

Preliminary Study on the Authenticity of Acacia, Multifloral and Linden Honey from Cluj County

**Loredana M. MARC, Liviu Al. MĂRGHITAȘ, Otilia BOBIȘ,
Victorița BONTA, Cristina MIHAI**

University of Agricultural Sciences and Veterinary Medicine, 3-5 Mănăstur Street,
400372 Cluj-Napoca, Romania; loredanamaria_marc@yahoo.com

Abstract. Honey samples from two different types of locations from Cluj county were subjected to this study: the beekeeping store APICOLA and the main free markets in the county. Seventeen honey samples representing three different types of Romanian honey: acacia, multifloral and linden honey for the main physical-chemical parameters were analyzed. Water and hidroxy-methylfurfural (HMF) content, together with sugar spectrum were analyzed into the APHIS Laboratory, USAMV Cluj. All determined parameters were situated within the limits established by Romanian and International Standards. For water content, the values were found between 16.0-19.9% for all honey types. HMF parameter was situated between 1.49-29.4%, indicating freshness for some honey samples and some unsuitable storage conditions for other honey samples. The main sugars in honey are fructose and glucose. Their individual values as well as their sum or ratio, places all samples in the characteristics of the respective honey type. Turanose, maltose, isomaltose and erlose are also present in different amounts in almost all honeys. The samples were authentic and having the declared botanical origin in terms of the physical-chemical analysis.

Keywords: honey, marketing research, water content, hidroximethylfurfural, sugar content, HPLC

INTRODUCTION

Honey is a unique natural product obtained by processing flowers nectar or plants manna, being used as sweetener or in food industry due to its nutritive, therapeutic and dietetic quality (Vica *et al.*, 2009). If the nectar from which the bees will process the honey is gathered mainly from the flowers of one specific plant species in the foraging area of the beehive, the honey is called unifloral honey and it can be named after the plant from which the nectar originates. On the other hand, the source of the honey may be the mixed flora and in this case, the honey is called multifloral honey (Salonen and Julkunen-Tiito, 2012). The composition of honey depends mainly on the floral source and secondly on some external factors such as season, environmental factors, physiological stage of the bees and honey processing (Bobiș *et al.*, 2007). Honey is a product with minimal types and levels of microbes, due to its chemical composition from which derive their natural properties (Abel Adebayo and Davies, 2012). Chemical composition of honey consists of water, carbohydrates (glucose, fructose, sucrose), dextrin, vitamins, minerals and small quantities of microelements and proteins (Vica *et al.*, 2009).

The Romanian apiculture has had to suffer after 1989 until present. The production of honey has been around the value of 19.000 tons/year, except for the 2007 (less than 11.000 tons), 2008 (18.000 tons) and 2010 (about 15.000-18.000 tons) when the worst productions were recorded because of the unfavorable weather conditions (Vica *et al.*, 2009).

Marketing research is one of the functions of marketing knowledge management. According to American Marketing Association, the definition of marketing research is “the function that links the consumer, customer, and public to the marketer through information—information used to identify and define marketing opportunities and problems; generate, refine, and evaluate marketing actions, monitor marketing performance; and improve understanding of marketing as a process. Marketing research specifies the information required to address these issues, designs and method for collecting information, manages and implements the data collection process, analyzes the results, and communicates the findings and their implications (Bednall *et al.*, 2008). The transaction is a process when the bee products are exchange with money. Where transactions take place is named honey market. It can be a location, for example a village market, a retail shop, a roadside stall, a supermarket or a simple market (Pocol and Mărghitas, 2006).

The main objective of this study was to make an overview of the Transylvanian honey market, analyzing the quality and authenticity of honey. The goal is to analyze the honey produced and consumed in this area. The research has been carried out in Cluj County and includes two segments of the market: beekeeping shop and main food markets, directly from beekeepers.

MATERIALS AND METHODS

All analyzes were performed according to the methods proposed by International Honey Commission and in agreement with the European Union and even more in triplicate.

