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Effects of Botanical Origin and Ageing on HMF Content in Bee Honey

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AK, AC and ZH designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors AK, AC, ZH, AS and NI managed the literature searches, analyses of the study performed the spectroscopy analysis and AK managed the experimental process and ZH identified the species of plant. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: HMF (5-hydroxymethylfurfural) is considered an important quality parameter for honey. Elevated concentrations of HMF in honey provide an indication of origin, storage in poor conditions or age of honey. The objectives of this study were to investigate the effect of aging and botanical origin of honey on the HMF content in it, as well as to analyze the relationship between the content of HMF and fructose/glucose ratio.
Study Design: In this paper, the HMF levels in different botanical origins of sixty bee honeys from Bosnia and Herzegovina (Acacia: *Robinia pseudoacacia*, meadow, chestnut: varii, mountain) were analysed; the influence of ageing on HMF formation was also investigated.
Place and Duration of Study: Department of Chemistry, University in Tuzla, between January 2011 and Mart 2011.
Methodology: Determination of HMF content was done by spectrophotometric White method. Content analysis of fructose and glucose in honey samples was performed using the HPLC.
Results: Concentration of HMF in analyzed honey samples ranged from 0.28 mg/kg to 207.45 mg/kg. The HMF formation was correlated with botanical origin, age of honey and fructose/glucose ratio. Samples of 4 year old honey contains on average 52.44% higher

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HMF than fresh honey samples. These results clearly show that longer storage of honey increases the concentration of HMF. In addition, honey exposure to high temperatures affects content of HMF, which catalyzes the dehydration of fructose to form new quantities of HMF. The formation of HMF and its concentration in honey also depends on the botanical origin of honey. Samples of acacia honey showed the highest average content of HMF. The data obtained were statistically elaborated.

Conclusion: Botanical origin, high temperature and storage significantly affect the content of HMF in honey. There is a negative correlation between the F/G ratio and HMF content in analyzed honey samples.

Keywords: 5-Hydroxy methyl furfural; aldehydes; honey; UV/Vis spectroscopy; Photodegradation.

1. INTRODUCTION

HMF is the abbreviation for a compound more correctly called 5-hydroxymethylfurfuraldehyde. It is probably not a constituent of fresh pure honey immediately as stored by honey bees. Its content increases during conditioning and storage. It has been known for very many years to be formed from simple sugars, especially fructose, by the action of acid. The process involves the loss of three water molecules from fructose. HMF formation from glucose involves a prior isomerisation or rearrangement to fructose before the loss three water molecules. HMF (5-hydroxymethylfurfuraldehyde) measurement is used to evaluate the quality of honey.

HMF content in honey also depends on its botanical origin. Honey processing involves heating in air ventilated chambers, at 45–50°C for 4-7days or by immersion of honey drums in hot water. This is to prevent crystallysation or fermentation of the sugar solution [1]. Heating of unifloral honey leads to different HMF levels in honey [2]. HMF is formed during acid-catalysed dehydration of hexoses [3] wich is associated with the chemical characteristics of honey, like pH, total acidity, mineral content [4,5,6,7,8].

Codex Alimentarius [9] established that the HMF content of honey after processing and/or blending must not be higher than 80 mg/kg. The European Union [10] fixed a HMF limit in honey of 40 mg/kg with the following exceptions: 80 mg/kg for honey coming from countries or regions with tropical temperatures, 15 mg/kg for honey with low enzymatic level (3-8 Schade Units).

As early as 1933, it was written that ordinary storage of honey, as well as its heating, could lead to accumulation of HMF in honey [11]. By the 1950s, methods for the quantitative measurement of HMF in honey became available [12,13,14]. Using the new methods, studies were made on the various factors affecting the rate of accumulation of HMF in honey. Because this aspect is extremely important to the setting of HMF standards for honey, it will be necessary to investigate just how the HMF content is affected by the botanical origin and ageing of honey.

The botanical origin of nectar has an outstanding influence on the chemical composition of honey. Honey is a natural product varying not only in colour, taste and odour but also in its chemical composition. Its characteristic properties are depending on the floral source of the nectar. For declaration of a honey as unifloral, the ratio of fructose/glucose was also an important parameter. We have examined the influence of botanical origin and ageing at room temperature on HMF content in sixty samples of honey.

