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DETERMINATION OF HYDROXYMETHYLFURFURAL CONTENT IN NATURAL HONEYS IN POLAND

Agnieszka Obiedzińska, Elżbieta Biller, Mieczysław W. Obiedziński, Monika Tyszka

Faculty of Computer Science and Food Science
Lomza State University of Applied Sciences, Lomza, Poland

E-mail: aobiedzinska@pwsip.edu.pl

Abstract: Hydroxymethylfurfural (HMF) is a heterocyclic aldehyde, which is formed from saccharides, like fructose or glucose during the thermal processing of food and during its storage. In the case of natural honey, the HMF content is used to assess its quality and stability if it has been decrystallized using high temperatures. According to EU legislation, HMF levels in honey should not exceed 40 mg/kg. The purpose of this study was to examine selected domestic honey for HMF content before and after different heat treatments. For determination the high-performance liquid chromatography (HPLC) method with UV-VIS detector was used. On the basis of the obtained results, it was found that sixteen of the seventeen analysed honeys met the quality requirements for honey in terms of HMF content. One honeydew honey (MPS) exceeded the acceptable limit of EU requirements. In most cases, heating with ultrasonic marinator, in a microwave oven and in an incubator resulted in an increase in HMF content in honeys. Microwave heating resulted in the fastest and largest increase of HMF content. All honey samples regardless of the combination of heating methods were within the acceptable limit, except pine honeydew honey treated in a microwave oven.

Key words: honey, heating, decrystallization, hydroxymethylfurfural (HMF), high-performance liquid chromatography (HPLC) .

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Introduction

In recent years, the public is increasingly interested in food issues like where it comes from, how it is produced and processed and how its consumption affects human health. More and more attention is paid to the conscious choice of food products, including low-processed products. One such product is honey, which is a natural sweetener of animal origin used since the earliest times [1, 2]. It is a source of valuable nutrients and bioactive compounds. Due to the content of glucose and fructose it is easily absorbed by the human body [3]. However, the presence of these sugars influences the occurrence of the natural process in honey – crystallization, where honey passes from a liquid phase into a solid phase [4]. It should be emphasized that this process does not reduce the quality of the food product [5]. However, when buying, consumers choose liquid or partly crystallized honeys because they think that crystallized ones are characterized by low quality. Beekeepers or honey producers often adapt their products to the preferences of consumers; therefore, honey processing aims to keep the product as long as possible in a liquid phase, which requires the use of various stages of the technological process: the liquefaction/decrystallization of honey, filtering, heating at the right time and temperature, as well as cooling and storage under appropriate conditions [6–8]. Decrystallization of honey involves honey heating usually to 45°C. This treatment delays its re-crystallization for a long storage period. It may have a negative effect because an improperly carried out process may lead to the overheating of honey and, consequently, to changes in its quality parameters [4]. One of the quality parameters of honey is the content of hydroxymethylfurfural (HMF), which is a heterocyclic aldehyde, and it is formed as a intermediate product of the Maillard reaction or during the dehydration process of monosaccharides, i.e. simple sugars like glucose and fructose [9, 10]. In the case of honey, HMF quantity increases generally in three cases: (1) during long storage under inappropriate conditions; (2) due to improper decrystallization, i.e. excessive heating or (3) by adulterating honey with invert sugars. It is believed that the hydroxymethylfurfural content in honey depends primarily on the variety of honey, chemical composition and acidity [11]. Monitoring of HMF content applies not only to honey but also to other food products, especially carbohydrate-rich products - cereal products (pasta, biscuits, bread, and breakfast cereals), fruit and vegetable preserves (tomato sauces, apple sauces, jellies, and fruit jams), coffee or nuts and other products that required production processes with high temperatures [11–14].

