Determination of Moisture Content and its Correlation with other Parameters in Honey Quality Control

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Honey is the natural sweet and viscous food produced by bees - the genus Apis- exclusively from the nectar of plants or from sweet secretions of green parts of plants in order to have it for their energetic needs. To become honey, the nectar and sweet secretions which the bees collect are enriched with specific substances of their own, are processed in a specific way, are stored and leaved in honeycombs of the beehive to ripen and mature. Honey of the same floral source can vary due to seasonal climatic variations or due to a different geographical origin. According to international and national food regulations, "honey stipulates a pure product that does not allow for the addition of any other substance". Unfortunately honey has long been a prime target of adulteration. Honey authenticity cannot be detected easily, by visually examining or by tasting; the only real evidence is coming from analysis using defined analytical techniques internationally certified. There is no single procedure that can certify that any product is pure honey, but a number of analyses are able to verify that nothing has been added to or substituted for the real thing. In the present contribution we have investigated the significance of the moisture content for honey quality and its correlation with other honey authenticity parameters, especially with the fructose-glucose and sucrose content. For the determination of moisture content it was applied the refractometric method and for fructose-glucose and for sucrose content a high performance liquid chromatographic method.

Keywords: hony, adulteration, moisture content, fructose, glucose, sucrose, HPLC, refractometry

Honey is one of the most complex mixtures of carbohydrates produced in nature. It is the valuable natural sweet and viscous fluid produced exclusively by honeybees (the genus *Apis*) from the nectar of flowers, stored by bees as a food source in winter or when food sources are scarce [1]. With a similar content alike human blood, honey is a source of readily available sugars, organic acids (aliphatic), enzymes, some amino acids and proteins, macro- and microelements and biologically active substances [2]. Honey has been used from antiquity, either as a high nutritional food or as a remedy for many diseases, being a topical anti-microbial agent for treatment of infected wounds [2]. In the same time, honey is a source of antioxidants, having natural antioxidant properties and can destroy biologically the toxic chemical agents which have been linked to many diseases, such as cancer [3].

As one the very few food pure natural which is offered today, honey does not allow the addition of any other food ingredient, including food additives [1,2]. Honey must, as far as possible, be free from organic or inorganic matters foreign to its composition [4,6]. But unfortunately, for economic gain, honey has long been a target of adulteration, which means the addition of foreign substances to honey, especially the addition of less expensive carbohydrates. Nowadays, as a result of the number of abuses observed, a priority issue is to ensure that honey is authentic food in respect of international and national legislative requirements. It is of particular importance to detect the honey authenticity and to declare that a specific honey is adulterated. In order to do this, a number of analyses are necessary [6,8]and the confirmation is done by the special method of Carbon Isotope Ratio Mass Spectrometry [9,10].

According to the European Council Directive 2001/110/EC [1], to the Codex Standard for Honey [4] and to the Romanian Regulations [5,6], the honey quality is defined by specific organoleptic characteristics and by composition criteria. As organoleptic characteristics, for honey can be mentioned: the aspect, the color, the taste, the consistency, the flavour and the aroma. The composition criteria for honey are the following: sugars content, moisture content, water-insoluble content, electrical conductivity, free acid, diastase activity, and hydroxymethylfurfural (HMF) content.

The purpose of this work has been to establish a correlation between the moisture content and the sugars content from different varieties of honey samples, being well known that these two parameters are of major importance in honey quality assessment.

Honey moisture is that quality criterion that determines the capability of honey to remain stable and to resist spoilage by yeast fermentation and is one of the criterion that can be correlated with honey quality and authenticity in connection with the other precision parameters, as mentioned [7,8].

Experimental and Reagents

All the reagents used were of analytical purity grade and bi-distilled water was used for the sugars standards (prepared in 25 methanol and 75 water, v/v) and for the mobile phase (a mixture of 75 volumes of acetonitrile with 25 volumes of water). The standards and sample solutions have to be stored in the refrigerator at a temperature of 4^0 C.

The moisture contents (W) of honey samples were determined by refractometric method [11], using a refractometer Mettler Toledo RE 40**.** This refractometer can be thermostated at the temperature of 20^0 C and permits regularly calibration with distilled water or with another certified reference material.

After a perfect dissolution and homogenization of honey sample in a beaker, it is transferred a drop of honey on the perfect clean prism surface of the apparatus and measured

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the refractive index, after waiting for 3 min for equilibration. The respective refractive index is then correlated with moisture content of honey using standard tables [2, 8]. This table is obtained from a graph that represents the variation of the logarithm of the refractive index minus unity plotted against the honey water content determined by a very precise and difficult technique, the vacuum drying [7].

