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Effect of country origin on physicochemical, sugar and volatile composition of acacia, sunflower and tilia honeys

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ABSTRACT

The aim of this study was to evaluate the influence of country (Spain, Romania, and Czech Republic) and botanical origin, on the physicochemical (HMF, diastase activity, moisture content, electrical conductivity), color (Pfund scale and CIEL*a*b*), principal sugars (glucose, fructose and sucrose) and volatile composition of acacia, sunflower and tilia honeys. PCA analyses considering these variables showed that honey type had a far greater influence on the differentiation of samples (above all due to the presence of certain volatile compounds such as carvacrol and α-terpinene for tilia honey; α-pinene and 3-methyl-2-butanol for sunflower honey, and cis-linalool oxide for acacia honey) than geographical origin. Discriminant models obtained for each kind of botanical honey (classified 100% for acacia and tilia honeys and 93.8% for sunflower of the cross-validated cases) confirmed that differentiation of honeys according to their country was mainly based on volatile compounds (for instance: 2-methyl-2-butenal and 2-methyl-2-propanol, for acacia honeys; 1-hexanol and α-pinene, for sunflower honeys and 3-methyl-1-butanol and otrienol, for tilia honey) and to a lesser extent on certain physicochemical parameters such as diastase, sucrose and conductivity, respectively. Correct classification of all samples was achieved with the exception of 10% of the sunflower honeys from the Czech Republic. The results suggest that the presented models are potentially useful tools for the classification of acacia, sunflower and tilia honeys according to the country of origin.

1. Introduction

Consumers appreciate the possibility to choose between different unifloral honeys as they have specific organoleptic characteristics and different attributable therapeutic properties. Since these unifloral honeys are part of the import–export market, they offer beekeepers and the industry the opportunity to obtain higher prices in comparison to those without a determined botanical origin. Physicochemical properties and color are taken into account when the market price of honey is fixed, and they can be measured to classify and typify the raw batches before entering the packaging process. Specifically, color is one of the most valuable attributes since it is considered to represent the preferred honey flavor, and therefore directly contributes to consumer acceptability (Visquert, Vargas, & Escriche, in press).

The physicochemical properties of honeys with the same floral source can vary to some extent as a consequence of different climatic conditions or different geographical origins (Anklam, 1998). The use of botanical appellation of honey together with geographical origin is becoming a good option to protect and promote this traditional food in different countries.

Melissopalynological characterization is commonly used for the classification of honey according to its uniflorality, and sometimes its geographical origin. However, in some cases the percentage of pollen is not always decisive because the production of pollen and nectar by flowers is not always simultaneous, varying between countries and even within the same country according to the geographical area (Feás, Pires, Estevinho, Iglesias, & Pinto de Araujo, 2010; Feás, Pires, Iglesias, & Estevinho, 2010). For this reason, in addition to the quantification of pollen, the combination of multi-component analysis and chemometric techniques is now the most efficient approach to guarantee the authentication of honey (Anklam, 1998; Ruoff et al., 2007; Terrab, Gustavo-González, Díez, & Heredia, 2003; Kropf et al., 2010). Among these procedures, physicochemical (electrical conductivity, diastase activity, moisture, etc.), color and chemical analyses (such as sugars, among others) have been widely used in the characterization of unifloral honeys (Escriche, Kadar, Juan-Borrás, & Domenech, 2011; Oroian, Amariei, Escriche, & Gutt, 2013; Persano-Oddo & Bogdanov, 2004; Ruoff et al., 2007).

However, the discriminative power of the physicochemical properties and color varies according to the botanical origin, and the geographical and climatic conditions as a consequence of their influence on the flowering or secretions of plants. For this reason, as suggested by Persano-Oddo and Bogdanov (2004), the broader the analytical scope considered, the more accurate the classification of a specific honey.

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Hence, considering that the flavor and aroma of honey are directly related to its volatile compounds, it is reasonable to consider that volatile fraction analysis could be of great importance to reach a better understanding of the intrinsic characteristics of honey (Aliferis, Ttarantilis, Harizanis, & Alissandrakis, 2010; Cuevas-Glory, Pino, Santiago, & Sauri-Duch, 2007). The importance of this analytical determination on its own or as a complement to the information provided by other methodologies is reflected in different studies published in the last decade (Radovic et al., 2001; Serra Bonvehí & Ventura-Coll, 2003).

There are many works focused on the characterization of honey from different botanical or geographical origins. However, to our knowledge there are no publications about the combined use of physicochemical, sugar and volatile composition for this purpose, or the comparison of specific unifloral honeys (with the same botanical origin), from different countries. For this reason, the aim of this study was to determine the influence of the country (Spain, Romania, and Czech Republic) on the physicochemical, sugar and volatile composition of acacia, sunflower and tilia honeys.

2. Materials and methods

2.1. Honey samples and their classification

A total of 80 raw unifloral honey samples (collected from beekeepers in 2011) with different botanical origins: acacia (*Robinia pseudoacacia*), sunflower (*Helianthus annuus*) and tilia or lime (*Tilia* sp.), and from different countries (Spain, Romania, and the Czech Republic) were analyzed. The acacia and sunflower honeys came from the three countries mentioned above, whereas tilia honey was only from Romania and the Czech Republic since it is practically inexistent in Spain. In summary, of the 80 raw samples, 30 came from Romania (10 acacia, 10 sunflower and 10 tilia, all of them from the Transylvanian region); another 30 came from the Czech Republic (10 acacia, 10 sunflower and 10 tilia, all of them from the Central Bohemian region) and 20 from Spain (10 acacia from northern Spain and 10 sunflower from central Spain).

