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QUALITY OF CHESTNUT HONEY MODIFIED BY THERMAL TREATMENT

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ABSTRACT
Honey is a natural product of animal origin very important in human diet from many points of view. Thermal treatment is used for processing reasons, the filling being facilitated by the decrees of viscosity. In the same time heating delays the crystallization process and prevents fermentation. Some of the minor constituents of honey, very important for its quality, are affected by heating. That is the case of HMF witch level is an indicator of freshness and proper storage. The present paper investigates the influence of thermal treatments, temperatures and period and a possible relation between HMF formation and pH alteration in Chestnut honey during this process. The samples of Chestnut honey purchased on the market in Oradea, Bihor and their main physico-chemical characteristics were determined mainly according to IHC methods: moisture, electrical conductivity, ash, HMF, ph, acidity (total, free and lactones), diastase activity, sugars, proline. The experimental values meet the European Council criteria. The samples were heated at 70°C for 1, 4, 8, 12, 24, 36 and 48 hours. Then they were cooled down to 4 °C by immediately plunging the tubes in an ice bath and analyzed for HMF and pH. The formation of HMF is strongly dependent on time at all testing temperatures, direct correspondence improves by the rising of temperature for R^2 from 96.36% at 50^0 C to 98.65% at 70^0 C and 90^0C. The very significant difference between initial values and those found after treatment appears before the allowed limit for HMF (40 mg/kg – Council Directive, 2001) is reached for 50, 60 and 70^0C unlike the situation recorder at 90 ^{0}C. The decreases of pH depend on the applied temperature from 12.7% at 50^0C to 41.6% at 90^0C, regarding the initial value. The effect is very strong in the first day at all temperatures ranging between 77% at 50^0C to 89% at 90^0C, from the total decrees. The results lead to the conclusion that Chestnut honey can be conditioned until 60^0C without over crossing the admitted limit for HMF and the acidity of the environment influences the formation of HMF especially in the first 24 h of thermal treatment.
Keywords: chestnut honey, HMF, thermal treatment, pH

1. INTRODUCTION

According to their origin, there is blossom honey or nectar honey obtained by bees (*Apis mellifera)* 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. Honey is a natural product of animal origin very important in human diet from many points of view. 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, the pharmaceutical value of honey was recognized from the beginnings of the medicine for wounds treatment**,** including burns (Simon et al., 2008; Molan 1999). A lot of researchers reveal its antibacterial and antimicrobial role (Molan 1995; Varga, 2006; Gomes et al, 2010) or potential role in cancer cure (Tsiapara et al, 2009). Antioxidants constituents polyphenols such as flavonoids and phenolic acids, may function as natural antioxidants in human diet (Meda et al., 2007; Blasa et al., 2005; Nagai et al., 2001)).

The main component of honey is sugar but the special qualities of this important food are due mainly to its minor constituents of which some can be affected by heating (Turhan et al., 2008; Tosi et al., 2008). It is the case of HMF and enzymes such amylase and invertase (Fallico et al., 2004; White, 1994). But thermal treatment is used for processing reasons, the filling being facilitated by the degrees of viscosity. In the same time heating is delaying the crystallization process and prevents fermentation (Tosi et al., 2002; Nozal et al., 2001; Singh et al., 1988). The great majority of the consumers do not accept crystallised honey because of its waxy aspect although for others it is considered a good quality indicator meaning the product was nor treated (Tosi et al., 2004).

HMF (5-hydroxymethyl 2-furaldehyde) is considered the most important degradation product acid-catalyzed dehydration of hexoses in food (Belitz and Grosch, 1999). Honey, with its content in glucose and fructose and the content in free acids meets the conditions required for the HMF formation during heating process or storage. So it is considered an excellent indicator of the honey's freshness and proper storage. According to ANNEX II - Composition Criteria for Honey of the Council Directive 2001/110/Ec, HMF content of honey should be under 40 mg kg^{-1} in general, except baker's honey and under 80 mg kg^{-1} for honeys of declared origin from regions with tropical climate.

