoleptic properties have detrimental effects on its quality (Pereyra-Gonzales et al., 1999). Honey colour depends on the potential alkalinity and ash content, as well as on active antioxidant compounds, such as carotenoids and flavonoids (Baltrusaityte et al., 2007).

All the Tabasco honeys analysed were found to be acidic in character. Their pH values ranged between 3.25 and 3.97. In general, honey is acidic in nature irrespective of its geographical origin (Saxena et al., 2010). These low pH values would be due to the presence of organic acids, mainly gluconic acid, in equilibrium with their corresponding lactones or internal esters, and to inorganic ions, such as phosphate, sulphate and chloride (Terrab et al., 2004). The pH values of Portuguese and Argentinean honeys have been found to vary from 3.45 to 4.70 and 3.1 to 4.1, respectively (Baroni et al., 2009; Silva et al., 2009).

As regards electrical conductivity, the results obtained for the honey samples studied varied between 0.23 and 0.28 mS cm\(^{-1}\). A linear relationship is known to exist between the ash content and the electrical conductivity, which is expressed as \( C = 0.14 + 1.74 A \), where \( C \) is the electrical conductivity and \( A \) is the ash content (Bogdanov et al., 2000). The electrical conductivity of honey is closely related to the concentration of mineral salts, organic acids and proteins. This parameter shows great variability according to the floral origin and is important for differentiating honeys of different floral origins. Water activity in Tabasco honey samples ranged from 0.498 to 0.614. This parameter is the crucial factor that determines enzyme activity and growth as well as the survival of micro-organisms in honey (Abramovic et al., 2008). Since osmophilic yeasts are only able to grow above a water activity of 0.6, the honeys under investigation had to be regarded as safe with respect to fermentation, except samples A, D and J. The water activity is mainly determined by the presence of soluble chemical species, of which sugars represent the largest portion in honey. The water activity is largely determined by the presence of monosaccharides, while disaccharides have a smaller influence on water activity (Zamora et al., 2006).
Sugar composition of Tabasco honeys

Sugars represent the main components of any type of honey. Reducing sugars (invert sugar), mainly fructose and glucose, have been found to be the major constituents of honey (Küçük et al., 2007). The total glucose and fructose content of all the samples exceeded the 60 g per 100 g of honey required for natural honey, in accordance with the EC Directive (110/2001). The predominant sugar (Table 3) was fructose with values ranging between 37.65 and 43.96% (samples D and C, respectively), which agrees with the values reported by Al et al. (2009) for Rumanian honeys and Baroni et al. (2009) for Argentinean honeys.

Glucose is the other major sugar present in honey. In the Tabasco honeys glucose values varied from 34.95 to 40.17% (samples I and H respectively). The fructose/glucose ratio was calculated for all samples. This ratio gives information about the crystallisation state of honey: when the fructose/glucose ratio is 1.14 or less there is a tendency to crystallisation, while values over 1.58 are associated with no such tendency (Tosi et al., 2004). The result obtained for the Tabasco honeys analysed confirmed the fluid state of all honey samples although following this criterion, samples B, D, E, F, G and H present a high tendency to crystallise.

The sucrose content was low in all the honeys, ranging between 2.88 and 3.13 g per 100 g of honey (samples D and E, respectively). The mean percentage of sucrose in all the honeys was below the maximum allowable limit of 5% proposed by the Codex Alimentarius Commission standard for honey (Codex Alimentarius Commission Standards, 2001). A high sucrose concentration in honey usually reflects an early harvest because the sucrose has not been fully transformed into glucose and fructose by the action of invertase (Küçük et al., 2007).

Table 3 Sugars content of analysed honeys

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fructose (g)</th>
<th>Glucose (g)</th>
<th>F/G</th>
<th>Sucrose (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>42.22 ± 0.05a</td>
<td>35.45 ± 1.33a</td>
<td>1.19</td>
<td>2.99 ± 0.07ab</td>
</tr>
<tr>
<td>B</td>
<td>43.54 ± 0.24b</td>
<td>40.02 ± 0.38b</td>
<td>1.09</td>
<td>2.95 ± 0.03a</td>
</tr>
<tr>
<td>C</td>
<td>43.98 ± 0.03c</td>
<td>37.87 ± 0.12c</td>
<td>1.16</td>
<td>2.94 ± 0.03a</td>
</tr>
<tr>
<td>D</td>
<td>37.65 ± 0.06d</td>
<td>35.83 ± 0.04*</td>
<td>1.05</td>
<td>2.88 ± 0.02d</td>
</tr>
<tr>
<td>E</td>
<td>38.16 ± 0.14e</td>
<td>35.29 ± 0.05*</td>
<td>1.08</td>
<td>3.13 ± 0.02c</td>
</tr>
<tr>
<td>F</td>
<td>42.60 ± 0.06f</td>
<td>38.98 ± 0.15d</td>
<td>1.09</td>
<td>2.96 ± 0.02a</td>
</tr>
<tr>
<td>G</td>
<td>39.51 ± 0.10g</td>
<td>36.96 ± 0.09*</td>
<td>1.07</td>
<td>2.95 ± 0.03a</td>
</tr>
<tr>
<td>H</td>
<td>41.49 ± 0.05h</td>
<td>40.17 ± 0.10b</td>
<td>1.03</td>
<td>3.01 ± 0.01h</td>
</tr>
<tr>
<td>I</td>
<td>40.70 ± 0.04i</td>
<td>34.95 ± 0.09</td>
<td>1.15</td>
<td>2.84 ± 0.02d</td>
</tr>
<tr>
<td>J</td>
<td>40.07 ± 0.11j</td>
<td>36.79 ± 0.08</td>
<td>1.16</td>
<td>2.94 ± 0.02j</td>
</tr>
</tbody>
</table>

