BOTANICAL ORIGIN AUTHENTICAFITION OF BLACK LOCUST (*ROBINIA PSEUDOACACIA*) HONEY, BY MEANS OF SUGAR SPECTRUM DETERMINATION

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Abstract. The quality of honey is established by its botanical or floral origin and chemical composition. Sugar spectrum is an important criterion for authenticity determination. High fructose content is a characteristic of locust honey. 64 honey samples originating from three different zones of Romania were investigated for sugar spectrum and water content in order to establish the botanical origin declared by the suppliers. Excepting few samples (3), all other honeys were in the range of standard values for water content, fructose, glucose and other sugars. Different calculated parameters (fructose/glucose F/G ratio, glucose/water G/W ratio and (glucose-water)/fructose (G-W)/F ratio) were used also for honey authentication.

Keywords: acacia honey, authenticity, sugars, HPLC, analysis

INTRODUCTION

Honey is a source of natural sugars as the product itself is a natural substance is produced by the bees from flower nectar, or from sweet substances left by aphides on other part of the plants than flowers, being also a natural sweetener.

Honey is considered a potential complete food, regarding nutritional standards, being a natural product, rich in simple, easy assimilable sugars (fructose, glucose), enzymes (invertase, glucose oxidase, catalase, phosphatase), amino and organic acids (proline, gluconic acid, acetic acid), vitamins (ascorbic acid niacin, riboflavin), volatile oils, phenolic acids and flavonoids, minerals and carotenoid like substances (Sudhanshu *et al*., 2010). High consumer demand and cost of production make this product susceptible to fraud by adulteration with sugars (invert sugar syrups) or by false declaration of floral origin. Council Directive 2001/110/EC related to honey (2002) includes general and specific characteristics to test the authenticity of botanical origin (Chudzinska and Baralkiewicz, 2010). Until now, many physicochemical parameters were analyzed for testing honey adulteration like: melisopalinological profile, sugar profile, amino acid profile, volatiles, organic acids, mineral content and enzyme activities (Persano Oddo and Piro, 2004).

The quality of honey is established by its botanical or floral origin and chemical composition. Traditionally, the floral origin of honey is established by the analysis of bee pollen present in the sediment of honey, but chemical approaches can be more accurate and more easily undertaken in the characterization of the floral source of honey (Yao *et al.* 2003). All these analysis require a lot of time and specialized personnel to perform all of them.

One of the simplest honey quality criteria is water content, which determine the capability of honey to be stable, to avoid or to promote spoilage or to initiate the fermentation process: the higher the water content, the higher the probability that honey will ferment upon storage.

The carbohydrate profile of honey consists of monosaccharides: fructose and glucose; disaccharides: sucrose, maltose, turanose, isomaltose, melibiose and trisaccharides: melezitoze, raffinose, panose.

Fructose and glucose, the major sugars present in honey (Mărghitaş 2002; Da Costa *et al.* 2000) can be determined titrimetrically or spectrophotometrically (Gonnet 1973), but the content of minor carbohydrates in honey can be determined accurately by liquid chromatography (Swallow & Low 1990; Weston and Brocklebanc 1999 Bobis *et al.* 2006).

Acacia honey is one of the most demanded honey type by the consumers, due to its gold-yellow bright color, sweet taste and flower flavor. Also the ratio of price and quality, make this type of honey to be highly demanded by the purchasers and implicitly by the consumers.

It is known that acacia honey have the highest content of fructose, which make this type of honey to remain fluid for as long period of time. Also, fructose/glucose ratio must be above 1.2. Also glucose/water ratio influence the crystallization process, values above 2.1 will conduct to faster crystallization processes.

The main objective of this study was to evaluate the composition of sugars from Romanian declared acacia honeys, the total sugar content, and to see if according to this composition, the botanical origin and authenticity may be established.

MATERIAL AND METHOD

Honey samples were purchased from three main regions of Romania (zone 1 North western part of Romania – Transylvania - , zone 2 South of Romania – Muntenia – and zone 3 Eastern part of Romania – Moldova). All samples were declared as acacia honey by the supplier.

The determination of moisture by refractometry is a very simple and reproducible method, successfully used up to the present time and thus there is no need for alternative methods. The harmonized method is based on a measurement with an Abbe refractometer (Bogdanov *et al.*, 1997).

Ultra pure water was used to dilute honey samples. Sugar standards and acetonitril were purchased from Sigma Chemical Company.