The present paper investigates the influence of thermal treatments, temperatures and period, and a

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possible relation between HMF formation and pH alteration in Chestnut honey during this process.

2. MATERIALS AND METHODS

2.1 Materials

The samples consist in a monofloral type of honey, that is Chestnut (*Castanea sativa L.*) which was purchased in the market in Oradea city. The source was three different beekeepers. The samples were purchased from local beekeepers in glass bottles of 1 kg for each one and were stored at 4^0C. All samples were from the 2010.

2.2 Methods

The quality parameters were determined mostly according to the instructions of the International Honey Commission (Bogdanov, 2002).

All physicochemical tests were performed in duplicate.

2.2.1. Moisture content

The determination of moisture was performed by refractometry, using an Abbé refractometer (Abbé Digital refractometer Krüss 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)

2.3.2. Electrical conductivity

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.

2.3.3. 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.

2.3.4 pH

The pH of the honey was measured in 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.

2.3.5 Free, lactonic and total acidity

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)

2.3.6 HMF content

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. Fife g 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.

2.3.7 Diastase activity

Diastase was determined after Shade method using a buffered solution of soluble starch and honey incubated in a thermostatic bath at 40 ^{0}C. There after, 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.

2.3.8 Sugars

Reducing sugars were 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 whit aldehyd function are 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.

2.3.9 Proline

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.

Thermal treatment

The honey samples were heated at 70°C for 1, 4, 8, 12, 24, 36 and 48 hours. Then they was cooled down to 4 °C by immediately plunging the tubes in an ice bath and analyzed for HMF, free acidity and lactones. Total acidity results by calculation. The samples were filled into tubes hermetically sealed. After the adjustment of the water bath to the process temperature, thermal treatment process was started with redrawing samples for analysis at the required time.

3. RESULTS AND DISCUSSIONS

Table 1 presents the values for the chemical characteristics of the tested chestnut 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 (Persano-Oddo and Piro, 2004), a reliable source of a large work from The International Honey Commission and Apimondia (IHC). As for Chestnut honey the number of data taken into consideration was between 69 for proline value and 406 for electrical conductivity.

From the legislative point of view (parameters 1, 2, 5, 8, 9, 10 and 14) the tested samples show a good frame to the admitted values. Regarding to the reference values, the statistic analysis (Student test) shows insignificant differences ($p > 0,05$; $-1.0078 < t < 1.854$) between the obtained experimental values and those from the Chestnut honey sheet.

Most of the present experimental values are comparable with those reported for Chestnut honey in Europe (Küçük et al., 2007; Zappala et al., 2005; Devillers et al., 2004; Fallico et al., 2004; Marini et al., 2004). Thus moisture value 18.2% range between 17.9 – 19.7%, electrical conductivity value 1.21 mS/cm range between 1.12 – 1.48 mS/cm, pH value 5.10 range between 5.22 – 5.9, ash content of 0.63 mg/kg range between 0.50 – 0.929 mg/kg. Regarding the sugar content only Devilliers and Fallico reported detailed values in witch the present ones fit, being closer to Fallico's: fructose 37.4% (36.8%), glucose 27.9% (25.1%). For the sucrose content only Küçük found a very high value (2.87% over the reference) meaning an early harvest of honey so sucrose has not the time to be yet inverted by invertase. For total acidity. the content expressed as 11.7 MEQ/1000 g is very close to those reported by Fallico (11.4) or Marini (16.22), but far away from Küçük with 36.7 MEQ/1000 g or one of Zappala's value (23.7).

