PHYSICO –CHEMICAL CARACTERISATION OF ARTISANAL LIME HONEY FROM BIHOR COUNTY

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Abstract

This paper present the characterization by physical-chemical properties of some samples of Lime honey from the Bihor county. The investigated parameters were: pH, moisture content, ash, free, lactonic and total acidity, proline and sugars, The diastase activity and HMF value was also investigated in order to verify the proper storage conditions and the eventual thermal treatment supported by the tested samples. All tested honey samples were found to meet European Legislation (EC Directive 2001/110) for all parameters and very close from references values for this type of honey. In the mean time, the values for HMF and diastase show that the tested honey did not suffer improper treatments. The reference values proceed from the descriptive sheets of the main European monofloral honeys (Persano- Oddo and Piro, 2004), a reliable source of information in our opinion.

Key words: lime honey, legislative and reference values.

INTRODUCTION

Among the animal origin food, honey is a natural product of great importance in human diet from many points of view. First of all, due to its carbohydrates content, it is an important energetic source (1,272 kJ (304 kcal) /100 g) having a high absorption rate. Besides its nutritional role and together with other bee products, honey has a great role in human health (Yoirish, 2001). Honey is important for human health in many issues: wounds treatment**,** (Simon et al, 2008), antibacterial and antimicrobial role (Osato et al, 1999, Varga, 2006), antioxidant properties (Corona and Robinson, 2006), children cough (Paul et al., 2009) or potential role in cancer cure (Tsiapara et al, 2009).

According to Council Directive 2001/110/EC **"**Honey is the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature". Thus, according to their origin, we have blossom honey or nectar honey obtained from the nectar of plants and honeydew honey obtained mainly from excretions of plant sucking insects (*Hemiptera*) on the living part of plants or secretions of living parts of plants. Baker's honey is a type of honey which is suitable for industrial uses or as an ingredient in other foodstuffs which are then

processed and may have a foreign taste or odor, or have begun to ferment or have fermented, or have been overheated.

First of all, the composition and properties of the honey depend on the floral origin in relation with the geographic area and the climate regime (Turhan et al., 2007). But the processing methods used together with the storage condition can affect the honey quality (White, 1994) especially regarding the temperature of the treatments applied (Nagai et al, 2001).

For that, the present paper investigates some physical-chemical properties of artisanal Lime Honey from Bihor County. This type of honey is obtained from the blossoms of Linden or Basswood trees of several species of Tilia and is known as Linden or Basswood honey in North America and Lime honey in the UK and Europe. It is a premier honey that has been enjoyed for thousands of years. It is also found in central and eastern European countries, Russia and China as the Small-leaved Lime (*Tilia Cordata*)

MATERIALS AND METHODS

Materials

The samples consist of monofloral type of Lime honey (**(***Tilia SPP***)** which were purchased in the market in Oradea town. The Lime honey was labeled as "bio". The samples were purchased from local beekeepers in glass bottles of 1 kg for each honey type.

All samples were from the year 2011.

Methods

All over the world, the characterization of honey is made by physical-chemical characteristics (Gomes et al., 2010, Meda et al., 2005, Rodrigues et al., 2004). The quality parameters were determined according to the instructions of the International Honey Commission (Bogdanov, 2002) except for the glucose content. All physicochemical tests were performed in duplicate.

Moisture content was performed by refractometry, using an Abbe refractometer (Digital refractometer Kruss Germany). All measurements were corrected for temperature and the corresponding % moisture (g/100 g honey) was obtained from the table for the purpose (Bogdanov, 2002).

Electrical conductivity was determined for a solution of 20 g dry matter of honey in 100 ml distilled water by conductimetric assay, using a HACH Conductometer sensiIon 378. The cell constant was determined by measuring the conductivity of a 0,1M potassium chloride solution and the results were corrected for the temperature of the sample.

Ash content - After the removal of water from the samples on an electric plate, the honey is burnt to ashes at 600°C and the residue is weighed.

The pH of the honey was measured in a solution of 10 g honey in 75 ml of CO_2 free distilled water (Bogdanov, 2002) using a HACH electronic pH-meter SensiIon378 with a precision of ±0.01 pH units.

