

Evaluation of 5-Hydroxymethylfurfural (HMF) levels in Honey Produced in Western Libya

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Abstract

Eighteen honey samples were collected from the local market of Libyan cities (Zawya, Zahra, Al jable- Al ghrbi, Surmman, Sabratha, Al-Ajeelat, Zuwara, Al-Jameel), at the period between 10/2/2019 to 3/3/2019. Three types of honey were collected from the different regions (Rabiee, Sader, and Zater) to evaluation of 5-Hydroxymethylfurfural (HMF) levels in samples. Samples were prepared in the chemistry laboratory of the faculty of science at the University of Sabratha, the determination of HMF was performed in Zawia medical research center using the white method, which is based on UV-Vis Spectroscopy measurement at wavelengths of 284nm and 336nm, and the results were compared with Libyan Standard No. 1988. Results showed that the concentration of the MHF in the eighteen samples were within the allowed range according to the Libyan standard specification. The highest levels of the MHF level found in the Rabiee Honey (6.60 ± 19.55 mg/kg) and Sader Honey (5.54 ± 13.45 mg/kg). While the lowest levels of MHF was found in Zater Honey (1.82 ± 7.13 mg/kg).

Keywords: 5-Hydroxymethylfurfural, honey, carcinogen, antioxidant, anti-allergen.

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Introduction:

Honey is a sweet natural product produced by honeybees (*Apis Mellifera*), which gather nectar from flowers before converting it to nutritious food. Honey is mainly composed of water (15–20%) and two sugars (dextrose and levulose), with the presence of small amounts of at least 22 other more complex sugars (80–85%, wt/wt) (Doner, 1977; White, 1980). Honey has also been reported to contain an intricate mixture of nitrogenous compounds, lactones, proteins, antibiotic-rich inhibit, enzymes, phenol antioxidants, aroma compounds, amino and organic acids, gluconic acid, phenolic acids, flavonoids, minerals, vitamins, 5-hydroxymethylfurfural (HMF) and other photo chemicals (Ball, 2007; Gheldof, 2002; White, 1967; White, 1975). Honey composition varies depending on its floral, geographical, and entomological sources Anklam, (1998); Gheldof, (2002) Besides, external features such as seasonal and environmental factors, honey processing, and storage time and conditions have crucial effects on honey's composition (Gidamis, 2002; Islam, 2012; Mehryar, 2013). Honey is considered both nutritional and medicinal, although the presence of certain constituents, for example, heavy metals (even in trace amounts), some alkaloids, and HMF and its derivatives may contribute to honey's toxicity. Islam, (2014); Sanna, (2000). HMF is a cyclic aldehyde produced by sugar degradation through the Maillard reaction (a non-enzymatic browning reaction) during food processing or long storage of honey (Markowicz, 2012). The presence of simple sugars (glucose and fructose) and many acids, as well as minerals, in honey, can further enhance the production of this substance (Kuster, 1990).

HMF is an organic compound formed from reducing sugars in honey. HMF is easily absorbed from food through the gastrointestinal tract and, upon being metabolized into different derivatives, is excreted via urine. In addition to exerting detrimental effects (mutagenic, genotoxic, organotoxic, and enzyme inhibitory), HMF, which is converted to a non-excretable, genotoxic compound called 5-sulfoxymethylfurfural, is beneficial to human health by providing antioxidative, anti-allergic, anti-inflammatory, anti-hypoxic, anti-sickling, and anti-hyperuricemic effects. Therefore, HMF is a neo-forming contaminant that draws great attention from scientists. This review compiles updated information regarding HMF formation, detection procedures, mitigation strategies, and effects of HMF on honeybees and human health (Ummay Mahfuza Shapla, 2018). HMF concentration is widely recognized as a parameter affecting honey freshness because it is typically absent (or is present in only very small amounts in fresh honey), while its concentration tends to rise during processing and/or because of aging. Previous studies have reported that honey stored at low temperatures and/or under fresh conditions has low or minimal HMF concentrations, while aged and/or honey stored at comparatively higher or medium temperature has high HMF concentrations. In addition to storage conditions, the use of metallic containers and honey floral sources are critical factors affecting HMF levels. Hence, higher HMF concentration is indicative of poor storage conditions and/or excess heating of honey (Fallico, 2004; Khalil, 2010). Therefore, the Codex Alimentations Standard commission has set the maximum limit for HMF in honey at 40 mg/kg (with a higher limit of 80 mg/kg for honey originating from tropical regions) to ensure that the product has not undergone extensive heating during processing and is safe for consumption (Alimentarius, 2001).