HPLC sugar analysis were carried out in a system equipped with a LC-10AD pump, DGU-14A degasser, CTO-10AS VP column oven, SIL-10AV VP auto injector, SCL-10A VP system controller and a RID-10A refractive index detector. Separation of the sugars was made on an Alltech (Altima Amino 100a 5 µm, containing modified amino silica gel), and acetonitril/water mixture (75/25 v/v) a mobile phase, in 1.3 ml/min flow rate.

Standard solutions were used for identification and quantification from the samples. Each standard was injected separately, to register the retention time and than in mixture, to see if all standards were baseline separated. Quantification was obtained by

peak integration in comparison with standards. Results were expressed as % for each sugar.

RESULTS AND DISCUSSION

Water content of honey samples

Refractometric method used for water content determination show that most of the honey samples were situated in the limits of standard values for this parameter. Three honey samples present moisture values above 20% (outside the standard limit) and three honey samples present moisture values higher than 19% (within the limit, but rather high content). Average content of all 64 samples was 17.06±1.35. Eliminating the outliers (3 samples), the average value of water content is 16.90, so not a significantly difference.

Saccharide composition of honey samples

With the HPLC method used for determining the profile of sugars from honey, we could identify and quantify 7 types of sugars.

In Fig. 1 is presented a chromatogram obtained at HPLC determination of sugars in analyzed honey samples.

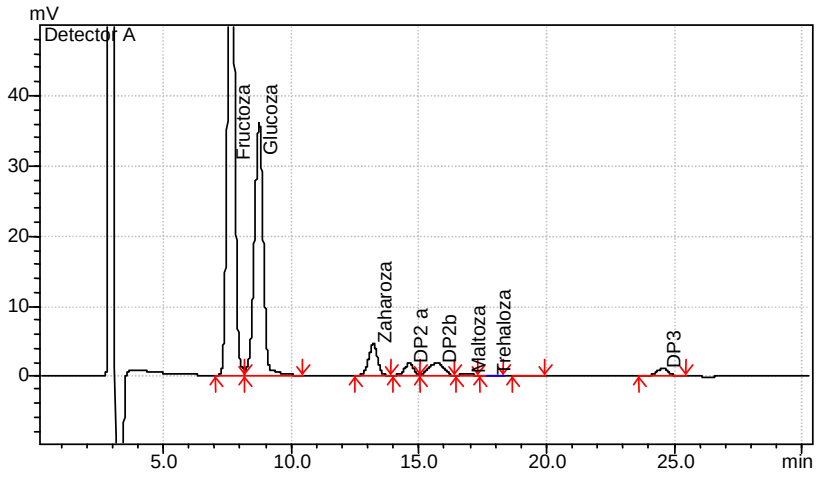

Fig. 1. Sugar profile of one Black locust honey

Fructose (43.34±2.81) and glucose (28.90±3.04) are the most abundant monosaccharides present in all honey samples. Following all investigated honey samples, we observed that four samples were outside the usual range of values for this type of honey. One of the four samples had glucose content higher than fructose, this sample could not belong to this type of declared origin. Similar results were obtained from other honey samples from Romania, Switzerland, Slovenia etc. (Marghitaş *et al*. 2010; Ruoff, 2006; Kropf *et al.*, 2010).

Disaccharides maltose (2.91±0.69) and turanose (2.07±0.52 %) are the most abundant from this class of sugars. Erlose, sucrose and isomaltose were also quantified in small amounts. This sugar profile was reported also by Persano Oddo and Piro (2004), Ruoff (2006) and Bobiş *et al.* (2007).

Fig. 2 presents the moisture content and distribution of these sugars between all the samples, together with the concentrations quantified.