Free, lactonic and total acidity were determined as follows, by titrimetric method: the addition of 0.05 M NaOH was stopped at pH 8.50 (free acidity), immediately a volume of 10 ml 0.05 M NaOH was added and, without delay, back-titrated with 0.05 M HCl to pH 8.30 (lactonic acidity). Total acidity results were obtained by adding free and lactone acidities. (Silva et al, 2009)

For the determination of HMF, the spectrophotometric White method (White, 1979) was used. This method involves measurement of UV absorbance of clarified aqueous honey solutions with and without sodium metabisulphite. Five gr. of honey were dissolved in 25 ml of distilled water, transferred quantitatively into a 50ml volumetric flask, added by 0.5 ml of Carrez solution I and 0.5 ml of Carrez solution II and made up to 50 ml with water. The solution was filtered through paper. After rejecting the first 10 ml of the filtrate aliquots of 5 ml were put in two test tubes; 5 ml of distilled water were added to one tube; 5 ml of sodium metabisulphite solution 0.2% (reference solution) were added to the second. The absorbance of the solutions at 284 and 336 nm was determined using an UV-Visible mini – 1240 Shimadzu spectrophotometer.

Diastase was determined after Shade method using a buffered solution of soluble starch and honey incubated in a thermostatic bath at 40 ^{0}C. Afterwards, 1 mL aliquot of this mixture was removed at 5 min intervals and the absorption of the sample was followed at 660 nm in a UV-Visible mini – 1240 Shimadzu spectrophotometer. The diastase value was calculated using the time taken for the absorbance to reach 0.235, and the results were expressed in Gothe degrees as the amount (mL) of 1% starch hydrolyzed by an enzyme in 1 g of honey in 1 h.

Sugars

Reducing sugars was determined by reducing Soxhlet's modification of Fehling's solution by titration at boiling point against a solution of reducing sugars in honey using methylene blue as an internal indicator. Glucose was determined by Auerbach and Bodlander method when only sugar with aldehyd function is reduced by iodine in basic environment (Popescu et al, 1986). The difference between inverted sugars and glucose gave fructose. The difference in concentrations of inverted sugar before and after the hydrolysis procedure (inversion) was multiplied by 0.95 to reach the apparent sucrose content.

Proline was determined based on the reaction of the proline with ninhydrin in an acidic medium, measuring the absorbance of the resulting product at 517 nm. Note that the coefficient of extinction is not constant. Therefore, for each series of measurements the average of the extinction coefficient of the proline standard solution was used.

RESULTS AND DISCUSSIONS

The obtained values are shown in Table 1 and Table 2. Each determination was performed as a triplicate. The results presented the chemical characteristics of the tested Lime honey samples in comparison with those accepted by European Union (Council Directive 2001/110/EC, 2001) and with the reference ones for this type of honey. The reference values proceed from the descriptive sheets of the main European monofloral honeys (Oddo and Piro, 2004), a reliable source of a large work from The International Honey Commission and Apimondia (IHC). As for Lime honey the number of data taken into consideration was between 34 for proline value and 248 for electrical conductivity.

Table 1

Characterization of the artisanal Lime honey (n = 3), 2011

Nr.	Parameter	UM	Experimental values Mean ±SD	Legislation limit	Reference value mean±SD	Ss
1	Moisture	g/100 g honey	16.2±1.1	Max 20	15.9±0.9	ns
2	Electrical conductivity	nS/cm	0.37±0.05	Max 0,8	0.40±0.07	ns
3	Ash content	mg/kg	0.20±0.06	-	-	-
4	pH	-	4.2±0.3	-	3.8±0.1	ns
5	Free acidity	MEQ/1000 g	29.3±3.5	Max 50	37.2±6.3	**
6	Lactones	MEQ/1000 g	2.2±0.9	-	2.4±2.2	ns
7	Total acidity	MEQ/1000 g	31.5±4.2	-	39.6±5.7	**
8	Fructose + Glucose	g/100 g	72±2.5	Min 60	72.7±2.9	ns
9	Glucose	g/100 g	31.4±2.3	-	30.3±1.8	ns
10	Fructose	g/100 g	40.6±1.1	-	42.4±2.4	ns
11	Fructose/Glucose	-	1.29±0.13	-	1.41±0.12	ns
12	Sucrose	g/100 g	0.5±0.4	Max 5	0.3±0.4	ns
13	Glucose/water	-	1.94±0.09	-	1.90±0.13	ns
14	Proline	mg/kg	474±122	-	956±196	***

