Alqalam Journal of Medical and Applied Sciences. 2025;8(4):2728-2731
https://doi.org/10.54361/ajmas.258490

Short communication

Estimation of Free Acid Content and the Effect of Thermal Treatments on HMF Levels in Samples from the Local Market in Derna, Libya

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Abstract
This study was conducted on five types of honey available in the local market in the city of Derna, including Dryas honey extracted from the plant *Thapsia garganica*. Caper honey, extracted from the plant *Capparis spinosa L.*, Sidr honey, extracted from the plant *Ziziphus lotus*, Zaater honey, extracted from the plant *Thymus compactus*, and Rabie honey is extracted from various plants during the spring season. The content and effects of thermal treatments on hydroxymethylfurfural (HMF) were evaluated, and the conformity of local honey to the Libyan Standard Specification Law No. 281 of 1988 was verified through spectral and quantitative analyses of the studied samples. Using White's method, which is based on measuring in the UV range at two wavelengths (284 nm, 336 nm) using a 6305 UV/VIS spectrophotometer, the results of the study showed that the free acid content recorded the highest value for Sidr honey, with a value of 31.16 mg/kg. HMF values were less than 40 mg/kg. HMF values exceeded the permissible values of 40 mg/kg after three hours of heat treatment at 40°C, 65°C, and 70°C, respectively, in almost all samples. The most affected samples by heat treatment were thyme, and the least affected was spring honey.
Keywords: Honey, HMF, UV, White's Method, Free Acid Content.

Introduction

Honey is a sweet liquid made by bees using the nectar of flowering plants. There are approximately 320 different types of honey, varying in color, aroma, and flavor. Sidr honey is considered one of the best types of honey [1], distinguished by its antibacterial and anti-inflammatory properties. The water content in honey increases when exposed to humidity, so containers must be tightly closed, as high humidity leads to honey fermentation. When temperatures rise, this leads to the decomposition of vitamins and the loss of their enzymes. When honey is exposed to strong light, it loses the substance contained in it that prevents the formation of cholesterol, and the germ-killing substance decomposes [2]. Honey contains a complex mixture of proteins, antibiotics, inhibitors, organic acids, phenolic acids, vitamins, 5-hydroxymethylfurfural (HMF), and other chemicals [3].

The crystallization property of honey is a natural characteristic of honey. The crystallization, or solidification, of honey is the formation of tightly packed glucose crystals. This characteristic is natural in all honeys collected by bees from flower nectar. The consistency of honey is subject to rapid changes, even before it is packaged in airtight containers. However, honey maintains its consistency throughout its time inside the hive, especially when these cells are covered with a thin layer of wax that protects it from natural and chemical changes. When beekeepers rush to remove the honey from the cells before it has fully matured naturally inside the hive, they make a mistake that is difficult to correct, because honey is naturally moisture-absorbing and tends to retain moisture. Thus, rushing to harvest the crop prematurely increases the water content of the honey, which accelerates its crystallization [4].

Fresh honey is subjected to heat treatments during the sorting process or during manufacturing to maintain quality characteristics. However, it has been found that excessive heat treatment leads to the formation of HMF, which negatively impacts honey quality [5]. Fresh honey is subjected to heat treatment during the sorting or processing process to maintain its quality. However, it has been found that excessive heat treatment leads to the formation of HMF, which negatively impacts the quality of honey. The presence of large amounts of HMF indicates that the honey has been exposed to high temperatures. HMF is a cyclic aldehyde produced by the decomposition of sugars via the Maillard reaction (non-enzymatic browning reaction) during food processing or long-term storage of honey. It's an organic compound composed of the reducing sugars in honey, with the chemical formula C6H6O3. HMF is a yellow, six-atom solid with a low melting point, but it is highly soluble in water [6].