If the determination is made at temperature other than 20° C, the correction reading to standard temperature of 20° C has to be made, according to AOAC Method 969.38 B/J [11].

The fructose and glucose contents (the sum of both) and sucrose were determined by high performance liquid chromatography (HPLC). The present method is based on the method described by Swallow and Low [12], which originated in the published method by Bogdanov and Baumann [13].

The honey sugars analyses have been performed with an Agilent 1200 Series liquid chromatograph. This liquid chromatograph is equipped with temperature regulated Diode-array Detector SL, temperature regulated column oven, autosampler and quaternary pump.

The following chromatographic conditions have been found to give good separation for honey sugars: analytical column 4.6 mm in diameter and 250 mm in length, containing lichrospher 100 RP 18, temperature detector and column of 35° C, mobile phase -acetonitrile:water (75:25, v/v), flow rate 1.3 mL/min and sample volume of 10 µL.

In the applied chromatographic method a relatively little sample preparation is required: 5 g of honey sample is dissolved in 40 mL water into a beaker and it is transferred quantitatively into 100 mL volumetric flask where were pipetted 25 mL of methanol. After the filling of the volumetric flask with water, it was filtrated the obtained solution sample through a membrane filter and collectd in a sample ampoule.

The honey sugars were identified by comparison of the retention times and were quantified by the peak area of the honey sugars with those of the standard sugars concentrations. Then the mass percentage of the sugars (fructose, glucose and sucrose) was calculated, as presented in table 1.

Table 1
LINEARITY IN THE CONCENTRATION OF SUGARS

Crt. No.	Compound	Range (mg/mL)	R^2 values	Equation curve
1.	Fructose	0.5-50	0.998	y=564198x -73578
2.	Glucose	0.5-50	0.998	y= 421350x - 42986
3.	Sucrose	0.5-50	0.999	y = 579957x - 10765

Table 2
MOISTURE AND CONTENT OF SUGARS IN HONEY SAMPLES

Crt. No.	Honey variety	Water %*	Sugar content (g/100g)	
			(Fructose + glucose) %*	Sucrose %*
1.	Multifloral honey	18.4	76.64	2.51
2.	Multifloral honey	18.4	76.86	2.38
3.	Multifloral honey	17.4	76.34	2.52
4.	Multifloral honey	17.2	76.56	2.55
5.	Multifloral honey	17.8	76.65	2.40
6.	Acacia honey	17.8	75.95	2.42
7.	Acacia honey	17.8	76.08	2.11
8.	Linden honey	17.8	76.97	2.54
9.	Linden honey	17.8	76.99	2.47
10.	Multifloral honey	18.8	76.05	3.40
11.	Sun flower honey	16.4	77.02	2.67
12.	Sun flower honey	16.6	76.30	2.56
13.	Sun flower honey	16.5	76.82	2.60
14.	Sun flower honey	16.8	76.50	2.65
15.	Multifloral honey	20.2	74.99	2.35
16.	Multifloral honey	20.6	74.45	3.94
17.	Multifloral honey	20.6	75.12	2.66
18.	Multifloral honey	20.0	75.20	1.52

*The values represent the mean of two determinations obtained in rapid succession by the same method, on identical honey sample, under the same conditions.

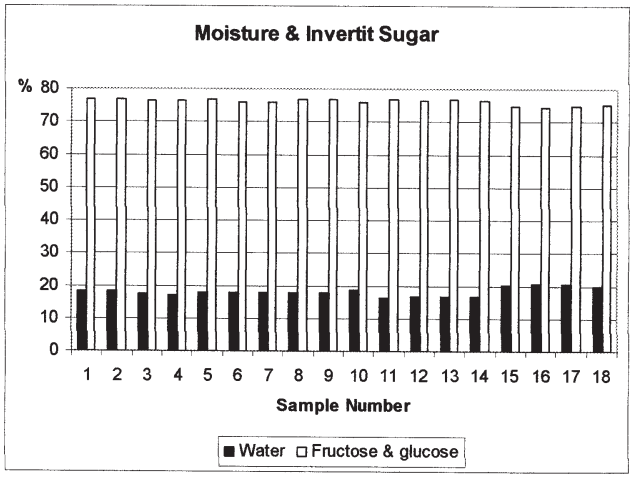

Fig. 1. The correlation between moisture content and invertit sugar in different varieties of honey. (Black is for moisture content and white is for invertit sugar)

Results and discussions

The study was carried on 18 samples of four varieties of honey (multifloral, acacia, linden and sun flower honey) originated from Prahova district. All the samples had a seal labeled on them, according to international regulations [1].