HMF is not only present in honey; it is nearly ubiquitous in our daily heat-processed, sugar-containing foodstuffs, from our breakfast cereals, bread, dairy products, and fruit

juices to liquors at different concentrations (Bachmann, 1997; Claeys, 2003 ; Murkovic, 2006 ; Ramirez-Jimenez, 2000 ; Schultheiss, 1999 ; Teixidó, 2006). Therefore, HMF is considered one of the main quality indexes of different commercial whey proteins, molasses and many other products (Dogan, 2005). In most previous studies, HMF has been reported to have negative effects on human health, such as cytotoxicity toward mucous membranes, the skin and the upper respiratory tract; mutagenicity; chromosomal aberrations; and carcinogenicity toward humans and animals (Glatt, 2005; Lee, 1995; Monien, 2012). However, in more recent extensive studies, HMF has been proved to have a wide range of positive effects, such as antioxidative Zhao, (2013) anti-allergic Yamada, (2011) anti-inflammatory Kitts, (2012) anti-hypoxic Li, (2011) anti-sickling Osheiza, (2005) anti-hyperuricemic effects Lin, (2012). It has been reported that humans may ingest between 30 and 150 mg HMF daily via various food products; however, safe levels of HMF consumption are not well clarified. The reason is that HMF's metabolism, biotransformation, and excretion and thus clearance rate from the body depend on the organ function of an individual Ulbricht, (1984) that has not been considered.

This study aims to describe the effects of HMF present in honey, which, if broadly analyzed, can be used to promote the more widespread application of honey with special medicinal implications. Upon highlighting the HMF content in honey, a general discussion of the HMF content in other foods and HMF's detection, optimized formation, mitigation, and eradication from food provided. For this purpose, most related articles relevant to the topic of "honey and other foods, HMF: its toxicity and therapeutic effects" were included, regardless of the time of publication. HMF is a six-carbon heterocyclic organic compound containing both aldehyde and alcohol (hydroxymethyl) functional groups. The ring of the structure is centered on furan moieties, whereas the two functional groups, i.e., formyl and hydroxy-methyl groups are linked at the second and fifth positions, respectively (Fig.1). HMF is a solid, yellow substance that has a low melting point but is highly soluble in water (Rosatella, 2011).

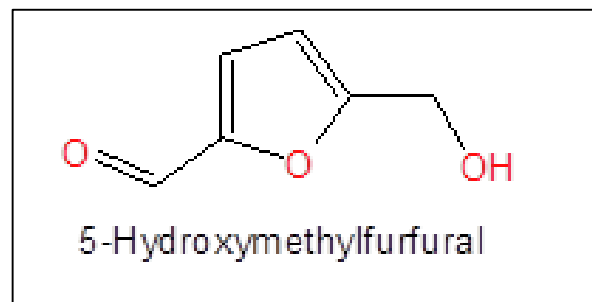


Figure (1): formation of HMF in honey

HMF is considered the most important intermediate product formed during two reactions (1) acid-catalyzed degradation of hexose and (2) decomposition of 3-deoxyosone in the Maillard reaction (Fig.2) (Fallico, 2008). HMF formation is correlated with chemical characteristics such as pH, free acid content, total acidity, lactone content, and mineral content, which in turn are related to the floral source of collected honey samples. The presence of simple sugars such as glucose and fructose and of many acids has been reported to be favorable for honey production (Fallico, 2004).