Recent and more thorough research has demonstrated that HMF has an assortment of beneficial effects, including anti-inflammatory, anti-allergy, anti-hypoxic, anti-sickle-cell, anti-hyperuricemic, and antioxidant properties [7]. In addition to honey, HMF is present in nearly all of the heat-processed sugar-producing foods we eat regularly, including bread, breakfast cereals, dairy products, and fruits. According to most of the earlier research, HMF has detrimental effects on human health, including cytotoxicity to the pores, mucous membranes, and the upper airway tract [8]. Prior investigations have documented that honey preserved at lower temperatures or in optimal conditions exhibits minimal or inconsequential levels of hydroxymethylfurfural (HMF), whereas honey subjected to moderately elevated or high temperatures displays significant concentrations of HMF. Consequently, an elevated concentration of HMF serves as an

Received*: 25-09-2025 -* ***Accepted****: 23-11-2025 -* ***Published****: 01-12-2025*

indicator of inadequate storage conditions or the overexposure of honey to thermal processing. [9]. Chemical characteristics, including pH, free acid content, and mineral content, are linked to the composition of HMF and are subsequently linked to the honey samples' floral source [10]. HMF is produced directly when sugar produced by the decomposition of hexoses is heated under high-temperature acidic conditions or during the Maillard reaction [11-15].

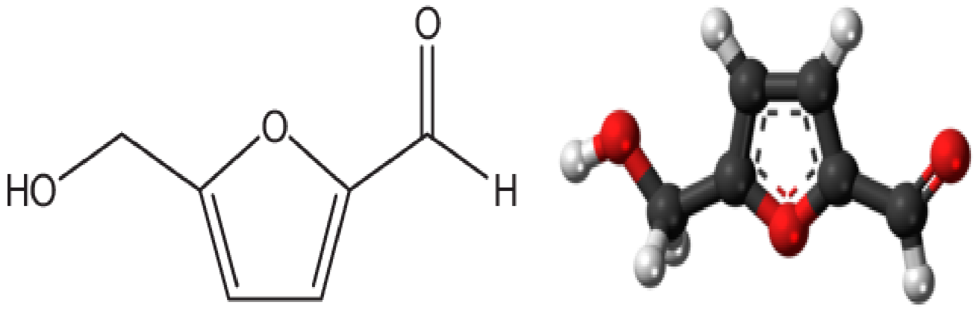

Figure 1: Molecular formula of HMF

Honey's quality decreases with longer storage periods and is impacted by heat treatment. The production and safety of honey depend on heat treatment. It is used to stop fermentation and pasteurize honey. Additionally, it has been demonstrated to prevent crystallization and enhance the look and feel of honey [16]. Furfural is formed as a result of caramelization reactions involving carbohydrates, especially hexoses, in an acidic medium [17]. It is also produced from the Maillard reaction, which is found to be formed from the breakdown of ascorbic acid. Honey quality is determined through sensory, physical, and chemical testing. Sensory monitoring includes odor, color, and taste. Biological tests identify yeasts [18]. While water activity, moisture, total and pathogenic bacteria, and viscosity are determined within the physical tests. Finally, chemical tests include determining the sugar level and ash content. Among these tests is the HMF test [19]. In this study, White's method was applied, as it is a reference and constitutional method for measuring HMF levels at two wavelengths (284 nm-336 nm).
Analyzing HMF levels in honey serves as a measure of honey quality, and assessing this compound's levels is vital for health due to its adverse effects on consumer well-being. This research aims to monitor the quality of locally available honey by analyzing HMF using spectral and quantitative methods to verify its conformity with the Libyan standard specification (40 mg/kg).

Method and materials

Materials and equipment

A set of equipment available in the laboratories of the Faculty of Science (University of Derna) was used, including the (JENWAY 6305 UV/Vis) and the standard chemical materials, which are potassium hexacyanoferrate (Carrez 1), zinc acetate (Carrez 2), sodium bisulfite (Carrez 3), sodium hydroxide, and distilled water.

Samples

Five samples of honey were gathered from different types of honey found in the markets of Derna. According to beekeepers, the samples were produced between June and November 2024, and the samples were analyzed in May 2025. All samples were stored at 25°C after collection until analysis (Table 1).

Analytical methods

The White method was used, which relies on using a spectrophotometer and measuring absorbance in the ultraviolet range at wavelengths (284 nm, 336 nm). Then, the concentrations of the (HMF) compound were calculated utilizing the subsequent equation:

$$\textbf{HMF}\ (mg/kg) = (A284 - A336) \times 149.7 \times 5 / \textbf{W}$$

HMF (mg/kg): The number of milligrams of HMF compound in kg of honey. A284: Absorbance at a wavelength of (284nm). A336: Absorbance of impurities at a wavelength of (336nm). W: The weight of the honey sample measured in grams.

