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SPECTROPHOTOMETRIC DETERMINATION OF 5-HYDROXYMETHYLFURFURAL IN HONEY SAMPLES OF VARIOUS BOTANICAL ORIGINS

Improper processing, storage conditions, or adulteration of honey promotes the formation of 5-hydroxymethylfurfural (HMF). HMF is detrimental to human health if the consumption level exceeds the limit established by regulatory bodies. The Codex Alimentarius Commission and the European Union have set the HMF limit in honey at 40 mg/kg, with a higher limit of 80 mg/kg for countries or regions with tropical temperatures. The International Honey Commission recommends three methods for HMF determination: two spectrophotometric methods (the White method and the Winkler method) and High-Performance Liquid Chromatography (HPLC). However, the regulated Winkler method is predominantly used for HMF determination in research conducted in Ukraine. In this study, 17 honey samples collected between 2022 and 2025 and having various botanical origins were analyzed using the White method. This method is based on the cleavage of the HMF chromophore system by the action of sodium metabisulfite. The difference in light absorption at a wavelength of 284 nm between the honey sample treated with metabisulfite and the untreated sample is proportional to the HMF concentration. To eliminate the effects of light scattering and light absorption by the honey matrix, measurements were conducted at two wavelengths: 284 nm and 336 nm. Monofloral honey samples (sunflower, linden, acacia, rapeseed, and amaranth), polyfloral wildflower honey, and blended honey samples were analyzed. The HMF content in almost all tested honey samples was significantly lower than the recommended limit. The lowest HMF content was found in monofloral honeys such as linden and sunflower, ranging from 0.63 to 2.8 mg/kg. A slightly higher HMF content was observed in acacia honey samples, ranging from 10.8 to 14.6 mg/kg. The HMF content in polyfloral wildflower honey and blended honey samples fell within the same range. Analysis of sunflower, rapeseed, and amaranth honey harvested in 2025 demonstrated that their HMF content was significantly below the normative values.

Keywords: honey quality, 5-hydroxymethylfurfural, spectrophotometry.

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СПЕКТРОФОТОМЕТРИЧНЕ ВИЗНАЧЕННЯ 5-ГІДРОКСИМЕТИЛФУРФУРОЛУ У ЗРАЗКАХ МЕДУ РІЗНОГО БОТАНІЧНОГО ПОХОДЖЕННЯ

Неправильні умови обробки, зберігання або фальсифікація меду сприяють утворенню 5-гідроксиметилфурфуролу (ГМФ), який шкідливий для здоров'я людини, якщо рівень споживання перевищує ліміт, встановлений регуляторними органами. Комісія Codex Alimentarius та Європейський Союз встановили межу ГМФ у меді 40 мг/кг, а для країн або регіонів з тропічними температурами 80 мг/кг. Міжнародна медова комісія рекомендує три методи визначення ГМФ: два спектрофотометричні методи визначення за методом Уайта та Вінклера і високоефективну рідинну хроматографію. Проте в дослідженнях, які проводяться в Україні, для визначення ГМФ в основному використовують регламентований метод Вінклера. В даному дослідженні 17 зразків меду 2022–25 років збору та різного ботанічного походження були проаналізовані методом Уайта, який ґрунтується на руйнуванні хромофорної системи ГМФ дією метабісульфіту натрію. Різниця світлопоглинання при довжині хвилі світла 284 нм меду, який оброблений метабісульфітом, та без обробки пропорційна концентрації ГМФ. Для усунення ефектів світлорозсіювання та поглинання світла матрицею меду вимірювання проводили при двох довжинах хвиль: 284 нм і 336 нм. Для визначення були взяті монофлорні зразки меду (соняшниковий, липовий, акацієвий, ріпаковий та амарантовий), поліфлорний мед із різнотрав'я та купажовані зразки меду. Вміст ГМФ майже у всіх протестованих медах був значно нижчим рекомендованої норми. Найнижчий вміст ГМФ був у таких монофлорних медах як липовий та соняшниковий і знаходився в межах 0,63–2,8 мг/кг. Деяко вищий вміст ГМФ був у зразках акацієвого меду і знаходився в межах 10,8–14,6 мг/кг. В таких же межах знаходився вміст ГМФ у поліфлорному квітковому меді та у зразках купажованого меду. Аналіз соняшникового, ріпакового та амарантового меду 2025 року збору, показав, що вміст ГМФ був у них значно нижче нормативних значень.