The International Honey Commission [15] recommends three methods for the determination of HMF. These methods include two spectrophotometric methods widely used in routine analysis, White determination (1979) and Winkler determination (1955), as well as the HPLC. The method described by White involves measurement of UV absorbance of clarified aqueous honey solutions with and without bisulphite while that of Winkler involves measurement of the UV absorbance of honey solutions with added barbituric acid and p-toluidine [15]. White's repeatability r and the reproducibility R and the HPLC method are better than Winkler's method [15].

Profile of carbohydrates in honey is the subject of numerous investigations. Qualitative and quantitative determination of carbohydrates in honey is made during routine quality assessment, determination of counterfeiting honey or determining the botanical and/or geographical origin of honey. Nectar honey, according to the Rules on the quality of honey, must contain a minimum of 65% of reducing sugars calculated as invert sugar and honeydew at least 45% of reducing sugar [16,17]. Ratio of fructose and glucose (F/G) is typical for certain types of honey and in most cases is greater than 1.00 [18].

2. MATERIALS AND METHODS

2.1 Samples

Analysis was performed on sixty honey samples belonging to the following botanical origin: blossom (acacia, meadow and mountain) and honeydew (chestnut). The samples were collected in cooperation with Beekeepers Association of Tuzla Canton. All samples were stored in plastic bottles at room temperature and at the same conditions. The botanical origin of the samples is presented in Table 1.

Table 1. Botanical, geographical origin and year of production of the analyzed Honey Samples

Sample No.	Botanical origin	Geographical origin	Year
1.			2009
2.			2009
3.		*Tuzla*	2009
4.			2013
5.			2009
6.			2009
7.			2013
8.		*Laništa*	2009
9.			2009
10.		*Kladanj*	2013
11.			2009
12.		*Miričina*	2009
13.	*Blossom (Meadow)*	*Bosanski Šamac*	2009
14.		*Derventa*	2009
15.		*Srebrenik*	2013
16.		*Čelić*	2013

Table 1 Continued …..

17.		*Zvornik*	2013
18.		*Mramor*	2009
19.		*Sarajevo, Rajlovac*	2013
20.			2013
21.		*Stara Majevica*	2013
22.		*Gradačac*	2013
23.		*Pelješac*	2009
24.		*Banovići*	2013
25.		*Velika Kladuša*	2009
26.		*Banovići*	2013
27.		*Kladanj*	2013
28.		*Kladanj*	2013
29.		*Tuzla*	2009
30.			2013
31.	*Blossom (Acacia)*		2009
32.		*Čelić*	2009
33.		*Gradačac*	2013
34.		*Koraj*	2009
35.		*Lopare*	2013
36.		*Živinice*	2013
37.		*Kalesija*	2013
38.		*Teočak*	2013
39.		*Banovići*	2013
40.	*Blossom (Mountain)*	*Kladanj*	2013
41.			2013
42.			2013
43.			2013
44.		*Banovići*	2013
45.		*Careva ćuprija*	2009
46.		*Srebrenik*	2013
47.		*Banovići*	2009
48.			2009
49.		*Živinice*	2013
50.		*Zvornik*	2009
51.	*Honeydew (chestnut)*	*Tuzla*	2013
52.		*Koraj*	2013
53.		*Bosanski Šamac, Kornica*	2009
54.		*Bosanska Bijela*	2013
55.		*Zagreb, Croatia*	2009
56.		*Ljubljana, Slovenia*	2009
57.		*Velika Kladuša*	2013
58.		*Vlašić*	2013
59.		*Romanija, Sokolac*	2013
60.		*Bosanski Šamac, Kornica*	2009