In order to guarantee the botanical origin of the samples, the percentage of pollen was measured for each one, following the recommendations of the International Commission for Bee Botany (Von Der Ohe, Persano, Piana, Morlot, & Martin, 2004). A light microscope (Zeiss Axio Imager, Göttingen, Germany) at a magnification power of ×400 with DpxView LE image analysis software attached to a DeltaPix digital camera was used in this analysis. According to this analysis, a honey was considered to be from acacia trees if the pollen from *R. pseudoacacia* L. was not lower than 45%; from sunflower, if the pollen from *H. annuus* L. was not lower than 60% and from tilia trees if the pollen from *Tilia* spp. was not lower than 45% (Gómez-Pajuelo, 2004; Persano-Oddo & Piro, 2004; Sainz-Laín & Gómez-Ferreras, 2000; Von Der Ohe et al., 2004). Samples were classified on arrival at the laboratory and were preserved at 12 °C until they were analyzed. None of the samples exhibited signs of fermentation or granulation before initiating the analyses.

2.2. Physicochemical and color analyses

Diastase activity (*Phadebas method*), 5-hydroxymethylfurfural content "HMF" (*White method*), electrical conductivity (by *conductimetry*), and moisture content (by *refractometry*) were analyzed in accordance with the Harmonized Methods of the European Honey Commission (Bogdanov, 2002). Color was determined using a millimeter Pfund scale C 221 Honey Color Analyzer (Hanna Instruments) and a spectrocolorimeter Minolta CM-3600d (Osaka, Japan). Translucency was determined by applying the Kubelka–Munk theory for multiple scattering of the reflection spectra (Hutchings, 1999). Color coordinates were obtained from R∞, between 400 and 700 nm for D65 illuminant and 2° observer. All tests were performed in triplicate.

Chromatic parameters, chroma (Eq. (1)) and hue (Eq. (2)), were calculated from L*, a* and b* coordinates.

$$C^*_{ab} = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h^*_{ab} = \operatorname{arctg}\frac{b^*}{a^*}. \quad (2)$$

2.3. Sugar determination

Sugar (fructose, glucose and sucrose) analysis was carried out as described by Bogdanov, Martin, and Lüllman (1997). Separation of carbohydrates took place in a HPAEC-PAD high-resolution ionic chromatograph with a pulsed amperometric detector (PAD) (Bioscan, Methrom, Switzerland) and a Metrosep Carb chromatographic column (styrene divinylbenzene copolymer, 4.6 × 250 mm). Carbohydrates were eluted with NaOH 0.1N at a flow rate of 1 mL min^{-1}. Quantification of sugars was carried out using external standards. The corresponding calibration curves were constructed covering the values of the three sugars which were expected to be found in the honey samples. For fructose, glucose and sucrose, respectively, the correlation coefficients (R^2) were: 0.995, 0.996 and 0.996; the LODs (limits of detection) were: 0.01 g/100 g, 0.01 g/100 g and 0.05 g/100 g and the LOQs (limits of quantification) were: 0.05 g/100 g, 0.05 g/100 g and 0.1 g/100 g.

All analyses were carried out in triplicate.

2.4. Volatile compound analysis

2.4.1. Extraction

Volatile compounds were extracted by purge and trap at 45 °C for 20 min and trapped in a glass tube packed with Tenax TA (20–35 mesh), bubbling purified nitrogen (100 mL min^{-1}) through the sample (Escriche et al., 2011). Next, the compounds were thermally desorbed at 220 °C for 10 min (at 10 mL min^{-1} helium flow) (TurboMatrix TD, Perkin ElmerTM, CT-USA), then cryofocused in a cold trap at −30 °C and transferred onto the capillary column by heating the cold trap to 250 °C (at a rate of 99 °C/s).

2.4.2. GC–MS analysis

A GC–MS (Finnigan TRACETM MS, TermoQuest, Austin, USA) with a DB-WAX capillary column (SGE, Australia) (60 m length, 0.32 mm i.d., 1.0 μm film thickness) was used to separate the volatile compounds. The carrier gas was helium at a flow rate of 1 mL min^{-1}. The temperature programme was: from 40 °C (2-minute hold time) to 190 °C at 4 °C min^{-1} (11-minute hold time) and finally to 220 °C at 8 °C min^{-1} (8-minute hold time). Electron impact mass spectra were recorded in impact ionization mode at 70 eV, with a mass range of m/z 33–433. A total of 3 extracts were obtained for each sample.

2-Pentanol was used as an internal standard. The identification of isolated volatile compounds was performed by comparing their mass spectra, retention times and linear retention indices against those obtained from authentic standards: acetic acid (ethanoic acid); nonanal; decanal; benzaldehyde; 6-methyl-5-hepten-2-one (6-methyl-hept-5-en-2-one); 2-methyl-3-buten-2-ol (Sigma-Aldrich, San Louis, Missouri and Acros Organics, Geel, Belgium); 2-methyl-1-propanol (2-methylpropan-1-ol); 3-methyl-3-buten-1-ol; octane; 3-hydroxy-2-butanone (3-hydroxybutan-2-one); 2-furanmethanol (furan-2-ylmethanol); furfural (furan-2-carbaldehyde); dimethyl sulfide; β-linalool (3,7-dimethylocta-1,6-dien-3-ol) (Fluka Buchs, Schwiez, Switzerland). The compounds for which it was not possible to find authentic standards were tentatively identified by comparing their mass spectra (m/z values of the most important ions) with spectral data from the National Institute of Standards and Technology 2002 library as well as retention indices and spectral data published in the literature