Differentiation of *Acacia*, *Sunflower* and *Tilia* Honeys from Different Countries Based on Sugar Composition, Physicochemical and Color Parameters

Melinda KÁDÁR(1), Marisol JUAN-BORRÁS(1), Magdalenka HELLEBRANDOVA(2), Eva DOMÉNECH(1), Isabel ESCRICHE(1)

1) Instituto de Ingeniería de Alimentos para el Desarrollo (IUIAD), Universidad Politécnica de Valencia, Spain.
2) Faculty of Agrobiology, Food and Natural Resources, Department of Quality of Agricultural Products. Czech University of Life Sciences Prague. Czech Republic, e-mail: iescrich@tal.upv.es

Abstract. The purpose of this work was to characterize three different types of honey (sunflower, tilia, and acacia) commercialized in Spain, Romania, and Czech Republic from the point of view of their values on physical-chemical (HMF, diastase activity, moisture, electrical conductivity), sugar composition and colour parameters. A multivariate analysis made it possible to differentiate these three types of honey according to the parameters analysed. However, it was found that the country had less influence in its differentiation than the variety of honey.

Keywords: honey, physicochemical parameters, sugars.

INTRODUCTION

Characterisation of unifloral commercial honeys is a priority research objective for the European apicultural industry. Appearance plays a key role in the consumer’s choice and acceptance of honey. Sugar composition and moisture of honey are two essential factors in its crystallization (Cavia *et al.*, 2002). Colour, besides playing an important role in the consumers perception, it is also useful in the classification of unifloral honeys, varying from white tones, to amber tones.

Among other physicochemical parameters, electrical conductivity is one of the most useful for the classification of unifloral honeys, since it can be determined using relatively inexpensive instrumentation (Persano-Oddo and Piro, 2004).

Water content and HMF are two important quality parameters. Unifloral honeys show some typical difference in water content, depending on the production season, the climate and the beekeeping practices. Moisture affects the physical properties of honey (viscosity, crystallisation) and may be the main cause of its fermentation. The shelf life of honey is established worldwide according to its HMF content as it is set down by national regulations.

HMF content is practically absent in freshly collected honey, however, it increases during beehive handling, extraction, conditioning and storage operations (Bogdanov, *et al.*, 2002; Tosi *et al.*, 2002)

The physicochemical analytical methods used for honey classification are basically the same as for the routine honey control. They were validated and harmonised by the International Honey Commission (Bogdanov *et al.*, 1997) and can be used within the scope of the Codex Alimentarius Honey Standard (Codex Alimentarius, 2001) and the European Union Honey Directive (European Commission, 2002).
The aim of this work was to contribute to the characterization of three different types of honey (sunflower, tilia, and acacia) commercialized in three European countries (Spain, Romania, and Czech Republic) with the purpose of comparing their different physical-chemical (HMF, diastase activity, moisture, electrical conductivity), sugar composition and colour parameters, with those established in the European Commission Council Directive relating to honey.

MATERIALS AND METHODS

Honey Samples
Samples were collected in 2009 in three different European countries (Spain, Romania, and Czech Republic; in every country 5 batches from acacia (Robinia pseudoacacia), 5 from sunflower (Helianthus annuus) and 5 from tilia (Tilia sp).

Physicochemical analysis
The Harmonized Methods of the European Honey Commission (Bogdanov, 2002) were used in order to analyse 5-hydroxymethylfurfural content (HMF), diastase activity, electrical conductivity, and moisture content.

HMF content was determined by spectrophotometry (Thermo model UV1, Cambridge, UK). Each of the honey samples was divided into 2 clarified aliquots; water was added to one of the aliquots and absorption was read at $\lambda=284$ and 336 nm. This was compared to a second solution in which this absorption was eliminated by the addition of sodium bisulphate. Results were expressed in milligrams of HMF per kilogram of honey.

Diastase activity was analyzed using a buffered solution of soluble starch and honey, which was incubated in a thermostatic bath until the endpoint was determined photometrically at 660 nm.

Electrical conductivity was measured at 20°C in a 20% (w/v) honey solution (dry matter basis) in deionised water, and measurements were taken using a conductimeter (Crison Instrument, model C830, Barcelona, Spain).

