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Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon

Olga Escuredo [a,*], Irina Dobre [b], María Fernández-González [a], M. Carmen Seijo [a]

[a] Department of Vegetal Biology and Soil Sciences, University of Vigo, Faculty of Sciences, Ourense, Spain
[b] Department of Biochemistry, University Dunarea de Jos, Faculty of Food Science and Engineering, Galati, Romania

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ABSTRACT

The present work provides information regarding the statistical relationships among the palynological characteristics, sugars (fructose, glucose, sucrose, melezitose and maltose), moisture content and sugar ratios (F + G, F/G and G/W) of 136 different honey types (including bramble, chestnut, eucalyptus, heather, acacia, lime, rape, sunflower and honeydew). Results of the statistical analyses (multiple comparison Bonferroni test, Spearman rank correlations and principal components) revealed the valuable significance of the botanical origin on the sugar ratios (F + G, F/G and G/W). *Brassica napus* and *Helianthus annuus* pollen were the variables situated near F + G and G/W ratio, while *Castanea sativa*, *Rubus* and *Eucalyptus* pollen were located further away, as shown in the principal component analysis. The F/G ratio of sunflower, rape and lime honeys were lower than those found for the chestnut, eucalyptus, heather, acacia and honeydew honeys (>1.4). A lower value F/G ratio and lower water content were related with a faster crystallization in the honey.

1. Introduction

Crystallization, a natural process that occurs in honey, is gaining increased interest from the scientific community, while for consumers this can be sometimes a controversial phenomenon. Honey is a natural product elaborated by *Apis mellifera*, its composition varies mostly with the floral and/or honeydew sources utilised by honeybees, as well as with the geographical origin and climatic conditions (Dobre, Georgescu, Alexe, Escuredo, & Seijo, 2012; Escuredo, Míguez, Fernández-González, & Seijo, 2013).

Essentially, honey contains a concentrated water solution of two main sugars: fructose and glucose, with small amounts of various complex sugars. Generally, fructose is the dominant component and only in very few honey types, such as rape (*Brassica napus*) and dandelion (*Taraxacum officinale*), the glucose fraction could be greater than the fructose (Persano-Oddo & Piro, 2004). Many other substances, such as acids, protein, minerals, pigments, flavour and aroma substances, sugar alcohols, colloids, phytochemicals and vitamins also occur in honey (Zamora & Chirife, 2006).

Honey sugars have been intensively analysed by various researchers over time (Bentabol, Hernandez, Rodriguez, & Rodriguez, 2011; Dobre et al., 2012; Escuredo et al., 2013; Kaskoniene, Venskutonis, & Ceksterytè, 2010; Ouchemoukh, Schweitzer, Bachir, & Djoudad-Kadji, 2010; Persano-Oddo & Piro, 2004; Was, Rybak-Chmielewska, Szczesna, Kachaniuk, & Teper, 2011a, 2011b), and some of them have emphasised the influence of sugars and water on crystallization (Gleiter, Horn, & Isengard, 2006; Laos, Kirs, Pall, & Martverk, 2011; Manikis & Thrasivoulou, 2001; Marghitas et al., 2009; Zamora & Chirife, 2006).

Some previously published works have evaluated the crystallization tendency of honey (Laos et al., 2011; Manikis & Thrasivoulou, 2001; Venir, Spaziani, & Maltini, 2010; Zamora & Chirife, 2006). Other works have provided information in terms of crystallization being focused on the ratios formed by the main sugars with the moisture content of different honey types (Dobre et al., 2012; Marghitas et al., 2009; Ouchemoukh et al., 2010; Persano-Oddo & Piro, 2004).

The time required for honey to crystallize depends mostly on the ratio of fructose to glucose (F/G) (Gleiter et al., 2006; Laos et al., 2011). Honey samples, which do not crystallize for a long time have F/G ratio greater than 1.33, and if the ratio is less than 1.11, the honey crystallizes quickly (Smanalieva & Senge, 2009). The rate at which glucose crystallization occurs in honey also depends on the glucose/water ratio (G/W). Manikis and Thrasivoulou (2001) and Dobre et al. (2012) indicated a slow crystallization of honey when G/W ratio was less than 1.7, and when the ratio was greater than 2.0 the phenomenon was fast and complete.

In spite of all this available information through the scientific community, in order to have an exhaustive comprehension regarding crystallization, further investigation is required. Nevertheless,

* Corresponding author. Tel.: +34 988387048; fax: +34 988387001.
E-mail address: oescuredo@uvigo.es (O. Escuredo).

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statistical treatment, including sugar ratios, could make it possible to examine of this tendency in honey. The main goal of the present work was to assess the influence of the most important factors (pollen and sugar composition) on the occurrence of crystallization in various types of honey. Therefore, the sugar profiles, pollen composition, moisture content and also the ratios between the major sugars were quantified, calculated and using advanced statistical techniques the crystallization tendency was examined.

2. Materials and methods

2.1. Honey samples

The present study was carried out on 136 honeys collected directly from beekeepers during the years 2008, 2009 and 2010. The geographical origins of the samples are the Northwest of Spain and different regions from Romania. A total of nine unifloral honeys were evaluated: bramble, chestnut, eucalyptus, heather, acacia, honeydew, lime, rape and sunflower honeys. The botanical origins of the samples were established by palynological analyses.