Both negative and positive effects of hydroxymethylfurfural activity on the human body were found [13, 15–17]. As positive effects, it is believed that HMF shows antioxidative, anti-allergic, anti-inflammatory, anti-hypoxic, and anti-sickling activities. It can also be used in the therapeutic treatment of cancer or to treat influenza caused by the virus type A – H1N1. The negative effects include cytotoxi-

city of mucous membranes, skin and upper respiratory tract as well as mutagenicity, carcinogenicity and chromosomal aberrations [16, 17].

It is not fully confirmed whether human exposure to HMF poses a potential health risk. That's why many countries, like the European Union have put restrictions on the maximum levels of HMF in food and beverages [15]. In European Union countries, and thus in Poland, honey can be marketed when it complies with General Food Law requirements and relevant sensory/organoleptic and physicochemical requirements that are included in Council Directive 2001/110/EC of 20 December 2001 relating to honey and its subsequent amendments [18, 19]. In the case of 5-hydroxymethylfurfural, its content should be below 40 mg/kg of honey. The exception are baker's honeys and honeys that originate from regions with a tropical climate, and mixtures of such honeys, where the HMF level can not exceed 80 mg/kg. According to Śliwińska and Bazylak [17], the quality (including HMF content) of bee honeys of different varieties, which are available in the Polish market, is very diverse. Honey of the same variety is different in terms of physical parameters, which may indicate that they were not properly obtained and stored. That's why the purpose of this research was the determination of hydroxymethylfurfural content in selected domestic honey before and after different heat treatments by the means of high-performance liquid chromatography.

Materials and methods

Seventeen samples of Polish honey of different botanic origin were obtained from the market or directly from the beekeepers (Table 1). Honey samples were analysed at the turn of the year 2016/2017. Among them, five honey samples for different heat treatment were chosen to examine how the hydroxymethylfurfural content would change. The heat treatment conditions used by the means of three different instruments are described below:

- ultrasonic marinator: U1 (1 min.), U2 (5 min.),
- microwave oven: M1 (1295W, 0,5 min.), M2 (1850W, 0,5 min.), M3 (1850W, 1 min.)
- incubator: C1 (40°C, 20 min.); C2 (40°C, 60 min.), C3 (50°C, 20 min.), C4 (60°C, 20 min.), C5 (60°C, 60 min.), C6 (80°C, 20 min.)

Sample preparation

About 2 g of honey was weighed into a beaker with an accuracy of 0.001 g. The sample was dissolved in distilled water and quantitatively transferred to a 25 ml graduated flask. The it was filled with distilled water to the mark,

Table 1: Characteristic of honey samples.

Honey sample	abbreviation	Geographical origin (town/voivodeship)
Polyfloral nectar honey	MW	- / Podlaskie Voivodeship
Rapeseed nectar honey	MR	Malbork / Pomeranian Voivodeship
Lime nectar honey	MNL	Barciany / Warmian-Masurian Voivodeship
Pinophyta honeydew honey	MSI	Gnatowo / Warmian-Masurian Voivodeship
Broad-leave honeydew honey	MSL	Nowy Sącz / Greater Poland Voivodeship
Forest nectar honey	ML	Barciany / Warmian-Masurian Voivodeship
Pine honeydew honey	MS	Stróże / Greater Poland Voivodeship
Fir honeydew honey	MSJ	Sokółka / Podlaskie Voivodeship
Honeydew honey	MSK	Wambierzyce / Lower Silesian Voivodeship
Broad-leave honeydew honey	MSLL	Nowy Sącz / Greater Poland Voivodeship
Broad-leave honeydew honey	MSSL	Bielsko Biała / Silesian Voivodeship
Pinophyta honeydew honey	MSE	Stróże / Greater Poland Voivodeship
Honeydew honey	MPS	Bielsko Biała / Silesian Voivodeship
Broad-leave honeydew honey	MSP	No information
Broad-leave honeydew honey	MSH	Nowy Sącz / Greater Poland Voivodeship
Pinophyta honeydew honey	MMM	Tomaszkowo / Warmian-Masurian Voivodeship
Honeydew honey	MSW	Stróże / Greater Poland Voivodeship

closed with a cork and mixed. Then the solution was filtered through a PTFE syringe filter, 25 mm diameter, 0.45 μm pore size. About 1.5 ml of the solution was transferred to the vial and placed in the HPLC autosampler.

