

THE INFLUENCE OF STORAGE CONDITIONS ON THE FRESHNESS OF SELECTED MONOFLORAL HONEY

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Abstract

Like for any foodstuffs, freshness of honey is one of the main quality parameters in order to put it on sale. This paper presents the results of different storage conditions on HMF content, as an indicator for freshness and proper thermal treatment of honey. Acacia and lime honey samples of different origin were stored during one year in three different conditions: refrigerated, room temperature at dark and direct sun light. HMF content was evaluated every four months by White spectrophotometrical method. All storage conditions lead to rising of HMF content but with different ratio depending on time and type of investigated honey. In some cases even linear correlation between time and HMF content was found.

Key words: acacia and lime honey, storage, HMF content

INTRODUCTION

Food quality is a subject of great interest not only as a marketable product but also because human health is closely related to it. As for food, it is not suitable to speak in terms of degree in what it concern quality. There are certain levels needed to be taken into consideration depending of the investigated parameter and usually legislation specifies the limit values as a maximum or a minimum, according to the case. Freshness as a quality parameter is mandatory and various foods require different investigation in order to asses it. Those parameters are directly correlated to food's specific composition.

Honey is an animal origin food produced by bees from two different sources, floral nectar and honeydew (Mărghitaș, 2005). The main constituent of honey are sugars, fructose and glucose representing minimum 60%, but more then 25 oligosaccharides were identified too (Anklam, 1998). Apart from water which must be under 20%, minor components of honey are antioxidants such as phenolic compounds, aminoacides as proline, vitamins (vitamin C) and enzymes as catalase, peroxidase, glucose-oxidase, amylase. Among those components, fructose and amylase are related to a specific freshness indicator or to an improper technological handling.

HMF content and diastase activity (ID) are the parameters of honey related to freshness and thermal treatments statutory in Romanian (SR

784/1-2009 and 784/2-2009) and European (CE 2001/110) legislation. HMF is the abbreviation for hydroxymethylfurfural, an aldehydic compound. Natural fresh pure honey does not contain HMF. It is formed by dehydration of some sugars, mostly fructose (Rosatella et al, 2011). Glucose too can form HMF, but through a more complex mechanism (White, 1994). Temperature and acidic environment influence HMF formation (Fallico et al, 2004, Turhan et al, 2007) so storage conditions need to be carefully supervised. Due to its viscosity, honey requires thermal treatment usually performed by keeping it for 4 to 7 days at 45-50⁰C or by immersing the honey drums in hot water (Fallico et al, 2004). Short time (15" to 60") thermal treatment at higher temperatures (80 to 100⁰C) affects HMF content and ID without falling under the permitted limits (Tosi et al.2004). On the other hand, honey must be stored before selling so in time the storage conditions, by default temperature, can affect HMF formation.. This is even a bigger concern in countries with natural hot climate (Nombre et al, 2010).

The present paper investigates the impact of different storage conditions during 12 month on HMF content (ID) in two different monofloral honey, Acacia and Lime honey from manufacturer and marketable.

MATERIALS AND METHODS

Materials

The tested materials consist in acacia (*Locust tree*) honey and lime (*Tilia sp.*) honey, two samples of each, coded as follows:

1. SPA – Acacia honey provided directly by beekeepers
2. SCA - Acacia honey purchased from general store
3. TPA – Lime honey provided directly by beekeepers
4. TCA - Lime honey purchased from general store

In this paper we refer to SP and TP as “manufacturer honey” and to SC and TC as “marketable honey”. All samples were divided and stored in three different conditions: 4⁰C (SPB and SCB), ambient temperature at dark (SPC and SCC) and directly in sun light, by the window (SPD and SCD).

All the used reagents were p.a. grade: sodium metabisulphite potassium hexacyanoferrate(II), K₄Fe(CN)₆.3H₂O, double sublimate Iodine and starch from Merck Germany, zinc acetate, Zn(CH₃.COO)₂.2H₂O from Chimopar Romania. The laboratory devices were ultrasonic bath Elma S 100H – Elmasonic and spectrophotometer UVMini-1240 (Shimatzu).

Methods

The HMF content was determined by the White method (White, 1979, Bogdanov, 2002). This spectrophotometric method involves measurement of UV absorbance of clarified aqueous honey solutions with and without sodium metabisulphite. A quantity as close as possible to five g (W, exactly weighted) of honey were dissolved in 25 ml of distilled water, transferred quantitatively into a 50 ml volumetric flask, then proteins were precipitated by adding 0.5 ml of Carrez solution I and 0.5 ml of Carrez solution II. The content was made up to 50 ml with water and the solution was filtered through filter 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 (sample solution); 5 ml of fresh 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 a UV-Visible mini – 1240 Shimadzu spectrophotometer. Dilutions (D) of both test and reference solution was used if the absorbance at 284 nm exceeded a value of about 0.6. HMF content, in mg/100g, was calculated as $(A_{284} - A_{336}) \times 149.7 \times 5 \times D/W$.

Procedures were applied on fresh samples and every four months, during a year, in all experimental storage conditions. The experiment was conducted between May 2013 and May 2014,

Statistical analysis - All tests were performed as triplicate for initial values and in duplicate for the following ones and are presented as mean \pm SD. The results of different experimental variants were compared by T-test for independent samples. Differences between means at 95% ($p < 0.05$) confidence level were considered statistically significant.

RESULTS AND DISCUSSIONS

The initial values of HMF content and ID are shown in table 1 for the initial samples.