Values followed by the same letter within the same column are not significantly different (P > 0.05) according to Tukey’s Multiple Range Test.

F/G, fructose/glucose ratio.

Volatile composition of Tabasco honeys

It is well established that the aroma of bee honey is highly dependent on the volatile fraction composition, which, in turn, depends on nectar composition and floral origin (Cuevas-Glory et al., 2007). Before starting to discuss the volatile composition of the studied samples, it is important to recall that the volatile compounds were quantified using simultaneous distillation–extraction (SDE). The main drawback of this technique is the formation of artefacts, especially hydrolysis reactions which may lead to sugar degradation products, such as furfural. This compound was present in all the samples analysed.

When the volatile fraction of the ten types of Tabasco honey was analysed, a total of 36 compounds were identified: 23 in A, B and D, 21 in G, 20 in J, 19 in H, 15 in I, 16 in F, 12 in E and finally 9 in C. Table 4 shows the relative areas for the mean values of the identified compounds. Most of them had been previously identified in honey. Of the thirty-six compounds identified, five were found in all the samples analysed, although in considerably different concentrations. These five compounds were 2,5,5-trimethyl-2-hexane, furfural, 2,2,4-trimethyl-3-penten-1-ol, 3-ethyl-1-octene and 2,3,4-trimethyl-2-pentene. Some other components were also common to several honey samples. Thus, 1-ethyl-3-methyl-cyclopentane, 3,5,5-trimethyl-2-hexane, 2,2-dimethyl-3-decene and dihydro tagetone were identified in eight honey samples; 4-penten-2-ol and linalool oxide in seven honey samples; pentenal, 3,5,5-trimethyl-1-hexane, 4,5-dimethyl-1-hexene, benzenacetaldehyde and perclene in six honey samples. The other volatile compounds were found in fewer honey samples.

In honeys A and B, 23 compounds were identified, the major constituents being benzenthanol (29.94 and 12.54%) and 4-cyclopenotenol (12.80 and 14.38%). In this sample, two norisoprenoids, such as isophorone (3,5,5-trimethyl-2-cyclohexene-1-one) and ketoisophorone (2,2,6-trimethyl-2-cyclohexen-1,4-diene) were present. These compounds have been suggested as markers for eucalyptus or heather honeys (Castro-Vázquez et al., 2007) although they are quite common in several types of honey.

In the D and E honey samples, the main compounds identified were 4-cyclopenotenol (15.78 and 21.60%, respectively) and 2,5,5-trimethyl-2-hexane (12.36 and 17.31%, respectively). Sample D contained three of the five terpenes identified in the samples as a whole: epoxifinalool (6.96%), trans-linalool oxide (0.95%) and linalool oxide (2.18%). Sample E contained a high concentration (9.89%) of linalool oxide, which is to be expected E since the sample corresponded to citrus honey. However, it is important to note the lack of some marker compounds of citrus honey, such as β-sinesal or anthranilic acid methyl ester (a compound specific to this variety of honey and considered a reliable marker)
as reported by Castro-Vázquez *et al.* (2006) and Escriche *et al.* (2009). The fact that none of these compounds were found in this sample is could be due to the different methods of extraction and analysis or the fact that E honey sample may be multifloral and not specifically a citrus honey. GC-MS analyses of the F honey sample identified 16 constituents, the main components being 6-methyl-1-heptanol (18.83) and 2,5,5-trimethyl-2-hexane.
 Analysed samples (except samples A, D and J for water activity and samples E, G and H for moisture) comply with the European honey directive for all the parameters, indicating adequate processing, good maturity and freshness.

Acknowledgment

Thanks are due to Dr. Cesar Vázquez-Navarrete (Colegio de Posgraduados, Cardenas, Tabasco) for facilitating collection of the honey samples by establishing links with honey and cocoa producers.

References


Conclusions

Artisanal honeys from Tabasco (Mexico) can be considered to present a good level of quality. All the analysed samples (except samples A, D and J) for water