2.2 Spectrophotometric Method (White)

In this paper, we chose the White’s method, because p-toluidine used in Winkler’s method may be carcinogenic. In this paper, the measurements were performed by the spectrophotometric method to White. Five grams of honey were dissolved in 25 ml of water, transferred quantitatively into a 50 ml volumetric flask, added by 0.5 ml of Carrez solution and 0.5 ml of Carrez II and made up to 50 ml with water. The solution was filtered through paper rejecting the first 10 ml of the filtrate. Aliquots of 5 ml were put in two test tubes; 5 ml of distilled water was added to one tube (sample solution); 5 ml of sodium bisulphite solution 0.2% (reference solution) was added to the second tube. The absorbance of the solutions at 284 and 336 nm was determined using a „UVmini-1240V SHIMADZU "spectrophotometer. The quantitative value of HMF was determined by using the proposed formula for the method reported by IHC [15]:

$$HMF[mg/kg] = (A_{284} - A_{336}) \cdot 149{,}7 \cdot 5 \cdot D/W$$

Where is:

A_{284}- absorbance on 284 nm
A_{336}- absorbance on 336 nm

$$149{,}7 = \frac{126 \cdot 1000 \cdot 1000}{16830 \cdot 10 \cdot 5}$$

2.3 Determination of Fructose and Glucose Content by HPLC (High Performance Liquid Chromatography) Method

Fructose and glucose contents of the honey samples were determined using the HPLC high performance liquid chromatogram Agilent 1100 with 100 NH_2 column lichrospher 5 μm, 250 x 4.0. The parameters determining the content of fructose and glucose in the samples of honey are given in Table 2 [14].

Table 2. The parameters determining the content of fructose and glucose in the samples of honey

Parameter	Value
Mobile phase	Acetonitrile (99.8 %)/ water (75:25),
Flow	1.3 ml
Detector	RI
Temperature	30°C
Sample volume	20 μL

3. RESULTS AND DISCUSSION

HMF content in honey is an important parameter for determining the quality of honey, its age, antioxidant activity, as well as its nutritional value [19,20].

HMF content in sixty analysed honey samples are given in Table 3.

Concentration of HMF in analyzed honey samples ranged from 0.28 mg/kg to 207.45 mg/kg. Allowed mass fraction of HMF in honey is 40 mg/kg. Exceptions are the honey samples from warmer climatic conditions, which allowed mass fraction of HMF to 80 mg/kg. In six analyzed honey samples, HMF concentration was above the allowable 80 mg/kg, and twenty analyzed samples showed concentrations greater than 40 mg/kg.

Table 3. Concentration of HMF [mg/kg] in analyzed honey samples

Sample No	Meadow	Sample No	Acacia	Sample No	Mountain	Sample No	Chestnut
1.	48.41	26.	9.63	37.	2.05	47.	10.06
2.	46.1	27.	101.34	38.	3.26	48.	15.03
3.	11.48	28.	22.11	39.	0.42	49.	19.29
4.	85.57	29.	41.24	40.	2.11	50.	43.13
5.	6.84	30.	33.31	41.	1.02	51.	22.07
6.	10.11	31.	101.02	42.	4.37	52.	41.22
7.	41.15	32.	42.34	43.	0.55	53.	11.07
8.	40.86	33.	19.19	44.	1.04	54.	18.26
9.	43.18	34.	48.2	45.	32.7	55.	10.18
10.	5.82	35.	45.2	46.	1.08	56.	40.06
11.	11.64	36.	61.2			57.	9.16
12.	107.45					58.	4.16
13.	8.39					59.	5.32
14.	82.12					60.	19.22
15.	2.41						
16.	0.28						
17.	5.58						
18.	107.5						
19.	2.91						
20.	4.46						
21.	2.41						
22.	3.07						
23.	6.25						
24.	2.18						
25.	43.52						

Table 4. Shows standard deviation for HMF concentration in analyzed honey samples.

Table 4. Standard deviation for HMF [mg/kg] content in analyzed honey samples

Meadow	Acacia	Mountain	Chestnut
33,53	28,77	9,35	12,74

Four samples of honey showed HMF concentration above 100 mg/kg, which is still considered an indicator of counterfeiting honey using invert syrup.

Three of the four samples with HMF concentration above 100 mg/kg were produced four years before analysis. In longer-term storage of honey, HMF in it grows, so the higher concentration of HMF in some of the analyzed samples can be explained by the influence of storage of honey for a period of 4 years.