Honey samples. Seventeen honey samples were harvested in 2011 and their declared type was 7 acacia honey, 7 multifloral honey and 3 linden honey. All honey samples (5 samples from APICOLA store and 12 samples from the main market from Cluj) were analyzed to determine the following parameters: sugar spectrum, water and HMF contents.

Procedures. HPLC determinations of sugars was performed on a Shimadzu system with a LC-10AD pump, DGU-14A degasser, SIL-10AV VP auto sampler, RID-10A refractive index, thermo stated at 30°C, with CTO-10AS VP temperature controller of separation column (Altima Amino 100A 5 μ m, 250mm x 4.6mm), with a mixture of acetonitrile/water as mobile phase with 1 ml/min flow rate.

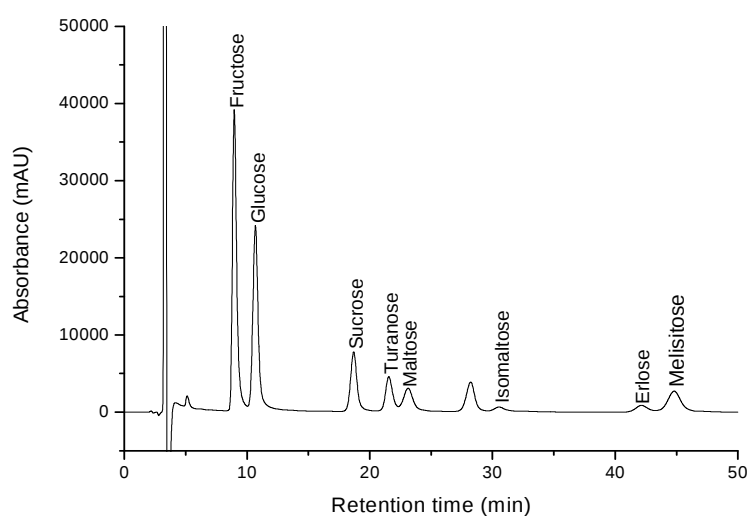


Fig.1. HPLC-IR chromatogram of the standard mixture solution

To perform the calibration curve on HPLC the sugar standards (glucose, fructose, sucrose, erlose, maltose, isomaltose and trehalose) were dissolved in ultra-pure water (1 mg/ml solution), mixed in equal volumes and diluted. To register the retention time and then in mixture, each standard was injected separately to see if all standards were baseline separated (Fig. 1). Quantification was obtained by peak integration in comparison with standards. Results were expressed as g/100g honey (Bonta *et al.*, 2007).

Water content was refractometrically determinate using an Abbe Refractometer with temperature correction. Refractive index corresponding to water content of honey was registered and where it was necessary temperature correction was made. A parameter that indicates the freshness of the sample is hydroxymethylfurfural (HMF). HMF content was determined by HPLC with a PDA (Photo Diode Array) detector. Chromatographic separation was carried out on Discovery HSC18 column (250 x 4,6mm, 5 μ m) using methanol: water (10:90 v/v) as mobile phase. This method is used for the first time in APHIS Laboratory, following the method described in International Honey Commission compendium of methods (Bogdanov *et al.*, 1999). HMF content was expressed in all samples as mg/kg honey, the amount being calculated according to the calibration curve of pure standard.

RESULTS AND DISCUSSIONS

After the market research regarding the bee breeds raised in this part of the country, the conclusion was that Carpathian bees form the bee families. The experiment, which involved the Carpathian breed Forti, and the Italian breed demonstrated that Carpathian breed is stronger than Italian breed. Mortality during winter was 35% in the Carpathian bee while Italian breed mortality was 52%. Even the honey consumption during winter is 11.5% higher to Italian bees than to Carpathian bees (Pârnu *et al.*, 2009). The results of physical-chemical analysis of acacia, multifloral honey and linden honey show good quality and authenticity for the samples.