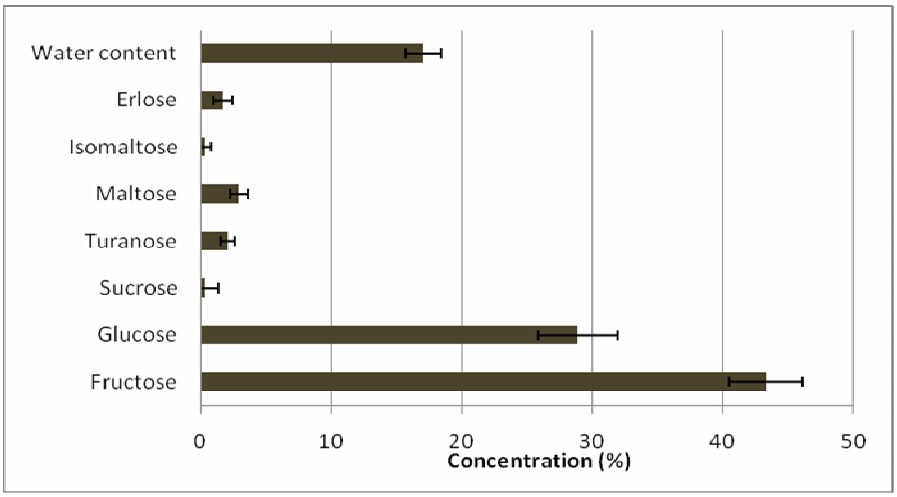

Fig.2. Distribution of sugars (mean ± SD) and water content in honey samples

From HPLC sugar spectrum can be observed that most of the analyzed samples present a characteristic profile for *Robinia pseudoacacia* honey. Generally accepted for this type of honey is high fructose content, the main reason that this type of honey remains fluid for a long period of time.

Botanical origin of locust honey is sustained also by the fructose/glucose ratio. In our case the majority of samples present values above 1.2 (Figure 3A). One sample had F/G ratio below 1 (other type of honey), three samples present values of the ratio below 1.1 and one sample below 1.2.

Glucose/water ratio is another parameter that shows the crystallization rate, knowing that locust honey crystallizes very late due to the high content of fructose. A ratio of <1.7 indicates that honey will remain fluid for a long period of time, meantime a ratio of >2.1 show an early crystallization process. Our samples present different ratios, most of them being <1.7 (Figure 3B). Four of investigated samples present a G/W ratio higher than 2, the same samples having also low fructose content, and F/G ratio under the value of 1.1. This finding confirms that this ratio also may be used in authentication of fluid honey (black locust honey).

Literature indicates that subtracting water content from glucose content and dividing to fructose content also a ratio of crystallization rate will be obtained. This calculation gave more or less the same outliers than the other ratios (Figure 3C). Looking carefully to the results obtained can be observed that these outliers came from individual outliers (from water content, from sugar content and other calculated ratios), indicating that this value have also confidence as the other calculated parameters (fructose/glucose ratio or glucose/water ratio). Four of the samples have (G-W)/F ratio above 0.4, samples that from the other analysis show doubt in respect of botanical denomination, being considered either falsified, or multifloral. Sample 34 present the lowest ratio (0.04), this sample presenting the highest fructose content, but one of the highest water content. Anyway, in all analysis all outliers (high values or small values) must be eliminated in order to have a confidence interval for the results.