Ключові слова: якість меду, 5-гідроксиметилфурфурол, спектрофотометрія.

Honey is a well-known unique beekeeping product characterized by a high content of active substances essential for the vital activity of the human body. It is distinguished by its botanical origin, consistency, color, and method of harvesting. Although the medicinal properties of honey are not yet fully understood, numerous researchers and scientists in various fields confirm that it is an indispensable consumer product with incredibly beneficial properties (Samarghandian et al., 2017; Sharaf et al., 2025). Given its use as a medicinal agent, honey must possess the highest quality, a requirement emphasized by the European Parliament Directive (Directive 2014/63).

As of the end of 2024, Ukraine ranked third globally in honey export volumes, surpassed only by China and India. Therefore, systematic quality control of Ukrainian honey is crucial for ensuring consumer trust and is a key factor in increasing the sector's export potential. According to the State

Standard of Ukraine DSTU 4497:2005 («Natural Honey. Specifications»), honey quality is determined by various organoleptic and physicochemical parameters. One of the quality indicators for honey is its content of 5-hydroxymethylfurfural (HMF). HMF primarily forms during the thermal processing of carbohydrate-containing foods through acid-catalyzed thermal dehydration from fructose, sucrose, and, to a lesser extent, glucose. The scheme for HMF formation from fructose is shown in Figure 1.

The Codex Alimentarius Commission and the European Union (Directive 2001/110) have established an HMF limit in honey of 40 mg/kg, with a higher limit of 80 mg/kg for countries or regions with tropical temperatures. The HMF content and diastase activity (Bhure et al., 2025) are considered key indicators used for honey quality control regarding its freshness and possible overheating.

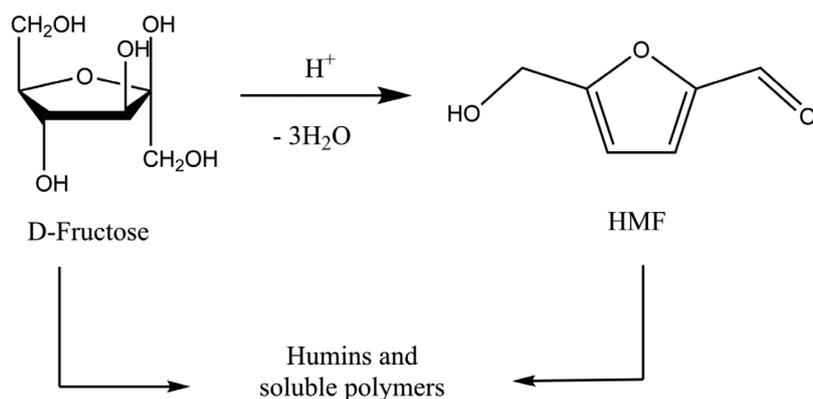


Fig. 1. Scheme of HMF formation from fructose

The study of HMF properties and the control of its content in food products began in the 1950s due to its harmful effects on the organism, including carcinogenic, mutagenic, genotoxic, and organotoxic effects. The most comprehensive reviews on the impact of HMF on the body are presented in the works (Abraham et al., 2011; Shapla et al., 2018; Greilberger et al., 2025). However, attributing HMF formation solely to the thermal processing of honey would be inaccurate, as several other factors also contribute to its elevated concentration in honey. High HMF concentration may indicate improper processing and storage conditions (Mouhoubi-Tafnine et al., 2018) or honey adulteration with invert syrup (Abdi et al., 2024). Furthermore, the botanical origin of honey (Vijan et al., 2023), pH, the concentration of divalent cations in the medium (Capuano & Fogliano, 2011), and the geographical location of the melliferous plants (Tasic et al., 2024) are factors that influence the HMF level.