Legend: Ss - Statistic significance: $p>0.05$= non-significant; $p<0.05$= * significant; $p<0.01$=** distinctly significant; $p<0.001$=*** very significant in comparison with the reference value

Table 2

HMF and Diastase activity for artisanal Lime honey (n = 3), 2011

Nr.	Parameter	UM	Experimental values			Legislation limit	Reference value mean±SD
			Mean ±SD				
1	HMF	mg/kg	1.744	0.802	0.564	Max 40	-
			1.040±0.620				
2	Diastase activity	Shade scale	5.3	7.8	8.4	Min 8	29.2±7.6
			7.167 ± 1.644				

From the legislative point of view we can observe different situations:

1. parameters 1, 2, 5, 8 and 12 from Table 1 and parameter 1 (HMF content) from Table 2 show a good frame to the admitted values. Regarding the reference values, the statistic analysis (Student test) shows insignificant differences ($p>0,05$) between the obtained experimental values and those from the Lime honey sheet for all parameters, except for free acidity (5) where the difference is distinctly significant ($p<0,01$).

2. parameter 2 (Diastase activity) is lower that the admitted value. Moreover, looking to the values for the three samples, the first one of them is lower, the next one is closer and the third one is higher that the admitted value. Regarding the reference values, the statistic analysis (Student test) shows very significant differences ($p<0.001$) between the obtained experimental values and those from the Lime honey sheet in. This situation is due most probably to an improper storage or thermal treatment (Tosi et al, 2002, 2004).

Looking to the parameters not included in the legislation in comparison with the reference values, the parameters referring to sugar content and lactones show insignificant differences (($p>0,05$) between the obtained experimental values and those from the Lime honey sheet in (table 1) and very significant differences ($p<0.001$) for proline content (14).

The great majority of the present experimental values are comparable with those reported for Lime honey in Europe (Persano 1995 and 2000, Ivanov Ts., 1997 and 2002, Golob T. and Plestenjak A, 1999, Mărghitaş et al., 2009).Thus ash content value 0.20±0.06 mg/kg range between (0.019-0.30), pH value (4.2±0.3) range between (4.1-4.4), lactone content of (2.2±0.9 MEQ/kg) in comparison with 2.1 MEQ/kg, free acidity

(29.3±3.5 MEQ/kg) range between (22,1-25,0 MEQ/kg), total acidity (31.5±4.2 MEQ/kg) range between (24.2-24.6 MEQ/kg, proline content (474±122 mg/kg) range between (420 -469 mg/kg), glucose content (31.4±2.3 mg/100 g) range between (27.3 -30.7 mg/100 g) fructose content (40.6±1.1 mg/100 g) range between (38.3 – 39.9 mg/100 g) and all ratios F+G, F/G and G/W.

As for sucrose content (0.5±0.4 mg/100 g) in comparison with 1.23 mg/100 g reported by Iva 1997, the difference can be due to the moment of harvest meaning an early harvest of honey so sucrose has not had the time yet to be inverted by invertase.

All the values founded in literature for Diastase number for Lime honey meet the legal criteria and they are much higher than the ones found in our study, being between 13.2±1.94 and 17.9±5.3. However, those values are much lower in relation with the reference values *(29.2± 7.6)*

CONCLUSIONS

The results lead to some conclusions:

- The tested physico-chemical parameters show that the tested samples of Lime honey are concordant with the European requirements for all criteria but Diastase number;
- The Diastase number value shows that the tested sample was subject to improper storage or thermal treatment;
- The value for HMF for the same sample is much higher than in the others, even if it is under the legal limit;
- The three tested samples show inhomogeneous values for HMF and Diastase number, so we can presume that different beekeepers use different procedures for storage and/or thermal treatment;
- The characteristics of the tested Lime honey are very close to those of the same kind of honey in other European counties;
- The values for HMF and diastase show that the tested honey was not submitted to improper thermal treatments.

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