The moisture content was determined by refractometric method, using the relationship between water content of honey to refractive index proposed first time by Wedmore [14]:

$$W = \frac{1.73190 - \log(R.I. - 1)}{0.002243}$$

where W is the water content in g per 100 g honey and R.I. is the refractive index. Based on the above relationship, there have been established tables showing directly the water content of honey to refractive index [2,4,8]. In this way, the determinations of the moisture content become an easy and rapid procedure in honey quality control.

Under the chromatographic conditions described above, a linear relationship between the concentrations of sugars (fructose, glucose and sucrose) and chromatographic peaks area was found up to 0.5 and 50 g/L as illustrated in table 1. For all these sugars, the method proved a good linearity with determination coefficients exceeding 0.99.

In table 2 are presented the obtained values of moisture content for 18 honey samples and the respective content of fructose, glucose and sucrose. It can be seen that 15 samples (No. 1-14 and 18) are in concordance with European Directive 2001/110/EC1[1] and with Romanian low [5,6], and that the samples no.15-17 do not meet the respective requirements for the moisture content (W>20 %). This content can be correlated with other quality honey parameters, especially with the content of fructose and glucose. From the table 2 it can be also seen that for the samples 1-14 and 20 the values of glucose and fructose meet the requirements of a very good honey quality, from the sugars content point of view. For the honey samples No.15 - 17, where the moisture content is higher than 20, the invertit sugar (the sum of glucose and fructose) decreases.

When the water content is above the admitted limit, the nourishing quality of the respective honey is decreasing proportionally, the organoleptic proprieties are modified in terms of the appearance and consistence and, in particularly, the honey is liable to fermentation. In our study, as it can be observed from table 2, for honey samples No. 15- 17, when the moisture content is > 20%, the invertit sugar has less value and as a consequence the nutritional value for these respective honey samples is reduced.

The entire discussed above are better illustrated by figure 1, where the moisture content and the invertit sugars are represented together for an easy comparison.

Conclusions

The moisture content of honey has a special significance and can give a primary honey quality assessment. High moisture content in honey may be due a direct or indirect adulteration. If the water content of a honey sample is greater than 20 %, that honey is possible going to ferment and lose its freshness. In this situation the yeast may survive and cause fermentation to begin in storage. As a consequence, it results an increase of honey acidity, which then becomes an important quality criteria. In the same time a high moisture content is a prime indicator for a possible honey adulteration, which must be confirmed by the special method of carbon isotope method.

In our study, the fifteen honey samples investigated complied with the Romanian and European Union Regulations in respect of water and sugars content and three of them may be suspected of adulteration. Although there are powerful methods to prove the honey adulteration, they have to be further improved in order to keep in mind the honey as authentic natural food.

References

1. *** Council Directive 2001/110/EC, Official Journal of the European Communities, 2002, L 10/47-L 10/52
2. N. POPESCU, S. MEICA, Mierea si produsele apicole, 2002, Editura Diacon Coresi
3. B. F. BECK, M.D. Smedley and D. Smedley, Health Resources Press, Inc., Silver Spring, MD, 1997.
4. *** Revised Codex Standard for Honey CODEX STAN 12-1981, Rev. 2001, 1.
5. *** ORDIN pentru aprobarea Normelor privind denumirea, descrierea, definirea, caracteristicile şi compoziţia mierii, Monitorul Oficial Nr. 650/12. 09. 2003
6. *** STAS, 784/3-89, Mierea de albine. Metode de analiza.
7. *** Official Journal of the European Communities, L10, 47-52, 2002.
8. BOGDANOV, S., Harmonized Methods of the International Honey Commission, 2002
9. *** AOAC International (1998). Official Method 991.41, C-4 plant Sugars in honey
10. J.W. WHITE JR., K. WINTERS, J. Assoc. Off. Anal. Chem. Int. **72**, nr. 6, 1989, p. 907
11. *** Determination of Moisture Content, AOAC 969.38 B/J. Assoc. Public Analysts, 1992, 28, 4, 183-187/MAFF
12. K.W. SWALLOW, N. H. Low, "Analysis and Quantitation of the Carbohydrates in Honey Using High-Performance Liquid Chromatography", J. Agric. Food Chem., 38, 1990, p. 1828
13. S. BOGDANOV, S. E. BAUMANN, Bestimmung von Honigzucker mit HPLC. Mitt.Gebiete Lebensm.Hyg., 79, 198-206, 1988.
14. E.B. WEDMORE, Bee World, 3, 6, 197, 1955

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