Table 1

Characterisation of the Chestnut honey (n=3)

Nr.	Parameter	UM	Experimental values Mean ± SD	Legislation limit	Reference value Mean ± SD
1	Moisture	g/100 g honey	18.2±0.2	Max 20	17.5±1.2
2	Electrical conductivity	nS/cm	1.21±0.03	Min 0.8	1.38±0.27
3	Ash content	Mg/kg	0.63±0.11	-	-
4	pH		5.10±0.02	-	5.3±0.5
5	Free acidity	MEQ/1000 g	11.2±0.2	Max 50	13.0±3.5
6	Lactones	MEQ/1000 g	1.5±0.3	-	3.1±2.4
7	Total acidity	MEQ/1000 g	11.7±0.5	-	16.1±4.1
8	HMF	mg/kg	1.028±0.44	Max 40	-
9	Diastase activity	Shade scale	21.1±0.8	Min 8	24.3±5.7
10	Fructose + Glucose	g/100 g	62.3±1.1	Min 60	68.7±2.5
11	Glucose	g/100 g	27.9±1.1	-	27.9±2.5
12	Fructose	g/100 g	37.4±1.1	-	40.8±2.6
13	Fructose/Glucose		1.34±0.08	-	1.48±0.19
14	Sucrose	g/100 g	0.3±0.1	Max 5	0.2±0.3
15	Glucose/water		1.53±0.09	-	1.62±0.13
16	Proline	mg/kg	600.20±65	-	585±167

As for the HMF content, the low value of 1.028 mg/kg is in concordance with the reported ones ranging between 0 and 4.1 mg/kg excepted for Küçük which put the high value of 28.6 mg/kg in correlation with the high acidity content of the tested honey. In the same time we must make the observation that in the present paper. HMF was determined by the White method which is considered to give values comparable with the HPLC method, used by other researchers. at low HMF levels under 5 mg/kg (Bogdanov, 2002; Zappala et al., 2005). Although these two methods give similar results, Zappala considers that the uncertainty associated with the spectrophotometric determination in chestnut honey is very high.

Tables 2 shows the variation of HMF content and pH at 50^{0}C, 60^{0}C, 70^{0}C and 90^{0}C from the initial values to them reached after 48 h of thermal treatment. The admitted value (40 mg/kg) was exceed after 36 hours at 70^{0}C and after 1 hour at 100 ^{0}C.

Table 2

Chestnut honey. HMF and pH variation with time at different temperatures

Time. Hours	Treatment temperatures							
	50°C		60°C		70°C		90°C	
	HMF, mg/kg ±SD	pH ±SD	HMF, mg/kg ±SD	pH ±SD	HMF, mg/kg ±SD	pH ±SD	HMF, mg/kg ±SD	pH ±SD
0	1.028 ±0.44	5.10±0.02	1.028 ±0.44	5.10±0.02	1.028 ±0.44	5.10±0.02	1.028 ±0.44	5.10±0.02
1	1.041 ±0.11	5.05±0.01	1.064 ±0.07	5.07±0.03	2.215 ±0.21	4.88±0.04	32.021 ±5.22	4.62±0.03
4	0.961 ±0.44	4.98±0.06	1.919 ±0.04	4.76±0.02	2.814 ±0.06	4.71±0.12	56.058 ±3.89	4.22±0.07
8	1.215 ±0.29	4.89±0.03	2.408 ±0.23	4.66±0.05	6.711 ±0.17	4.50±0.08	174.601 ±8.22	3.91±0.03
12	2.241 ±0.08	4.66±0.08	5.191 ±0.81	4.55±0.05	11.744 ±0.10	4.42±0.09	695.113 ±11.09	3.41±0.14
24	1.041 ±0.11	4.58±0.06	7.311 ±0.59	4.51±0.04	26.166 ±0.41	4.26±0.12	944.207 ±41.22	3.11±0.11
36	8.205 ±0.12	4.48±0.11	11.511 ±0.31	4.41±0.10	85.209 ±0.35	4.11±0.07	1915.31 ±34.28	2.94±0.18
48	11.221 ±0.52	4.45±0.09	18.302 ±0.46	4.40±0.08	128.554 ±0.21	3.93±0.10	2604.33 ±60.09	2.88±0.09

The formation of HMF is strongly dependent on time at all testing temperatures. as it can be seen in table 2. Figures 1 and 2 show that direct correspondence improves by the rising of temperature, R^2 is 96.36% at 50° C and 98.65% at 70° C and 90°C.