Mendes and colleagues analyzed 25 types of honey from the area of Portugal and Spain, and in 12 samples recorded a share of HMF above allowable, 40 mg/kg, while one sample even had 471 mg/kg [21].

Share of HMF in fresh honey is very small and is below 1 mg/kg. However, this share is growing rapidly if the temperature is above 20°C [19,20].

Average concentrations of HMF in honey samples of different ages in the analysis are shown in Fig. 1.

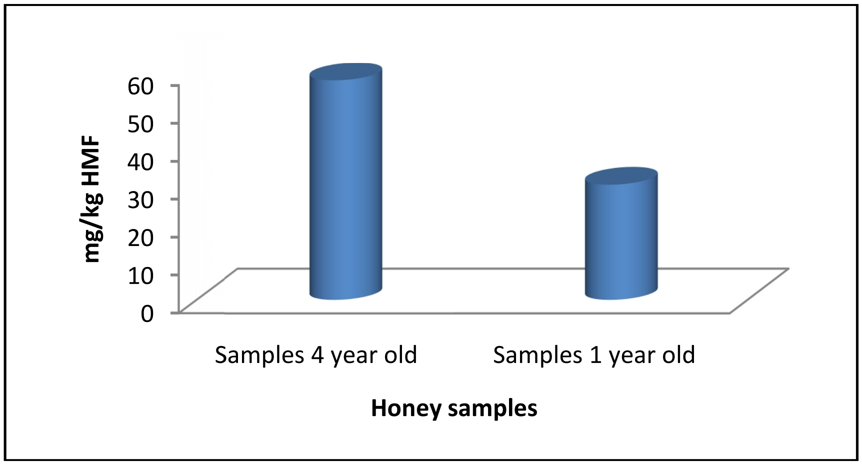

Fig. 1. The average concentration of HMF [mg/kg] in the samples of different ages in the analysis

Samples of 4 year old honey contains on average 52.44 % higher HMF than the fresh honey samples. These results clearly show that longer storage of honey increases the concentration of HMF. In addition, honey exposure to high temperatures affects content of HMF, which favors the dehydration of fructose to form new quantities of HMF. The formation of HMF and its concentration in honey also depends on the type of honey.

The concentration of HMF in honey samples from different botanical origin is shown in Fig. 2.

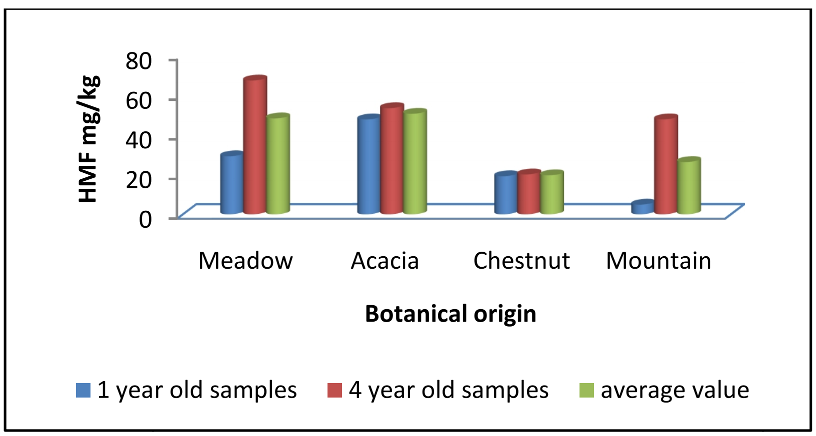

Fig. 2. The average content of HMF in honey samples from different botanical origins and of different ages in the analysis

All analyzed honey samples showed a higher content of HMF with longer storage. Samples of acacia honey showed the highest average content of HMF. Generating HMF in honey favors low pH of honey. Low content of HMF in chestnut honey over other honey samples, can be explained by significantly higher pH values in these honey samples [22].

These results are in accordance with the results of Fallico and his collaborators, who studied the influence of pH and temperature on the concentration of HMF in honey. Their studies have shown that the formation of HMF at low temperatures was correlated with the pH of honey. For example, in chestnut honey, which had the highest pH, there was no formation of HMF, even after 144 hours of heating at a temperature of 50°C, while in other types of honey a certain amount was formed, depending on the heating period, the proportion of acid and pH-values [5].