All the samples show water content below 20% (maximum level permitted by the standard), with the lowest value of 16.0%. The average value of water content in acacia samples was $17.56 \pm 1.36\%$, for multifloral honey the mean value was $17.97 \pm 1.18\%$ and for linden honey was $17.8 \pm 0.44\%$ (Tab. 1).

HMF content as indicator of honey freshness show these values: acacia honey between 2.085 and 7.605 mg/kg, multifloral honey between 1.49 and 25.185 mg/kg and linden honey between 3.39 and 29.04 mg/kg (see Tab. 1).

Gabor and Goian published in 2006 a paper on the methods of counterfeit honey and they consider three classes for HMF. Class 1 up to 10 mg/kg HMF content indicates that honey is fresh. Class 2 exceeding 30-40 mg/kg HMF content up to 100 mg/kg HMF content indicates that honey was kept in misfit conditions for a long period. Class 3 HMF content higher than 150 mg/kg indicates that honey contains artificial inverted sugar.

Our HMF interval for acacia honey sample ranged between 2.08-7.60 mg/kg, which indicates that acacia honey samples, fits in class 1. The samples MH7, MH9, and MH16 for multifloral honey have the HMF interval between 1.49-6.61 mg/kg, which indicates that samples fits in class 1. HMF interval for MH2, MH5, MH11 and MH13 samples is between 12.99-25.51 mg/kg that indicates that our multifloral honey samples fits in class 2.

HMF content for LH14 sample (linden honey) is 3.39 mg/kg, which indicates that this sample fits in class 1. LH4 and LH17 (Fig. 2) have the HMF content of 12.43-29.04 mg/kg that indicates that these honey samples fits in class 2. Sample AH1 has the lowest

value of fructose (39.94g/100g) and sample AH3 the highest value of fructose (44.53g/100g). Mean value for acacia honey was 40.88%±1.71.

Tab. 1

Water (%) and HMF content (mg/kg) for analyzed honey samples

Code	Water content (%)	HMF content (mg/kg)
AH1	19.90	7.60
AH3	18.30	5.78
AH6	17.00	3.67
AH8	17.80	2.49
AH10	16.00	2.08
AH12	16.10	3.24
AH15	17.80	3.69
Average	17.56	4.08
SD	1.36	1.95
MH2	19.80	25.18
MH5	18.00	25.51
MH7	16.40	1.49
MH9	18.70	6.61
MH11	18.30	15.55
MH13	16.60	12.99
MH16	18.00	3.54
Average	17.97	12.98
SD	1.18	9.78
LH4	18.10	12.43
LH14	17.30	3.39
LH17	18.00	29.04
Average	17.8	14.95
SD	0.44	13.01

Note: AH–acacia honey; MH–multifloral honey; LH–linden honey. SD–standard deviation.
All determinations were performed in triplicate.