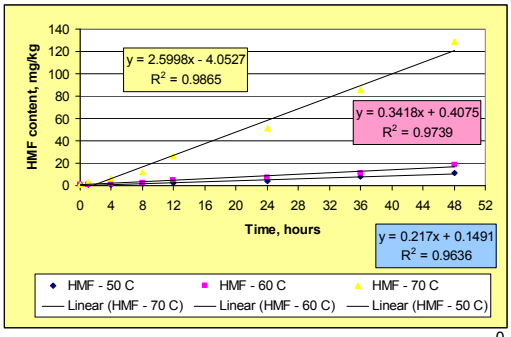

Figure 1 – Variation of HMF content / time, at 50°C, 60°C and 70°C

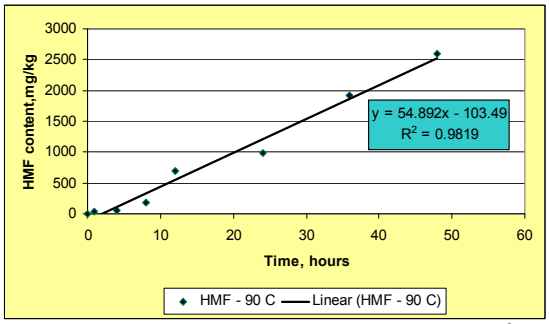

Figure 2 Variation of HMF content / time at 90°C

The dependence of HMF content with time at different temperatures is statistically interpretive by "t - test" (Student) using Prisma 5 for Windows (Table 3). The calculation was made until the very significant difference has been reached.

Table 3

Statistic analysis. t-test

Temperature		Time. Hours				
		1	**4**	**8**	**12**	**24**
50°C	t value	0.0496	- 0.1865	0.6146	2.3011	3.0429
	significance	ns	ns	ns	*	***
60°C	t value	0.1400	3.4538	4.8143	7.8223	14.7859
	significance	ns	*	**	**	***
70°C	t value	4.2169	10.8665			
	significance	*	***			
90°C	t value	10.2475				
	significance	***				

Legend, in comparison with the level without heating:

p>0.05= non-significant (ns);
p<0.05= * significant;
p<0.01=** distinctly significant;
p<0.001=*** very significant

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At 50^{0}C the growth is not-significant during the first 8 hours of treatment; it becomes significant after 12 hours and very significant after the first day. At 60^{0}C it is not-significant during the first hour of treatment. it becomes significant after 4 hours, distinctly significant after 8 hours and very significant after the first day. The rise of the temperature leads to a distinct result, so the differences gets very significant after 4 hours at 70^{0}C and from the beginning at 90 ^{0}C. The very significant difference appears before the allowed limit for HMF (40 mg/kg – Council Directive. 2001) is reached for treatment at 50, 60 and 70^{0}C unlike the situation recorder at 90 ^{0}C. Thus initial value of untreated honey becomes very important for choosing the right condition for thermal treatment without affecting the quality of the product, especially since this procedure is performed more often in air ventilated chambers at 40-50^{0}C for several days then by immersion of the honey drums in hot water (Fallico et al., 2004).

Since HMF is a cyclic aldehyde formed by fructose and glucose dehydration in an acid media we seek for a link of his levels and pH. Dates in table 2 show that the decreases of pH depend of the applied temperature. The higher the treating temperature, the more obvious is the effect on the pH, from 12.7% at 50^{0}C to 41.6% at 90^{0}C from the initial value. In the same time the effect is very strong in the first day at all temperatures ranging between 77% at 50^{0}C to 89% at 90^{0}C, from the total decrees. Therefore the acid environment seems to be the main cause of HMF formation in the first half of the thermal treatment, then the responsibility moves to Maillard type degradations.