The analytical methodology was based on Rój and Przybyłowski's work on the determination of HMF in fruit sorbets [20]. The determination of HMF content was performed by high-pressure liquid chromatography using Agilent Technologies 1260 Infinity with an automatic sampler and a spectrophotometric UV-VIS/DAD detector. Chromatograms were obtained with the wavelength range of 200 – 400 nm, separation on HPLC RP column ZORBAX Poroshell 120 EC-C18 4.6 × 100 mm, 2.7 μm. As a mobile phase, water: acetonitrile (80/20 v/v), which was acidified with formic acid, was used. The column and detector temperature was set at 30°C. The separation was performed at a flow rate 400 μl/minute. The injection volume was 10 μl. The water used in all stages of the analysis came from the SOLPURE 78 deionizer. The qualitative analysis was carried out by comparing the retention time of the determined component with the retention time of the standard and by comparing the UV spectra. The quantitative analysis was carried out using the external standard method (calibration curve). The HMF standard curve was determined by preparing solutions of the HMF standard at various concentrations and by chromatographic analysis. The HMF standard was detected at a retention time of 2.463. The ratio of matching points to a straight line was 0.994. The HMF content in honey samples were calculated by equating the peak height of the sample to the HMF standard curve, including the sample weight and the honey dilution in the sample. All the analyses were performed in triplicate (n = 3), and

the results are expressed as their means value. The limit of quantification LOQ was 0.10 mg HMF/kg of honey.

Results and discussion

The results obtained for the HMF content of the 17 honey samples are presented in Fig. 1. The analysed honeys were characterized by a significant differentiation of the HMF content. Eight honey samples were within the range of 0-10 mg/kg of honey; the next 8 were in range from 10 to 20 mg of HMF in 1 kg of honey. Two honey samples, MPS and MS, had HMF content above 30 mg/kg of honey.

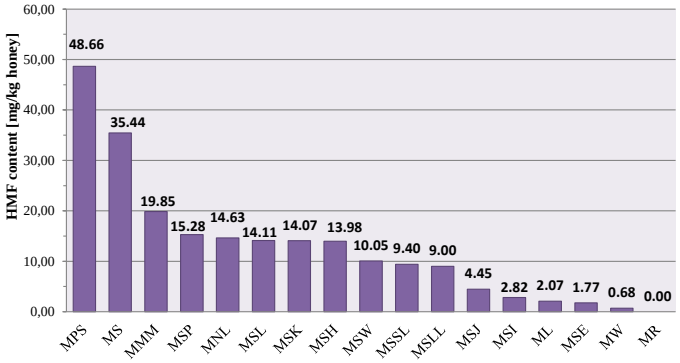

Fig. 1: The hydroxymetylofurfural content in honey samples.

Rapeseed nectar honey (MR) was the only one, which did not contain hydroxymethylfurfural. On the other hand, honeydew honey (MPS) contained the highest amount of HMF (48.66 mg/kg honey), which was 8.66 mg above the acceptable limit of EU requirements - Council Directive 2001/110/EC [18]. Martysiak-Żurowska and Borowicz (2009) investigated the content of HMF in honey using spectrophotometric Winkler method and HPLC technique [21]. After the chromatographic analysis they obtained following results: for forest honey – 45.32 mg/kg, for lime honey – 5.06 mg/kg and 2.23 mg/kg, for multifloral honey in range from 3.56 to 49.80 mg/kg and for honeydew honey – 45.00 mg/kg. These values were different from the results obtained in this work. Further, Bártáková et al. studied honey HMF content using HPLC. In their work the forest honey had 0.16 mg HMF per 1 kg of honey, honeydew honey – 4.05 mg/kg and multifloral honey – 1.69 mg/kg [22]. However, Piekut and Borawska in their research obtained different results: lime honey was characterized by 4.6 mg/kg HMF content, multifloral honey – 21.5 mg/kg, honeydew honey – 8.3 mg/kg and rapeseed honey 6.7 mg/kg [23]. As can be seen from the above data from different authors, the HMF content differs widely in honeys even for the same varieties. Kukurov et al. believe that this is influenced by non-uniform conditions of collection and storage of honey [24].