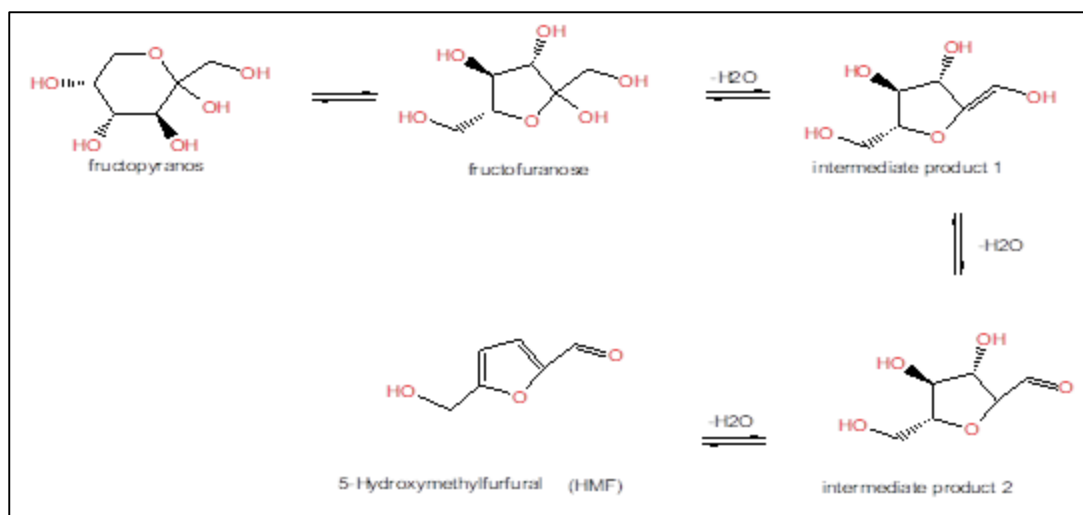


Figure (2): Chemical structure of HMF

Similarly, to honey, which is rich in glucose and fructose, most sugar-containing foods also contain HMF (Huber, 2006). Nevertheless, although HMF occurs at very low concentrations and can even be absent in both fresh honey and food products, heat treatment and/or prolonged storage conditions can enhance further HMF production. Moniruzzaman, (2013) reported the mean HMF concentration in Malaysian honey stored for 2 months at 5 °C to be 35.98 mg/kg. In contrast, Khalil, (2010) found that HMF concentrations in Malaysian honey stored at 25–30 °C for more than a year could reach very high levels (118.47–1139.95 mg/kg). Islam, (2012) also observed high HMF levels (3.18–703.10 mg/kg) in honey samples from Bangladesh stored for more than a year at room temperature (20–25 °C). Therefore, the HMF level is not only indicative of honey freshness but also storage duration and conditions.

In addition to being directly produced when heating sugar from the degradation of hexoses under acidic conditions at high temperatures and/or during the Maillard reaction (Arribas-Lorenzo, 2010). HMF is produced from the oligo- and polysaccharides that can yield hexoses upon hydrolysis. However, HMF appears to be more selectively produced from keto-hexoses such as fructose (Román-Leshkov, 2006). Interestingly, there are two reasons why higher yields of HMF are obtained from fructose (ketose) than from glucose (aldose). First, the reactivity of glucose is lower than that of fructose, with a lower enolization rate (Kuster, 1990). Enolization is believed to be the rate-determining step of HMF production. Second, fructose forms an equilibrium mixture of difructoses and dianhydrides and thus internally blocks most reactive groups, leading to the formation of certain by-products. In contrast, glucose forms true oligosaccharides that still contain reactive reducing groups, posing a higher risk for cross-polymerization with the reactive intermediates, including HMF (Kuster, 1990). Honey has been reported to contain many different types of sugars. In fact, a recent review by Solyman, (2015) indicated that honey contains approximately 39.44% fructose, 28.15% glucose and 3.19% sucrose. The cyclic aldehyde HMF is also produced in honey by the degradation of related sugars (Khalil, 2010). Heating of honey during its processing reduces its viscosity, which can prevent crystallization or fermentation (Singh, 1988). In addition to heating, several other factors influence the formation of HMF in honey, such as honey's physicochemical properties (pH, free acid content, total acidity, lactone content and mineral content), water activity (a_w), the use of metallic containers White Júnior, (1978) and thermal and photochemical stress (Spano, 2006).