Leaving honey at different temperatures significantly affects the concentration of HMF in it. Indian scientists heated two types of honey to 70°C for 60 min, and increased the share of HMF from 44.5 to 76.6 mg/kg and from 12.3 to 31 mg/kg and concluded that higher share of HMF in one type of honey can be related to its low pH [5].

Scientists Kubis and Ingr, in their research, came to the conclusion that the honey samples heated to 50°C slowly increase the share of HMF in samples, while heating at 82ºC increased the share of HMF ten times the limit value [19].

According to the research, the time required for the development of 30 mg/kg HMF in honey at a temperature of 30°C is up to 300 days and at a temperature of 80°C, it takes less than 2 hours to achieve the same amount of HMF [23,24].

Since HMF is formed by dehydration of fructose, we compared F/G ratio with the content of HMF in honey samples analyzed. HMF is formed from glucose as well. Rate constant in case of fructose as a substrate is higher then in case of glucose. The results of this analysis are shown in Table 5.

Table 5. Correlation of F/G ratio and the content of HMF in analyzed honey samples

Botanical origin of honey samples	%glucose	%fructose	F/G Ratio	HMF content [mg/kg]
Meadow	26.80	46.30	1.73	29.188
Acacia	30.70	47.50	1.55	47.705
Chestnut	23.80	44.70	1.88	19.160
Mountain	21.55	47.58	2.20	4.860
Pearson correlation (r)			-0,977	
Probability of errors (p)			0,023	
Standard deviation	3,43	1,08		

Based on these results, it is clear that there is a negative correlation between the F/G ratio and HMF content in analyzed honey samples. So in 97.7% of cases, honey that contains a high concentration of HMF has a smaller fructose/glucose ratio. So at higher HMF content in honey, concentration of fructose is smaller, which is expected considering that the HMF is formed by dehydration of fructose. This correlation is shown in Fig. 3.