Colour
Colour was determined using a millimetre Pfund scale (Fell, 1978) and a spectrocolorimeter Minolta CM-3600d (Osaka, Japan). Coordinates CIE L* a* b* were obtained from $R\infty$ between 400 and 700 nm for D65 illuminant and 2° observer (Hutchings, 1999).

Sugars
Sugar determination was carried out using the method of (HPAEC-PAD) high-resolution ionic chromatography, (817 Bioscan, of the Metrohm Company) fitted with a pulsed amperometric detector (PAD). The system was equipped with a CarboPac PA1 column (polyvinylbenzene column) suited for mono-, di-, tri-, and oligosaccharides analysis. All analysis were performed in triplicate.

Statistical analysis
The data of physicochemical, sugars and colour were analysed by analysis of variance (ANOVA) (significance level $\alpha=0.05$) and by least significant difference (LSD) test using Statgraphics Plus 5.1. Principal component analysis was performed (Unscrambler version 9.7; CAMO Process AS, Oslo, Norway) on the means of the data obtained in the samples.
RESULTS AND DISCUSSION

Figure 1 shows the average values and standard deviation obtained for the different physicochemical parameters evaluated: diastase activity, HMF, moisture, conductivity, colour Pfund and colour CIEL*a*b*. These values were compared with the limits established for honey in the international regulatory standards.

It is well known that HMF (hydroximethylfurfural) does not depend on the type of honey. On the contrary it is considered a good indicator of quality since warming or natural ageing causes a progressive increment in its level (Escriche, Visquert, Carot, Doménech & Fito, 2008). In this study, all samples comply with the internationally recognized maximum admitted value for this parameter of 40 mg/kg (European Commission, 2002).

Diastase, one of the most important enzymes in honey, is not only related to the quality but also to the floral origin of honey (White & Bryant, 1996). Diastase, like HMF,
increases with thermal treatments and time of storage. However, diastase as opposed to HMF, shows different values related to the botanical origin of honey (Escriche et al., 2009). In this study samples ranged from 9.39º to 19.01ºGothe for the acacia honey. Therefore, for this parameter all samples comply with legal requirements in accordance with the EU (Council Directive relating to honey, 2001).

In order to avoid fermentation during storage and subsequent commercialization of honey it is important to maintain its moisture content below 20g/100g (Council Directive relating to honey, 2001). In this work moisture values were adequate, ranging from 14.95 for the sunflower honey to 17.45 g/100g for the tilia honey.

Conductivity, related to mineral salt content, organic acids, proteins and poliols, is a stable value for the same variety of honey, within a range of variability that remains practically invariable with treatments or storage (Krauze & Krauze, 1991). As expected the tilia honey had the highest levels of conductivity 0.80 mS/cm, and acacia honey had the lowest levels 0.18 mS/cm.

With regard to colour, measured on the Pfund scale, values varied between 4 mm for the acacia honey and 67mm for the sunflower honey.

CIEL*a*b* colour is not regulated, however this parameter was measured in order to supplement the information that the Pfund scale provides. To characterize the honey samples by colour, they were plotted in their corresponding positions on the a*-b* and a*-L*colour spaces (Fig. 2 A and B). As it can be observed, the honey with the greatest purity colour was the acacia honey. Sunflower and tilia honey had the greatest yellow component (highest b* values) and sunflower honey had the greatest red component (highest a*values). Fig. 2 B shows that acacia honey was clearer (higher L* values) than other variety. Sunflower honey not only showed the less purity of colour of all the analysed honeys, but it also proved to be the darkest (lowest L* values). In general, the colour values obtained with Pfund scale, as well as CIEL*a*b*, was as expected for this varieties of honey (Persano-Oddo & Piro, 2004; Escriche et al., 2009).