Table 1

HMF initial content, mg/100g				
HMF, mg/100g	Acacia honey		Lime honey	
	SPA	SCA	TPA	TCA
HMF, mg/100g				
Sample 1	0.60	1.43	0.35	0.82
Sample 2	0.54	1.59	0.57	0.84
Mean \pm SD	0.57 \pm 0.03	1.51 \pm 0.08	0.46 \pm 0.11	0.83 \pm 0.01

I.

Romanian legislation (SR 784-1/2009 and SR 784-2/2009) stipulates different values for HMF content, depending on the origin: max 1 mg/100g for honey taken from beekeepers and maximum 1,5 mg/100g for the trade one when it is sold in jars, which cater for technological need of packaging this foodstuff. These values are lower than those from European Directive (CE nr 2001/110/CE) which is 4 mg/100g. The experimental values range from 46 to 57% in respect to the admitted limits for manufacturer's honey and from 55% to 100% for the marketable one. Moreover the HMF content is higher for acacia honey compared to lime honey, which is unexpected because acacia honey is very fluid so it doesn't need thermal treatment for liquefying in order to be put in jars. Our experimental values for acacia honey comply to those observed by other researchers in Europe by Golob and Plestenjak, 1999(0.52 \pm 0.04), Devillier et al, 2007 (1.848 \pm 0.069), Tucak et al, 2007 (0.391 \pm 0.145) Kasperova et al, 2010 (1.108 \pm 0.089), Isopescu et al, 2014 (0.83 \pm 0.77) as well as for lime honey: Golob and Plestenjak, 1999 (0.363 \pm 0.313), Tucak et al, 2007 (0.34 \pm 0.442) Zielinska et al, 2014 (1.31 \pm 1.22), Isopescu et al, 2014 (0.43 \pm 0.35).

The evolution of the hidroxymethylfurfural content of acacia honey, for all the storage conditions, during the 12 month of the experiment are in table 2 and for Lime honey in table 3, as mean \pm SD.

Table 2

Evolution of HMF content of Acacia honey, mg/100g

	HMF, mg/100g				Statistical significance	
	SPA	+ 4 month		+ 8 month		+ 12 month
SPB	0.57±0.03	0.55±0.07	ns	0.58±0.02	ns	0.6±0.03
SPC		0.8±0.08	*	0.87±0.09	*	0.92±0.12
SPD		0.99±0.09	**	0.98± 0.12	**	1.22± 0.15
	SCA	+ 4 month		+ 8 month		+ 12 month
SCB	1.51±0.08	1.5±0.10	ns	1.57±0.09	ns	1.55±0.02
SCC		1.65±0.19	ns	1.74±0.11	ns	1.74±0.07
SCD		1.99±0.1	*	2.15±0.13	**	2.44±0.2

Legend: ns Non-significant, * Significant, **Distinctly significant (p<0.01)

Data from table 2 emphasises the fact that no matter the storage conditions, during 12 months HMF content is growing in acacia honey, the highest value 1,22 mg/100g (manufacturer honey) and 2.44 mg/100g (marketable honey) being reached for the samples stored in direct sun light (D variant). The HMF content remains practically unchanged for the refrigerated samples (A variant), statistical analysis show insignificant differences. As for the one kept at ambient conditions (C variant) HMF rise more for manufacturer honey (61%) than for marketable honey (15%), but the value was still under the admitted limit due to the low initial HMF content. For C and D variants, the bigger increase was observed during the first laps of time of the experiment corresponding to the summer period of 2013, characterised by high temperatures, so even samples stored in the dark (B) were influenced. Anyway, only for the D variant the limit permitting the takeover from beekeepers was overpasses.

Statistical analysis disclosed some differences between the tested samples. For manufacturer's honey, C and B variants, the storage conditions had a greater influence than for marketable one. A linear correlation between HMF content and time was found for variant C ($R^2 = 0.8577$ and 0.8593) and D ($R^2 = 0.8735$ and 0.9578) for both acacia honey samples.

Table 3

Evolution of HMF content of Lime honey, mg/100g

	HMF, mg/100g					Statistical significance	
	TPA	+ 4 month		+ 8 month		+ 12 month	
TPB	0.46±0.11	0.45±0.07	ns	0.48±0.09	ns	0.52±0.05	ns
TPC		0.51±0.13	ns	0.54±0.11	ns	-	-
TPD		0.55±0.09	ns	-	-	-	-
	TCA	+ 4 month		+ 8 month		+ 12 month	
TCB	0.83±0.01	0.85±0.18	ns	0.89±0.10	ns	1.02±0.14	ns
TCC		0.91±0.07	ns	0.98±0.03	ns	-	-
TCD		-	-	-	-	-	-

Legend: ns Non-significant (p>0.05)

The “-“sign in Table 2 refers to the fact that the applied White method leads to inconclusive results in some cases due to the extent of the dilution needed in order to reach UA under 0.600. So the discussion concerns only the concrete results in B and C variants. As a general observation, for lime honey too the HMF content augmented during the 12 month of the experiment between 13% for manufacturer honey and 23% for marketable honey in B variant-12 month. Statistical analysis shown non-significant differences between initial values and all obtained results. For lime honey, unlike acacia honey, B variant showed linear correlation between HMF content and period in B variant. The lack of results does not permit a proper correlation in all variants.

CONCLUSIONS

The experiment leads to some conclusions, as follows:

Storage conditions have an important effect on HMF content of acacia and lime honey related to time and temperature. The magnitude of HMF rising depends on the kind of tested honey.

Refrigeration is not justified if initial values of HMF are not spoiled by brutal thermal treatments. Those initial values, on the moment of taking from beekeepers are essential for maintaining HMF within legal limits, even at ambient temperature, during a year.

Further testing on greater number of samples, with different initial HMF values are needed in order to extrapolate the mathematical correlation between HMF content and periods.

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