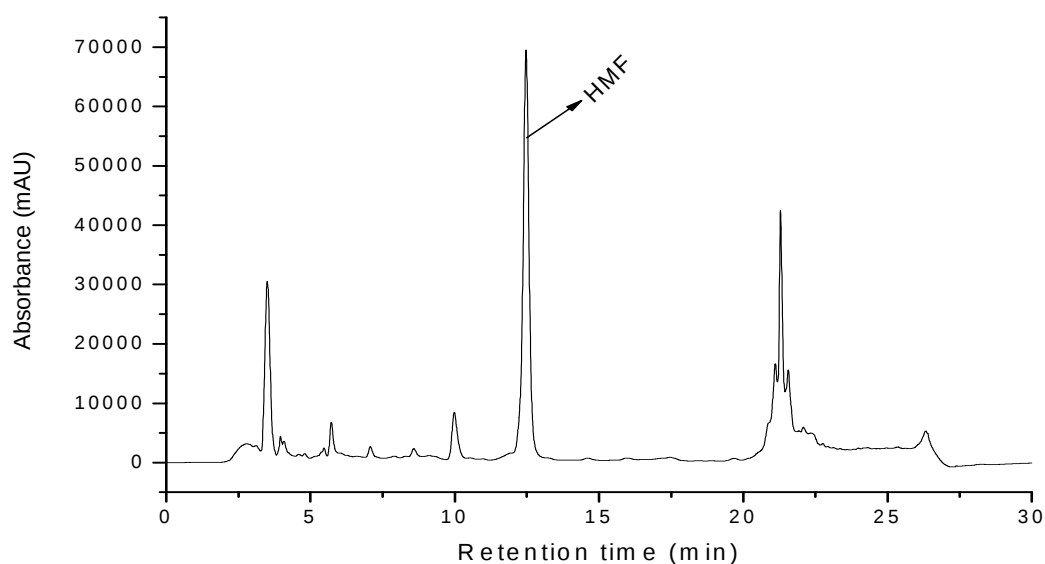


Fig. 2. HPLC-PDA chromatogram of honey sample LH17

Tab. 2

Sugar profile of acacia honey from Cluj County, expressed as g/100g (%)

Code	Fructose	Glucose	Sucrose	Turanose	Maltose	Isomaltose	Erlose
AH1	39.94	31.48	0.21	2.20	3.38	0.38	1.03
AH3	44.53	28.89	0.19	1.72	2.44	-	1.19
AH6	40.54	32.49	1.19	1.67	3.35	0.18	1.41
AH8	40.83	31.82	0.80	1.72	2.99	0.40	1.52
AH10	39.08	28.55	2.75	2.37	3.76	0.27	2.07
AH12	40.51	30.35	1.43	2.28	3.83	0.41	1.87
AH15	40.78	32.53	0.42	1.96	3.34	0.51	1.45
Mean value	40.88	30.87	0.99	1.98	3.29	0.30	1.50
±SD	±1.71	±1.64	±0.90	±0.29	±0.47	±0.11	±0.36

Sample AH12 has the lowest value of glucose (28.55g/100g) and the sample AH8 the highest value of glucose (32.49g/100g). Mean value of glucose was 30.87%±1.64.

All honeys present different amounts of sucrose, turanose, maltose, isomaltose and erlose excepting one sample. This is the standard sugar spectrum for acacia honey. The highest level of disaccharide was registered for maltose (Tab. 2).

The highest value of fructose in multifloral honey was registered in sample MH13 (39.07 g/100g), while the lowest value was registered in sample MH11 (34.40g/100g). The mean value of fructose in multifloral honey was 36.65%. High values of glucose were registered in samples MH16 (42.06 g/100 g) and MH2 (38.99 g/100g), these samples having the content of glucose, superior to those of fructose (high cristalization possibility).

The lowest value of glucose was registered in and MH7 (31.48 g/100g and 32.55 g/100g) (Tab. 3). Only turanose and maltose was quantified in all 7 samples, the rest of sugars being quantified in 5 respective 4 samples. Linden honey presents the same sugar spectrum as acacia and multifloral honey. In this type of honey, the main sugar is quantified generally in the same amounts.

Tab. 3

Sugar profile of multifloral honey from Cluj County, expressed as g/100g (%)

Code	Fructose	Glucose	Sucrose	Turanose	Maltose	Isomaltose	Erlose
MH2	34.74	38.99	-	0.92	1.55	-	-
MH5	38.04	31.48	-	2.17	3.62	1.04	0.29
MH7	36.27	32.55	0.48	1.63	2.76	0.30	1.01
MH9	37.38	36.48	-	0.99	1.72	0.28	0.23
MH11	34.40	33.90	0.07	0.56	4.86	0.25	0.11
MH13	39.07	36.23	0.47	1.44	2.91	0.17	0.87
MH16	36.70	42.06	0.26	0.81	-	-	-
Mean value ±SD	36.65 ±1.69	35.95 ±3.71	0.18 ±0.21	1.21 ±0.55	2.48 ±1.57	0.29 ±0.35	0.35 ±0.41

The samples of our study present similar values for fructose and glucose (sample LH14 –37.31 and 37.15%), or higher amounts of glucose over fructose (LH4 and LH17). Sample LH17 has the lowest value of fructose (35.35g/100g) and sample LH14 the highest value (37.31g/100g). Sample LH4 has the lowest value of glucose (34.87g/100g) and sample LH17 the highest value of glucose (39.52g/100 g). For all samples were quantified turanose, maltose and isomaltose, while erlose and sucrose was quantified only in one sample (Tab. 4).