HMF is easily formed at low temperatures in the presence of low-pH or acidic conditions Lee, (1990) while high temperature and long storage duration increase its concentration largely. Nevertheless, a different pathway is proposed in dry and pyrolytic conditions under which HMF is formed from fructose and sucrose. In addition to temperature and pH, the rate of HMF formation in honey is also dependent on honey's moisture content (Gökmen, 2007; Gökmen, 2008). Therefore, many steps are taken to maintain low moisture content in honey samples, including gamma irradiation and heat treatment to inhibit HMF formation.

The rate of HMF formation is also dependent on the fructose, glucose ratio and the type of sugars formed because it has been reported that at pH 4.6, fructose has five times more reactivity than glucose, and a high fructose, glucose ratio will accelerate the reaction (Lee HS, 1990). Turhan, (2009), showed that temperature and duration of heat treatment might both affect HMF formation in honey samples. Moreover, it was shown that heating honey samples collected from Anatolia in Turkey at 135 °C for 100 s produced similar amounts of HMF as that yielded by heating samples to 150 °C for 40 s. According to Sancho, (1992), there is a logarithmic relationship between the storage time and HMF levels in honey. In their study on Malaysian honey samples Khalil, (2010), showed that honey samples stored for 3-6 months had HMF values below the International Honey Commission (IHC) limit for tropical honey (< 80 g/kg); however, samples stored for 12-24 months had HMF concentrations above the recommended level.

Materials and Method:

1. Materials and Equipment:

- Crise solution (I): Dissolve 15 g of Potassium hexacyanoferrate (II) trihydrate ($K_4Fe(CN)_6 \cdot 3H_2O$) in Distilled water and makeup to 100 ml.
- Crise solution (II): Dissolve 30g of zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$) in Distilled water and makeup to 100 ml.
- Sodium metabisulfite solution $Na_2S_2O_5$ 0.20 g\100g: Dissolve 0.2 g of Sodium metabisulfite in 100 ml of distilled water, and it is recommended that you prepare for the experiment on the day of use.
- Filter paper (general purpose).
- Spectrophotometer (Jasco.V-530 UV) operating in a wavelength range including 284 and 336nm.
- Cuvettes (10 mm path length).
- Vortex mixer.
- Deionized water.

2. Collection of Samples:

Eighteen samples were randomly purchased from groceries, supermarkets, and pharmacies from different Libyan origins (Zawya/ Zahra/ west mountain/ Surmman / Sabratha/ Al-Ajeelat/ Zuwara/ and Al-Jameel) and divided in samples of 100g. Each sample was labeled to identify the source, site, and date of sampling then transferred to the laboratory of chemistry Department, Faculty of Science, Sabratha University, wherein the preparation of the samples was done to be ready for analysis of the concentration of 5-hydroxymethylfurfural. The results have expressed in milligrams per kilogram (mg/kg).