In addition, in this work, five honey samples were treated with different heat treatments for variable intervals, power and temperature parameters: in ultrasonic marinator (U1,U2), in microwave oven (M1,M2,M3) in incubator (C1,C2,C3,C4,C5,C6). The obtained results are presented in the Table 2.

Table 2: The hydroxymetylofurfural (HMF) content in honey samples before and after different kind of heat treatment.

Honey samples ID				MS	MR	MW	MSI	MSJ
HMF content [mg/kg honey]	Before treatment			35.44	0.00	0.68	2.82	4.45
	Ultrasound marinator [min]	U1	1	35.46	0.97	0.57	3.12	4.49
		U2	5	36.47	1.05	0.68	1.35	3.49
	Microwave oven [min, W]	M1	0.5, 1295 W	40.92	1.13	1.46	3.76	33.3
		M2	0.5, 1850 W	41.60	8.15	2.02	6.21	10.45
		M3	1, 1850 W	60.47	4.50	5.65	14.76	13.94
	Incubator [min, temp]	C1	20 min, 40°C	36.87	1.47	0.96	3.11	4.54
		C2	60 min, 40°C	36.63	0.90	0.72	2.80	4.32
		C3	20 min, 50°C	38.05	0.98	0.76	3.21	4.52
		C4	20 min, 60°C	36.88	0.00	0.96	1.60	3.62
		C5	60 min, 60°C	34.13	0.86	0.89	1.08	3.25
		C6	20 min, 80°C	36.54	0.91	0.78	1.12	3.28

The application of ultrasound in food technology influences the efficiency of several processes like tempering, extraction, mixing or bleaching [25]. One of the advantages is faster energy and mass transfer which positively affects the possibility to use lower temperatures in food processing. The use of an ultrasound marinator for a different period of time influenced bi-directionally the HMF content in the honey samples. In rapeseed honey, in which no compound was detected before treatment, the use of ultrasounds influenced the formation of HMF. After one-minute treatment the HMF content in rapeseed honey was 0.97 mg/kg and increased to 1.05 mg/kg after 5 minutes. In the case of two honeydew honeys (MSI and MSJ) the prolonged ultrasound treatment to five minutes reduced the HMF content.

One of the currently studied methods used to decrystallize honey is its heating in a microwave oven. In the present study when using microwave heating, regardless of the time and heating power, the HMF content increased in all samples (Table 2). For the honeys MS, MW and MSI, the highest increase occurred with the use of heating in a microwave at 1850 W for 1 minute, and the determined content was respectively 60.47 mg/kg, 5.65 mg/kg and 14.76 mg/kg. In the case of rapeseed honey (MR), heating shorter than 30 seconds influenced the formation of HMF, which content was 8.15 mg/kg, and longer heating affected a 50% decrease in content. For the MSJ honey, the use of

a lower microwave power of 1250 W resulted in a sevenfold increase in the HMF content in relation to the initial value (33.30 mg/kg), and the increase in power resulted in a three-fold reduction in the HMF content. Three samples of pine honeydew honey (MS) heated in a microwave exceeded the permissible by EU regulation HMF content (for treatment M1 – 40.92 mg/kg, M2 – 41.60 mg/kg, M3 – 60.47 mg/kg). Bath and Singh (2001) in their study showed that the level of HMF content increased with the increase of microwave power and duration of heating [26]. In our study, this relationship was demonstrated for honeys MS, MW and MSI. In any honey variety, Bath and Singh (2001) did not notice a decrease in the HMF content, which was the case in this research. Probably this was due to the use of low microwave power (70W, 140W, 210W, 280W) in relation to the microwave power used for this study (1295W and 1850W).