The equation indicates that the absorbance due to impurities (336 nm) was considered and deducted from the overall absorbance of the sample (284 nm) to reduce the error caused by impurities (International Honey Commission, 2002).

Statistical analysis

All analyses were conducted with three replicates using a completely randomized design. Differences between different honey varieties were assessed using one-factor analysis of variance (ANOVA) at a significance level of $P \geq 0.05$. Ten grams of honey was mixed into 75 mL of distilled water,

Received*: 25-09-2025 -* ***Accepted****: 23-11-2025 -* ***Published****: 01-12-2025*

and the mixture was stirred with a magnetic stirrer until fully dissolved. The solution was titrated with 0.1 N NaOH to a pH of 8.30, noting the volume of NaOH used.

Free acid contents (mg/kg) = 0.1× V NaOH×1000 / W

V NaOH: volume of NaOH (ml).

W: sample weight (g).

Results and discussion

The results of estimating the free acid substance of honey samples were recorded within the acceptable limits of Libyan standard specifications of 40 mg/kg. The greatest worth was recorded for the Sidr honey sample at 31.16, and the lowest value was recorded for the Caper honey at 15.22 (Table 1).

Table 1. Type of honey used, initial HMF values, and free acid content

Free acid content	Initial Value of (hmf)	Compilation data	Sample location	Sample code	Sample name
31.16	33.53	05/05/2025	Derna	**H1**	Sader
26.14	32.48	29/12/2024	Derna	**H2**	Rabea
15.22	57.18	24/04/2025	Derna	**H3**	Dereaas
20.32	61.22	27/01/2025	Derna	**H4**	Zaater
18.20	52.20	29/06/2024	Derna	**H5**	Gabar

The results of the statistical analysis showed that there is a relationship between the inducing effect of heat on the levels of HMF, as the significance (P value)was high at 0.001, the standard error(N/S value) was 0.5278, and the arithmetic mean of the treatment in the samples H1, H2, H3, H4, H5 was 66.33,54.76,108.05,118.67,91.12 respectively, There is a relationship between the induction effect of heat on the levels of HMF and time, as the longer the induction effect time, the higher the HMF values. The P value was high at P ≥ 0.0020, and the N/S value was 0.6835, and the arithmetic mean of time in the samples 3h,6h&12h was 64.85,81.95,116.56 respectively (Table 2,3,4). While there is no significant effect of temperature only on the levels of HMF without change in time [20], as the P value was P ≥0.5399 and the N/S value was 0.4541, and the arithmetic mean of temperature in the samples 3h, 6h&12h was 77.913,86.200,99.251, respectively.

Table 2. The effect of honey exposure to a temperature of 40°C.

SAMPLES	3H	6H	12H
H1	40	55	67
H2	35.1	44	48
H3	74	80.12	87.14
H4	74.85	78.58	80.1
H5	67.5	70.2	71.12

Table 3. The effect of honey exposure to a temperature of 65C°.

SAMPLES	3H	6H	12H
H1	51	69	80
H2	49.15	50.44	55.51
H3	92.2	96.31	120.1
H4	97.12	101.1	120.11
H5	**72**	**80.1**	**95.12**

Table 4. The effect of honey exposure to a temperature of 70°C.

SAMPLES	3H	6H	12H
H1	70	75	90
H2	60.32	71.2	79.15
H3	137.21	140.25	145.1
H4	145.1	160.95	210.15
H5	103.14	120.75	140.15

Conclusion

An increase in HMF content is considered evidence of honey being exposed to high temperatures, as the amount of HMF increases with increasing thermal coefficient and time, and the rate of increase also varies depending on the type of honey sample. It is preferable to avoid exposing honey samples to direct sunlight and heat treatment, and they should be stored in a cool place to prevent a decrease in their chemical

***Received**: 25-09-2025 - **Accepted**: 23-11-2025 - **Published**: 01-12-2025* https://doi.org/10.54361/ajmas.258490

components and the activity of enzymes and vitamins. Also, avoid toxic content.

Conflict of interest. Nil

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Received*: 25-09-2025 -* ***Accepted****: 23-11-2025 -* ***Published****: 01-12-2025*