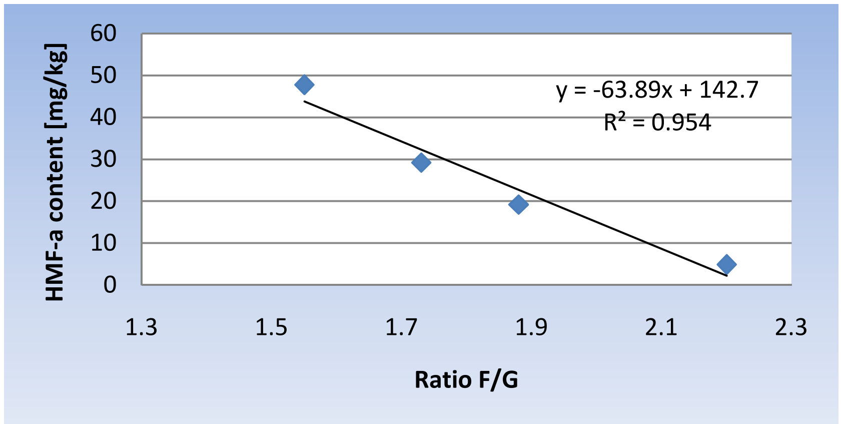

Fig. 3. Negative correlation of F/G ratio, and the concentration of HMF in honey

4. CONCLUSION

Botanical origin, high temperature and storage significantly affect the content of HMF in honey. Share of HMF in fresh honey is very small and is below 1 mg/kg. However, this share is growing rapidly if the temperature is above 20°C. Samples of 4 year old honey contains on average 52.44% higher HMF than the fresh honey samples. The formation of HMF and its concentration in honey also depends on the botanical origin of honey. Samples of acacia honey showed the highest average content of HMF. Generating HMF in honey favors low pH of honey. There is a negative correlation between the F/G ratio and HMF content in analyzed honey samples. So in 97.7% of cases, honey that contains a high concentration of HMF has a smaller fructose/glucose ratio.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Singh N, Singh S, Bawa A S, Sekhon K S. Honey– its food uses. Indian Food Packer. 1998;42:15–25.
2. Fallico B, Zappala M, Arena E, Verzera A. Effects of heating process on chemical composition and HMF levels in Sicilian monofloral honeys. Food Chemistry. 2004;85:305–313.
3. Belitz HD, Grosch W. Food chemistry. Berlin: Springer-Verlag; 1999.
4. Anam OO, Dart RK. Influence of metal ions on hydroxyl methyl furfural formation in honey. Analytical Proceedings Including Analytical Communications, 1995;32:515–517.
5. Bath PK, Singh N. A comparison between Helianthus annuus & Eucalyptus lanceolatus honey. Food Chemistry, 1999;67:389–397.
6. Hase S, Suzuki O, Odate M, Suzuki S. Changes in quality of honey on heating and storage. I. Changes in hydroxyl methyl furfural (HMF) content of honey. Journal of Food Science and Technology. 1973;20:248–256.

7. Singh N, Bath PK. Quality evaluation of different types of Indian honey. Food Chemistry. 1997;58:129–133.
8. Singh N, Bath P K. Relationship between heating & hydroxyl methyl furfural formation in different honey types. Journal of Food Science and Technology, 1998;35:154–156.
9. Codex Alimentarius, Alinorm 01/25. Draft revised standard for honey at step 8 of the Codex procedure; 2000.
10. EU Directive /110/2001 of 02/12/2001 (L10/47)
11. Boer HW DE. Het verbandtusschen de chemischesamenstelling en debotanischeherkomst van in Nederland gewonnenhonig. Chemische Weekblad, 1933;30(23):401-40. Nederland.
12. Schade JE, Marsh GL, Eckert JE. Diastase activity and hydroxyl methyl furfural in honey and their usefulness in detecting heat alteration. Food Research, 1958;23(5):446-463.
13. Winkler 0. Beitragzum Nachweis und zur Bestimmung von Oxymethyl furfural in Honig und Kunsthonig. Zeitschrift for Lebensmittel Untersuchung und –Forschung. German. 1955;102(3):161-167.
14. Bogdanov S. Harmonised methods of the international honey commission, Swiss Bee Research Centre FAM, Liebefeld, CH-3003 Bern. Switzerland; 2002.
15. Bogdanov S. Harmonised methods of the International Honey Commission, responsible for the methods. 1999;1–54.
16. Codex Alimentarius Commission. Revised Codex Standard for Honey, Codex STAN 12-1981, Rev.1 1987, Rev. 2; 2001.
17. CODEX STAN 12-1981. Adopted 1981. Revision 1987 and 2001. Available:http://www.codexalimentarius.org/standards/list-of standards/en/?provide=standards&orderfield=fullreference&sort=asc&num1=codex
18. Krell R. Value-added products from beekeeping. Ch. 2. FAO Agricultural Services Bulletin 1996;124.
19. Wunderlin DA, Pasce SF, Ame MV, Faye PF. Decomposition of hydroxymethylfurfural in solution and protective effect of fructose. J. Agr. Food Chem. 1998;(46)1855-1863.
20. Nozal MJ, Bernal JL, Toribio L, Jimenez JJ, Martin MT. High performance liquid chromatographic determination of methyl antranilate, hydroxyl methyl furfural and related compounds in honey. J Chromatogr. A. 2001; 917:95-103.
21. Mendes E, Brojo Proenca E, Ferreira I. M. P. L. V. O., Ferreira M A. Quality evaluation of Portuguese honey. Carb. Pol. 1998;37:219- 223.
22. Thrasyvoulou A, Manikis J. Some physicochemical and microscopiccharacteristics of Greek unifloral honeys. Apidologie 1995;26:441-452.
23. Spano N, Casula L, Panzanelli A, Pilo MI, Piu PC, Scanu R et al. An RP-HPLC determination of 5-hydroximethylfurfural in honey. The case of strawberry tree honey. Talanta. 2005;68:1390-1395.
24. Sarah W, Kristyn K, Peter B. The quantitation of hydroxymethylfurfural in Australian Leptospermum honeys. Journal of Pharmacognosy and Phytotherapy 2013;5(1):21-25.

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