The International Honey Commission recommends three methods for HMF determination: two spectrophotometric methods (the White method and the Winkler method) and High-Performance Liquid Chromatography (HPLC). In Ukraine, the HMF content in honey is determined using the Winkler method (DSTU 4497:2005). The Winkler method is based on the absorption at 550 nm of a colored complex formed by the reaction of *p*-toluidine with HMF in a barbituric acid medium. However, studies (Zappal et al., 2005) have shown that the Winkler method yields slightly overestimated results, whereas the White method and the chromatographic method produce results within the statistical error margin. Furthermore, the Winkler method employs *p*-toluidine, which possesses toxic and carcinogenic properties.

The White method, first described in 1979 (White, 1979), is based on the cleavage of the HMF chromophore system by the action of sodium metabisulfite. The difference in light absorption at a wavelength of 284 nm between the honey sample treated with metabisulfite and the untreated sample is proportional to the HMF concentration. The HMF content is calculated using the tabulated value of the molar absorption coefficient $\epsilon^{284} = 16,830$ (Zappal et al., 2005). To eliminate the effects of light scattering and light absorption by the honey matrix, measurements are performed at two wavelengths: 284 nm and 336 nm.

We have not found the use or detailed description of the White method for HMF determination in honey for quality control of domestic honeys in Ukrainian scientific publications. Therefore, the aim of this study was the spectrophotometric determination of HMF in honey samples of various botanical origins using the White method and the assessment of the quality of the studied samples based on their HMF content.

Seventeen honey samples, differing in botanical and geographical origin and year of collection, were selected for the study (Table 1). The samples were collected between 2022 and 2025. Samples 3, 10, 15–17 were provided by a beekeeper from a certified apiary in the Cherkasy region, while the blended samples 12–14 were supplied by a leading Ukrainian honey trading company. The remaining samples were purchased at agricultural markets. HMF determination was performed during 2024–2025. Sample No. 3 was analyzed in 2024 and then re-analyzed a year later in 2025 (listed as No. 9 in the table).

The following reagents were prepared for the analysis: Carrez solution 1 $K_4[Fe(CN)_6]$ 15 % solution), Carrez solution 2 ($Zn(CH_3COO)_2$

30 % solution), and a 0.2 % solution of sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$), which had to be freshly prepared for use. Absorbance measurements were performed on a Shimadzu UV2100 PC spectrophotometer (Shimadzu, Japan) in the range of 284–336 nm using 10 mm path length quartz cuvettes.

The determination methodology included the following procedures: 5 g of the honey sample was accurately weighed (with a precision of 0.001 g) into a 50 mL beaker. The sample was dissolved in approximately 25 mL of distilled water and quantitatively transferred to a 50 mL volumetric flask. 0.5 mL of Carrez solution 1 was added and mixed. Then, 0.5 mL of Carrez solution 2 was added, and the volume was adjusted to the mark with water. Any foam present was removed by adding a drop of ethanol. The solution was filtered through a paper filter into a clean, dry beaker, discarding the first 10 mL of the filtrate. Two 5.00 mL aliquots of the filtrate were measured into two test tubes (18 · 50 mm). To the first tube (test solution), 5.00 mL of distilled water was added and mixed. To the second tube (reference solution), 5.00 mL of a 0.2 % sodium metabisulfite solution was added and mixed. The absorbance of the test solution relative to the reference solution was measured at wavelengths of 284 nm and 336 nm in

a 10 mm quartz cuvette no later than one hour after solution preparation.

The result of the analysis was calculated using the formula:

$$w(\text{HMF}), \text{ mg/kg} = \frac{(A^{284} - A^{336}) \cdot 748,7 \cdot DF}{m},$$

where:

- A^{284} is the absorbance at 284 nm;
- A^{336} is the absorbance at 336 nm;
- DF is the dilution factor (if any), otherwise, it is one;
- m is the mass of the weighed honey sample, g;
- 748.7 is a constant, calculated as:

$$748,7 = \frac{126 \cdot 10 \cdot 50 \cdot 1,000 \cdot 1,000}{16,830 \cdot 5 \cdot 1,000},$$

where:

- 126 is the molar mass of HMF, g/mol;
- 1000 (numerator) is the conversion of mL to L;
- 1000 (numerator) is the conversion of g to mg;
- 1000 (denominator) is the conversion of g of honey to kg;
- 10 is the final volume of the test solution, mL;
- 5 is the volume of the honey sample aliquot taken for analysis, mL;
- 50 is the volume of the honey sample solution, mL.