As it can be seen in figures 3, 4, 5 and 6 the correlation HMF - pH belongs at a logarithmic scale and improves by the rising of temperature. R^2 have values from 90.55% (60^{0}C) and 89.64% (50^{0}C) to 98.20% (70^{0}C, 90^{0}C)

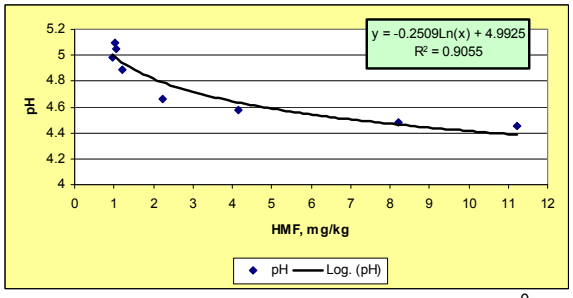

Figure 3 - Variation of HMF content / pH. at 50^{0}C

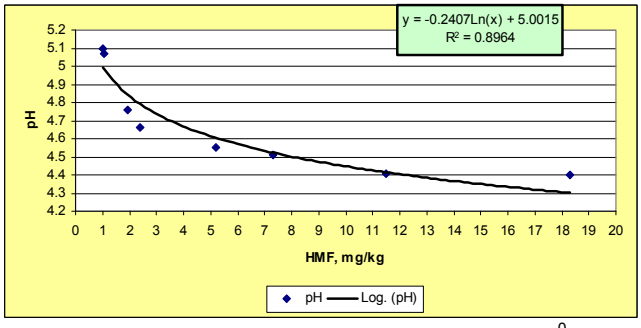

Figure 4 - Variation of HMF content / pH. at 60^{0}C

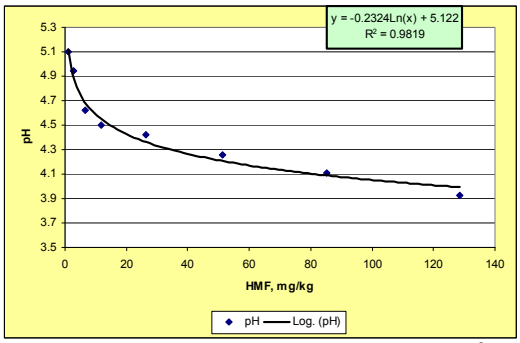

Fig 5 - Variation of HMF content / pH. at 70^{0}C

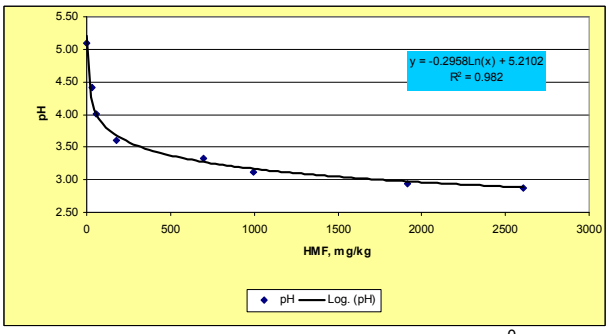

Fig 6 - Variation of HMF content / pH. 90^{0}C

We consider that a good comparison with the situation described by Fallico can not be made because in his case HMF was detectable in Chestnut honey only after 48 hours of heating at 70^{0}C showing a lack of HMF in the tested samples. But for pH value, in Chestnut honey it was reported the same trend of decreasing with time at every testing temperature.

5. CONCLUSIONS

- The tested physico-chemical parameters show that the tested samples of chestnut honey are concordant with the European legislative requirements and with them of the descriptive sheets for monofloral European honey.
- The chestnut honey contains low amounts of HMF at normal temperature. So it seems that the limit of 40 mg/kg could be too high to indicate thermal treatments or improper storage in the Chestnut honey and further modification

of current admitted values could be taken into consideration.

- The HMF level is strongly related to time of heating at temperature from 50°C to 90°C even if the initial values are very low.
- This type of honey can be conditioned until 60°C without over crossing the admitted limit for HMF. That is important for producers in order to provide an easy filling product with lower energy consume, what could have a good impact on prices.
- The acidity of the environment influences the formation of HMF especially in the first 24 h of thermal treatment, so this parameter should be kept under automatic control during the thermal treatment of the honey.

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