Tab. 4

Sugar profile of lime honey from Cluj County, expressed as g/100g (%)

Code	Fructose	Glucose	Sucrose	Turanose	Maltose	Isomaltose	Erllose
LH4	36.56	34.87	-	2.21	3.96	0.95	-
LH14	37.31	37.15	0.08	1.35	2.61	0.17	0.35
LH17	35.35	39.52		1.25	1.67	0.17	-
Mean value $\pm$ SD	36.40 $\pm$ 18.22	37.18 $\pm$ 18.68	0.026 $\pm$ 0.04	1.60 $\pm$ 0.91	2.74 $\pm$ 1.66	0.43 $\pm$ 0.42	0.11 $\pm$ 0.17

Sum of fructose and glucose quantified for acacia honey was 71.75 g/100g. Mean value for the sum of fructose and glucose quantified for multifloral honey was determined to be 72.6g/100g. Mean value for the sum of fructose and glucose quantified for linden honey is 73.58g/100g. These results are >60g/100g (UE Standards), which indicates the alignment to UE Standards.

Each type of honey has different values of sugars, values for the sum of fructose and glucose and a characteristic ratio of fructose/glucose, which is evident from Tab. 5. Ratio of fructose/glucose significantly differs between the types of honey. The literature studies reveal that fructose/glucose value for Romanian acacia honey is between 1.4-1.7 (Golob and Plestenjak, 1999). Our study shows that fructose/glucose values for Romania acacia honey (1.25-1.54) are comparable with values for French (1.32-1.56), Hungarian and Chinese (1.4-1.7) and for Polish (1.51-1.60) acacia honey (Golob and Plestenjak, 1999). Multifloral honey shows that the ratio of fructose/glucose is between 0.89 and 1.11, with a mean value of 1.01. Linden honey had a sub nominal mean value of fructose/glucose (0.97) (Tab. 5). This parameter is important in the setting of crystallization process. Honey with a high value of this ratio (level of fructose is superior to that of glucose), will crystallize later, after a long period of time, while a sub nominal value ratio (glucose in higher amounts than fructose), will make the honey to crystallize in a shorter period of time.

Tab. 5

Sum and ratio of fructose and glucose for acacia, multifloral and linden honey, calculated for quality and authenticity determinations

	Fructose	Glucose	Fructose+Glucose	Fructose/glucose
Acacia honey (n=7)				
Interval	39.08-44.53	30.35-32.53	67.63-73.42	1.25-1.54
Average	40.88	30.87	71.75	1.32
SD	1.71	1.64	3.11	1.40
Multifloral honey (n=7)				
Interval	34.40-39.07	31.48-42.06	68.30-78.76	0.89-1.11
Average	36.65	35.95	72.60	1.01
SD	1.69	3.71	5.4	0.45
Linden honey (n=3)				
Interval	35.35-37.31	34.87-38.52	71.43-74.87	0.89-1.04
Average	36.40	37.18	73.58	0.97
SD	18.22	18.68	36.9	0.97

CONCLUSION

The results of physical-chemical analysis show that all the parameters lie within the limits set by EU standards and Romanian legislation. Water content was found to be below the limit of 20%, necessary to have a good preservation of honey, without any danger of fermentation. HMF values were found below the limit of 40 mg/kg, our samples being situated below half of this value. Sugar spectrum presents the characteristic profile of each type of honey analyzed, with the individual amounts in the standardized limits. Acacia honey has a fructose/glucose ratio of 1.32, while linden honey has a ratio of 0.97.

In conclusion Romanian honey from Cluj County is authentic and possesses a high quality of composition.

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