Table 1

5-Hydroxymethylfurfural Content in the Studied Honey Samples

Sample №	Botanical Origin	Year of Collection	Year of Analysis	Geographical Origin	HMF Content, mg/kg ($P = 0.95, n = 3$)
1	Linden, liquid consistency	2022	2024	Cherkasy region	2.81 ± 0.64
2	Linden, crystallized	2022	2024	Cherkasy region	1.83 ± 0.63
3	Linden, crystallized	2023	2024	Cherkasy region	<0.65*
4	Sunflower, liquid consistency	2024	2024	Dnipropetrovsk region	2.15 ± 0.63
5	Sunflower, liquid consistency	2024	2024	Cherkasy region	<0.65
6	Wildflower, liquid consistency	2024	2024	Kyiv region	9.47 ± 0.72
7	Wildflower, liquid consistency	2024	2024	Dnipropetrovsk region	11.74 ± 0.74
8	Wildflower, liquid consistency	2023	2024	Cherkasy region	42.3 ± 1.1
9	Linden, crystallized	2023	2025	Cherkasy region	1.75 ± 0.63
10	Acacia, crystallized	2024	2025	Cherkasy region	10.8 ± 0.73
11	Acacia, liquid	2024	2025	Chernihiv region	14.6 ± 0.78
12	Blended, crystallized	2024	2025	Ukrainian honey trader company	9.30 ± 0.72
13	Blended, crystallized	2024	2025	Ukrainian honey trader company	10.3 ± 0.72
14	Blended, crystallized	2025	2025	Ukrainian honey trader company	11.9 ± 0.75
15	Sunflower, crystallized	2025	2025	Cherkasy region	2.03 ± 0.63
16	Rapeseed, crystallized	2025	2025	Cherkasy region	2.74 ± 0.64
17	Amaranth, liquid	2025	2025	Cherkasy region	±0.63

* limit of detection of the method.

The characteristics of the samples and the results of the 5-hydroxymethylfurfural determination are presented in Table 1.

As indicated by the results, the HMF content in almost all samples was significantly lower than the acceptable limit of 40 mg/kg. Only one sample, No. 8, purchased from a private entrepreneur, had a slightly elevated HMF content. This sample may have undergone thermal processing or even been adulterated with inverted sugar syrup. The lowest HMF content was found in monofloral honeys such as linden and sunflower (samples 1–5, 15), ranging from 0.65 to 2.8 mg/kg. No correlation was observed between the honey consistency (liquid or crystallized) and the HMF content. Linden honey Sample No. 3 (2023 harvest) with an HMF content of <0.65 mg/kg was re-analyzed in 2025 (Sample No. 9): the HMF content slightly increased to 1.75 mg/kg. This suggests that HMF content increases only slightly under proper storage conditions and in the absence of excessive thermal processing. A slightly higher HMF content among the monofloral honeys was observed in the acacia honey samples (samples 10–11), ranging from 10.8 to 14.6 mg/kg. The HMF content in polyfloral wildflower honey (samples 6–7) and blended honey samples (12–14) fell within the same range,

specifically 9.3–11.9 mg/kg. Regarding the 2025 harvest, unfavorable weather conditions resulted in a negligible collection of traditional Ukrainian honeys such as acacia and wildflower varieties. However, analysis of sunflower, rapeseed, and amaranth honey harvested in 2025 showed that their HMF content was significantly below the normative values (1.67–2.74 mg/kg).

The spectrophotometric method used in this study for the determination of 5-hydroxymethylfurfural in honey (the White method) is simple to execute, employs safe reagents, and meets the objective of the study. Given that honey quality determination is critically important for Ukraine to ensure consumer safety and protect the reputation of its export products, this method can be successfully utilized in food quality control laboratories. Honey samples from 2022 to 2025 of various botanical origins were analyzed using this methodology. The HMF content in almost all tested honey samples was significantly lower than the recommended standard, which may indicate that these samples have not undergone thermal processing. The lowest HMF content was found in monofloral honeys (linden, sunflower, rapeseed, and amaranth), with a slightly higher content in polyfloral wildflower honey and blended honey samples.

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