![Fig. 2. Colour spaces (A: a*-b* and B: a*-L*) showing position of raw honey (acacia, sunflower, and tilia)](image)

The level of fructose and glucose was high but within the limits established by the European legislation. Calibration curves were obtained for all the sugars (glucose, fructose, and sacarose) at three different concentrations.
Physicochemical parameters, identified in different honeys (acacia, sunflower and tilia); and Anova F-ratio for each of the two factors (type of honey and country)

<table>
<thead>
<tr>
<th>TYPE OF HONEY FACTOR (H)</th>
<th>COUNTRY FACTOR (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physicochemical Parameters</td>
<td>Acacia</td>
</tr>
<tr>
<td>Diastase activity (ID)</td>
<td>12.67b</td>
</tr>
<tr>
<td>HMF (mg kg⁻¹)</td>
<td>4.81b</td>
</tr>
<tr>
<td>Moisture (g/100g)</td>
<td>16.79a</td>
</tr>
<tr>
<td>Conductivity (µS cm⁻¹)</td>
<td>0.22c</td>
</tr>
<tr>
<td>Pfund colour</td>
<td>13.32c</td>
</tr>
<tr>
<td>CIE L<em>a</em>b* colour</td>
<td></td>
</tr>
<tr>
<td>L*</td>
<td>53.95a</td>
</tr>
<tr>
<td>a*</td>
<td>0.20c</td>
</tr>
<tr>
<td>b*</td>
<td>17.17b</td>
</tr>
<tr>
<td>Sugars</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>29.31b</td>
</tr>
<tr>
<td>Fructose</td>
<td>44.04a</td>
</tr>
<tr>
<td>Sacarose</td>
<td>1.77a</td>
</tr>
</tbody>
</table>

H: Type of honey; C: Country; ns: Non significant; * p<0.05; ** p<0.01; *** p<0.001. Different letters in each column indicate significant differences at 95% confidence level as obtained by the LSD test.

In order to know if the observed differences among the different honeys and the physicochemical parameters are statistically significant, an analysis of variance (ANOVA) multifactor (with 2 factors: type of honey and country) was conducted for each parameter (Tab. 2). The ANOVA results showed that colour Pfund and CIE L*a*b* next to conductivity and glucose are the parameters with higher significant differences among the three types of honey and diastase and glucose among countries.

Considering that a higher value of F-ratio means a more noticeable effect of the factor (type of honey and country) in a variable, color Pfund next to conductivity were the parameters more affected by the type of honey, and glucose next to diastase activity were the parameters more affected by the country.

With the aim to obtain an overview of the main effect of the analyzed parameters on the different types of analyzed honeys, a Principal Component Analysis was carried out.

256
Fig. 3. Biplot for the two principal components (PC1 and PC2) of the PCA model of the physicochemical parameters (diastase activity and electrical conductivity), sugars and colour on the three types of honey from three different countries. The analysed parameters are identified by an X before the name (conductivity, L*, a*, b*, Colour Pfund, glucose, sacarose, fructose). Sp: Spain; ChR: Czech Republic, Ro: Romania.

Fig. 3 shows the PCA bi-plot of scores and loading obtained considering the totality of the honey (3 varieties from three different countries) and the different parameters (sugars, colour, diastase activity, electrical conductivity). The values of HMF and moisture were not taken into account, as both are only related to the quality of honey and not to the botanical origin and therefore not useful for the differentiation among honeys.

It was found, that two principal components (PCs) explained 64% of the variations in the data set. The PC1 explains the 45% of variability, and the PC2 explains the 19%. First principal component clearly differentiates the three kinds of honeys; sunflower is located in the right quadrant, acacia on the left, whereas tilia is placed between both of them. This indicates that the botanical origin of honey has a marked influence on the parameters studied. On the other hand, the country exerts a minor effect on the analysed parameters as the samples were grouped principally according to type of honey and not to the country.

The PCA model showed that sunflower honey and acacia are the most different honeys studied. Sunflower is associated with the highest value of colour pfund, glucose, conductivity, a*, b* and the lower values of L* and sacarose; on the contrary, acacia has the lowest values of colour pfund, glucose, conductivity, a*, b* and the higher of L. Tilia honey has medium values of all parameters with the only exception of diastase activity, for which the value was the highest in this kind of honey. This corresponds with what can be observed in Fig. 1 and 2, and in Tab. 1.
CONCLUSIONS

The profile of physical-chemical (diastase activity, electrical conductivity), sugar composition and colour parameters made it possible to differentiate three different types of honey (sunflower, tilia, and acacia) that originated from Spain, Romania, and Czech Republic. Although it was found that the country led to significant variations in the levels of certain parameters, a PCA analysis proved that honey type had a far more greater influence in its differentiation than their geographical origin.

REFERENCES