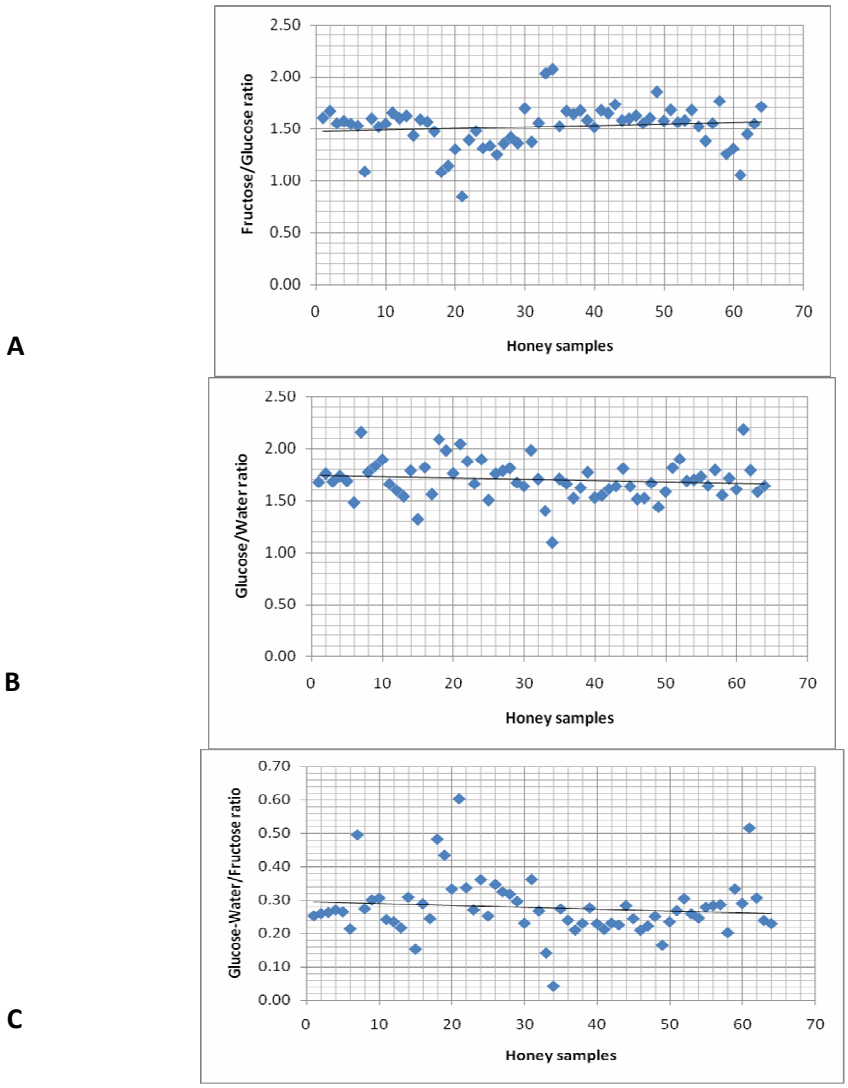

Fig 3. Different ratios determined from sugar spectrum and moisture content: F/G ratio, G/W ratio and (G-W)/F ratio

CONCLUSIONS

- The percentage of fructose is higher in all samples than the percentage of glucose, the average ratio between those two (1.52), showing that we can include the samples in acacia type.
- All honey samples studied, presented in different concentrations a pattern of 7 sugars: fructose, glucose, maltose, turanose, erlose and sucrose.
- Sugar spectrum determination may be used as botanical authenticity evaluation for acacia honey, but anyway additional analysis (especially pollen analysis and color measurement) must be performed to evaluate the uniflorality of honey.

- Different calculated parameters (fructose/glucose ratio, glucose/water ratio or (glucose-water)/fructose ratio may be used in unifloral locust honey evaluation, together with determination of other chemical parameters.

REFERENCES

1. Bobis, Otilia, L. Al. Mărghitaş, Victoriţa Bonta, D.Dezmirean, Oltica Stanciu, 2006, Determinations of sugars in Romanian honeys of different botanical origin, using High Performance Liquid Chromatography, Buletin USAMV-CN, 63/2006 (-), 143-148
2. Bogdanov, S., Martin, P., Lüllmann, C. Harmonised methods of the European Honey Commission, *Apidologie*, 1997, extra issue, 1-59, available online at: http://www.apis.admin.ch/host/doc/pdfhoney/IHCmethods_e.pdf
3. Chudzinska, M. and D. Baralkiewicz, 2010, Estimation of honey authenticity by multielements characteristics using inductively coupled plasma-mass spectrometry (ICP- MS) combined with chemometrics. Food Chem. Toxicol. 48:284-290.
4. Council Directive 2001/110/EC of the 20 December 20012 relating to honey (2002). Official Journal of the European 334 Communities. L10 47-52.
5. Da Costa Leite J.M., L.C.Trugo, L.S.M. Costa, L.M.C. Quinteiro, O.M. Barth, V.M.L. Dutra, C.A.B. De Maria, Determination of oligosaccharides in Brazilian honeys of different botanical origin, *Food Chemistry* 70 (2000), 93-98
6. Gonnet M., Deux methods colorimetriques simples permettant d'apprecier la teneur en glucose et en fructose des miels, Apidologie 4, (1973), 45-55
7. Kropf, U., M.Korosec, J.Bertoncelj, N.Ogrinc, M.Necemer, P.Kump, T.Golob, 2010, Determination of the geographical origin of Slovenian black locust, lime and chestnut honey, Food Chem., 121(3), 839-846
8. Mărghitaş L.A., (2002), Albinele şi produsele lor, Editura Ceres Bucureşti
9. Persanno Oddo, L. and R. Piro, 2004, Main European unifloral honeys descriptive sheets. Apidologie. 35:S38-S81.
10. Ruoff, K. , 2006, Authentication of the botanical origin of honey, Dissertation thesis, DISS.ETH NO.16857, ETH Zurich
11. Sudhanshu, S., G. Satyendra and S. Sarun, 2010, Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem. 118:391-397.
12. Swallow K.W. & Low N.H., Analysis and quantification of carbohydrates in honey using high performance liquid chromatography, *Journal of Agricultural and Food Chemistry* 38 (1990), 1828-1832
13. Weston R.J., Brocklebank L.K., The oligosaccharide composition of some New Zealand honeys, *Food Chemistry* 64 (1999), 33-37
14. Yao, L., Datta, N., Tomás-Barberán F.A., Ferreres, F., Martos, I., Singanusong, R., 2003, Flavonoids, phenolic acids and abscisic acid in Australian and New Zealand *Leptospermum* honeys, Food Chem., 81(2), 159-168