The use of thermal heating, by operating the temperature and heating duration, indicated the changes in the HMF content. Heating honey in laboratory incubator at 40°C, 50°C, 60°C and 80°C for different periods (20 min, 60 min) gave very similar results for individual types of honey. None of the specific parameters caused a significant increase in HMF content. Heating for 20 minutes at 40°C and 50°C influenced the increase of HMF in all honey samples. Extending the heating time to 60 minutes or increasing the temperature influenced the increase or decrease of the HMF content. Probably the origin of honeys had an impact on this result. For multifloral honey (MW) regardless of the incubation conditions, the HMF content increased. For rapeseed honey (MR) and pine honeydew honey (MS), five of the six heating conditions contributed to the increase in HMF content. For honeys MSI and MSJ, only heating for 20 min. at 40°C and 50°C affected the increase of HMF content. In other cases, this value decreased relative to the initial values of honey samples. The observed process coincides with the conclusions of Śliwińska et al., who found that the decrease in HMF content in some honey may be related to the intensification of complex processes contributing to HMF degradation and simultaneous slowdown of the formation of precursors of this compound occurring in these honeys [11].

Decrystalizing honey samples in an incubator also influenced HMF content but to a significantly lesser extent than microwave heating. This dependence was demonstrated by the studies of Piekut and Borawska regarding the effect of honey decrystallization on HMF content [23]. The decrystalization was carried out in a microwave for 5-11 seconds, obtaining honey temperatures from 45°C to 75°C and in a laboratory incubator from 2 to 4 hours, obtaining the same honey temperatures. The honey samples subjected to the decrystallization showed an increase in the initial average HMF content by 36.8% in a laboratory incubator and by 47.8% in the microwave. In the case of Kowalski research, it was shown that the increase in HMF content was faster for samples of honey treated with a microwave field in comparison with the conventional process heating in a water bath [27].

Bártáková et al. studied regional honey from the Czech Republic, including forest honeydew honey and multi-flower honey in terms of the impact of microwave heating on HMF content (from 90W by 800W, for 15-60 seconds) [22]. Their research shows that HMF increased and decreased to different levels when different conditions of heating was adjusted to individual samples. As a result, none of the HMF changes were significantly different. It was influenced not only by the different conditions of microwave time and power but also by the botanical origin of honey. The production of HMF by microwave heating does not take place in the same way in each type of honey. The longest heating times at all power levels did not cause significant changes in the HMF content although the honey was heated to around 80-90°C. The results of HMF content in honey heated in a microwave from our study are confirmed by the studies of Bártáková et al. [22]. The longest heating time at the highest power level did not cause the highest HMF growth in all honeys. Also, HMF levels increased to varying degrees depending on the honey variety.

Conclusions

The use of HPLC chromatographic analysis allowed to determine the content of HMF in honeys. Analysing the obtained results from this research, the following statements and conclusions can be formulated.

1. The honeys used for research purposes can be described as good quality in terms of meeting the HMF content requirements. Nectar honey contained less hydroxymethylfurfural than honeydew honey.
2. As it was stated before, it is HMF that is recognized as the determinant of honey quality. All honey samples regardless of the combination of heat treatment method were within the acceptable limit, except pine honeydew honey treated in a microwave.
3. In most cases, heating with ultrasonic marinator, in a microwave and in an incubator resulted in an increase in HMF content in honeys.
4. Microwave heating resulted in the fastest and largest increase with heating for 1 minute at 1850W gave the highest HMF content in most of honey samples.

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