2.2. Melissopalynological analysis

Microscopical analysis was carried out according to the method described by Louveaux, Maurizio, and Vorwohl (1978). 10 g of honey was dissolved in bi-distilled water and centrifuged at 4500 rpm (3373*g*) for 10 min. The obtained sediment (after eliminating the supernatant) was then re-dissolved and centrifuged for a further 10 min. The final volume of the sediment was used to prepare the slide. The pollen spectra were determined by counting and identifying a minimum of 800 pollen grains using a Nikon Optiphot II microscope (400× and 1000×, when needed). The results of the identified pollen types were expressed as percentages.

2.3. Moisture content

The moisture content was determined using a refractometer (ABBE 325, Auxilab, Navarra, Spain) at 20 °C. The samples were homogenised at room temperature and were directly deposited in the prism of the refractometer. The obtained refractive index in each sample was related to the water content of honey. The results of the measurement were expressed as a percentage.

2.4. Sugar composition by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

Using an ion Dionex ICS-3000 chromatography system (Sunnyvale, California, EEUU) incorporating an analytical column, guard column and a pulse amperometric detector (PAD) all the data for the identification and quantification of sugars in honey were provided. The separation of sugars from 10 μl of honey dissolved in water until a concentration of 10 mg/l was performed with a CarboPac PA1 column (3 × 250 mm). A pulsed amperometric detector was used to detect the sugars, with a gradient of two mobile phases (A and B). Phase A was ultrapure water, while phase B was 200 mM NaOH (HPLC grade, Merck). The sugar content of the honey samples was calculated using calibration curves of standard solutions for each pure sugar (Sigma–Aldrich). The identified sugars by this method were: fructose, glucose, sucrose, melezitose and maltose. The concentration of the standard solution of glucose and fructose was 25 mg/ml and for sucrose, melezitose and maltose of 0.2 mg/ml.

The acquisition of all the chromatograms was performed with CHROMELEON Chromatography Management System.

2.5. Statistical analyses

The statistical analyses were performed with both SPSS Statistic 19.0 and STATGRAPHICS Centurion 16.0 software for Windows.

In order to compare the quantified sugars and their rations according to each honey type an analysis of variance (ANOVA) using the Bonferroni test was carried out. On the other hand, the relationships between the sugars and pollen variables were investigated by Spearman's rank correlation coefficient in bivariate linear correlations. The significance was calculated for $P < 0.05$.

Multivariate statistical treatments as Principal Component Analysis (PCA) were carried out due to their efficiency of providing accurate graphical representations, which best integrate all the significant data. Also, they enabled us to represent objects or variables on a graph in order to study the proximity of the objects.

3. Results and discussion

3.1. Melissopalynological characteristics

The samples were classified according to their botanical origins in nine unifloral honeys: bramble, chestnut, eucalyptus, heather, acacia, lime, rape, sunflower and honeydew. The main pollen types identified by the melissopalynological analysis and the percentages of the principal pollen for each honey type are summarised in Table 1.

The pollen spectra of the honeys showed the principal plants for honey production in the regions studied. In the case of Northwest Spain, these were *Castanea sativa*, *Rubus*, *Eucalyptus*, *Cytisus* and *Erica*. In the Romanian honeys, *B. napus*, *Tilia*, *Helianthus annuus* and *Robinia pseudoacacia* were the best represented pollens.

In Europe more than 100 botanical species are known to produce unifloral honey (Persano-Oddo & Piro, 2004). Bramble honeys are very valuable in Northwest Spain, the principal pollen type being *Rubus*, which had a mean value in this group of 60% ranging between 43.3% and 91.3%. These honeys are produced from different bramble species that grow mainly in the Northern hemisphere and Central Europe. Progressive decline in agricultural activity had led to large areas of previously arable land that later became overgrown with shrubs, of which *Rubus* is the predominant taxa (Escuredo, Seijo, & Fernández-González, 2011; Seijo & Jato, 1998).

One of the main unifloral honeys in the Atlantic and Mediterranean coastal areas is *Eucalyptus* honey, while chestnut honey is abundant in mountainous areas. In the present study, *Eucalyptus* pollen had a mean value of 77.4% within this group, with a maximum value of 94.8% and a minimum value of 60.5% in the pollen spectra. Similar values were found for the *C. sativa* pollen in chestnut honeys with a mean value of 78.0%, and a range between 64.1% and 90.2%. Both pollen types are considered to be overrepresented in honeys (Persano-Oddo & Piro, 2004; Yang, Battesti, & Djabou et al., 2012).

In heather honeys the presence of *Erica* could be considered as an underrepresented pollen grain. This pollen rarely reached a percentage higher than 45% in the pollen spectra, due to its morphology and its dispersion in tetrads. The mean value of *Erica* pollen in honeys was 36.7%, ranging between 23.1% and 49.4%. These results are in accordance with the common representation of *Erica* pollen in heather honeys (Louveaux et al., 1978; Von Der Ohe, Persano-Oddo, Piana, Morlot, & Martin, 2004; Yang, Battesti, & Paolini, et al., 2012). Other underrepresented pollen in honeys was *R. pseudoacacia*, which was present in a low or very low percentage (Atanassova, Yurukova, & Lazarova, 2012; Dobre, Alexe, Escuredo, & Seijo, 2013; Persano-Oddo & Piro, 2004). In the studied acacia samples, the mean value of this pollen type was 17.2% (ranging between 5% and 57.9%). For lime honeys, *Tilia* had a mean percentage