3. Samples preparation:

Approximately 5 g of honey weighed into a 50 ml beaker. Samples have dissolved in approximately 25 ml of distilled water and then transferred quantitatively into a 50 ml volumetric flask (including washing the residue from the beaker with a small amount of distilled water). 0.5 ml of Crise solution (I) were added and mixed, followed by adding 0.5 ml of Crise solution (II), the mixture mixed and makeup to the mark with distilled water (a drop of ethanol may be added to suppress surface foam). Then the mixture has filtered through filter paper and the first 10 ml of the filtrate have rejected. 5 ml of the mixture has pipetted in each of the two test tubes. 5 ml of distilled water has pipetted to one of the test tubes and mixed well (the sample solution). While 5 ml of sodium metabisulfite solution (0.20 %) has added to the second, test tube and mixed well (the reference solution).

4. Spectrophotometric Analysis:

UV-Vis absorbance of the sample solution against the reference at 284nm and 336nm has measured in 10 mm quartz cells within one hour, at the Zawia Medical Research Center used precise XB220A sensor and photometric spectrophotometer systems Jasco.V-530 UV. If the absorbance at 284 nm exceeds a value of about 0.6, dilute the sample solution with distilled water and the reference solution with sodium metabisulfite solution to the same extent to obtain a sample absorbance low enough for accuracy.

5. Dilution:

Dilution of sample and reference solutions have carried out as follows:-

- Additions to test tube sample preparation reference solution
- Initial honey solution 5 ml in 5ml Water.
- 0.2 % sodium metabisulfite solution solute 5 ml.
- Dilution D = final volume of sample solution \10.

6. Calculation:

HMF (mg/100g of honey) = (A₂₈₄-A₃₃₆) * Factor / weight of the sample

A₂₈₄, A₃₃₆ = Absorbance reading

Factor= $126 * 100 * 1000 * 100 / 16830 * 1000 = 74.87$

M.W. of HMF = 126

16830 = Molar Absorptivity of the HMF

Results and Discussions

According to statistical analysis, the repeatability (for absorption value and wavelength shift) was corroborated acquiring the spectrum three times for each sample. According to the scan step, the error on the wavelength shift was ± 1 nm.. All spectra were recorded using deionized water as blank. Table (1) shows the analysis of variance (ANOVA) has been used to determine the coefficient of statistical measure p-value and prediction of the driven regression model was found to be satisfactory.

Table (1): Statistical data analysis using (ANOVA) statistical design.

ANOVA						
DEGREE						
Source of variance	Sum of squares	D.f	Mean square	F	p-value	Result
Between groups	376.018	2	188.009	0.693	0.516	Non-significant
Within Groups	4072.346	15	271.490			
Total	4448.364	17				

From table (1) above it is clear that the value of (P-value) is equal to (0.5160) which is a value greater than (0.05) Therefore, the zero hypotheses are not rejected. Rabiee, Sader and Zater and the difference between the 5-hydroxyl methyl furfural averages of the three types of honey (Rabiee, Sader and Zater) does not represent a significant difference, according to the following relationship when the value of the analysis of variance analysis (P) = 0.5160 and the significance level = 0.05 and thus we infer the value of P Greater than the significance level, therefore we cannot reject the null hypothesis and not accept the alternative hypothesis.

Resolution: the difference between the mean samples and the difference is not statistically significant.

It is clear that the average amount of 5-hydroxyl methyl furfural for the three types of honey ranged between (7.13 and 19.55), where they were the lowest value in Zater honey, which averaged (7.13), and a standard deviation of (1.82) compared to other types of honey as showed in table (2), while it is more concentrated in Rabiee honey with an average of (19.55) and a standard deviation (6.6).

Table (2): 5-hydroxyl methyl furfural (mg/kg) in honey types

Honey Type	mean and standard deviation (mg/kg)
Sader	13.45 ± 5.54
Rabiee	19.55 ± 6.6
Zater	7.13 ± 1.82

According to the previous descriptive measures (averages), it is clear that there are differences in the mean concentration of the compound 5-hydroxyl methyl furfural according to the type of honey since the normal range allowed for this compound in honey is 40 mg / 100 g.

HMF concentration listed in table (3) indicated that most of the studied samples conformed with the Libyan specifications except samples (Rabiee2, and Rabiee3) which have high level of HMF, this agreed with the results which found by Said, 2010, the concentration recorded 74.9 ± 2.34 mg/kg. The concentration of HMF varied significantly, while some samples were close to the maximum allowable of 40mg / kg. Sample (Sader 11) recorded a reading of 36.1 mg/kg; this is consistent with what Mouhoubi –Tafinine, 2018 found with a reading of 40 mg/kg. The different

concentration of 5-hydroxyl methyl furfural from one sample to another sample is attributed to several factors, as they are of different honey varieties and therefore the composition of honey and shelf-life affects the form of the compound HMF, and because of different ratios specimens are conflicting, but there is no information on the conditions under which the samples of honey, especially the temperature, whether we get them from the honey beehive, or while is storage them from the date of production. These conditions affect the form of the compound studied, where a similar study was conducted to measure the concentration of (HMF) in honey using the method White Ramadan, 2013 .While there were studies, the HMF concentration was below the allowed limit (Belay, & Haki, 2017).

Table (3): Concentration of HMF in the samples: -

Sample NO.	Honey Type	HMF Concentration (mg/kg)
1	Rabiee	22.30
2	Rabiee	51.80
3	Rabiee	53.90
4	Rabiee	7.20
5	Rabiee	11.60
6	Rabiee	12.50
7	Rabiee	2.02
8	Rabiee	4.70
9	Rabiee	9.10
10	Sader	23.60
11	Sader	36.10
12	Sader	4.30
13	Sader	10.00
14	Sader	5.2
15	Sader	1.5
16	Zater	9.9
17	Zater	3.7
18	Zater	7.8

Conclusions:

The eighteen samples were within the allowed range according to the Libyan standard specification. The highest levels of the MHF level found in the Rabiee Honey (6.60 ± 19.55 mg/kg) and Sader Honey (5.54 ± 13.45 mg/kg). While the lowest levels of MHF was found in Zater Honey (1.82 ± 7.13 mg/kg). Finally, it can be concluded that there is no significant difference between HMF averages of the three honey types (Rabiee, Sader, and Zater) at 5% level.

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تقييم مستويات 5-هيدروكسي ميثيل فورفورال (HMF) في العسل المنتج في غرب ليبيا

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المستخلص:

ثمانية عشر عينة عسل جمعت من السوق المحلي للمدن الليبية (الزاوية، الزهراء، الجبل الغربي، صرمان، صبراتة، العجيلات، زوارة، الجميل) في الفترة ما بين 2019/02/10 إلى 2019/03/3م. تم جمع ثلاثة أنواع من العسل من مناطق مختلفة (ربيعي، سدر، زعتر) لتقييم مستويات 5-هيدروكسي ميثيل فورفورال (HMF) في العينات. تم تحضير العينات في معمل الكيمياء بكلية العلوم بجامعة صبراتة، وتم قياس HMF في مركز البحوث الطبية بالزاوية باستخدام الطريقة البيضاء، والتي تعتمد على قياس الطيف بالأشعة المرئية وفوق البنفسجية بأطوال موجية 284 نانومتر و336 نانومتر، وتمت مقارنة النتائج بالموصفة الليبية رقم 1988. وأظهرت النتائج أن تركيز MHF في الثمانية عشر عينة كان ضمن النطاق المسموح به وفقاً للمواصفة القياسية الليبية. أعلى مستويات MHF وجدت في عسل الربيعي 19.55 ± 6.60 مغم / كغم والعسل السدر 13.45 ± 5.54 مغم / كغم، بينما ت أدنى مستويات MHF وجدت في عسل الزعتر 7.13 ± 1.82 مغم / كغم.

الكلمات المفتاحية: 5-هيدروكسي ميثيل فورفورال، عسل، مادة مسرطنة، مضاد للأكسدة، مضاد للحساسية