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DETERMINATION OF POLYPHENOLIC COMPOUNDS IN HONEY SAMPLES OF DIFFERENT OTANICAL ORIGIN

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A study was conducted on the quantitative determination of biologically active components of Ukrainian honey, in particular polyphenols and flavonoids, which are responsible for its antioxidant, antibacterial, and anti-inflammatory properties. Thirteen honey samples harvested in 2023–2024 from different regions of Ukraine were analyzed, including monofloral (sunflower, linden, rapeseed, black locust), polyfloral (wildflower), and blended varieties. The primary method for quantitative analysis of total polyphenol content was the Folin–Ciocalteu colorimetric assay (measured at 750 nm), while flavonoid determination was carried out using a photometric reaction with $AlCl_3$ in an alkaline medium (measured at 510 nm). The total phenolic content in the domestic samples was found to range from 108 to 770 mg GAE/kg. The highest values were characteristic of wildflower honey, while the lowest values were recorded in acacia and linden honeys. No direct correlation was found between polyphenol content and botanical origin for monofloral and polyfloral varieties. The flavonoid content in the studied samples ranged from 3 to 28 mg/kg. For rapid approximate estimation of total polyphenol content, spectrophotometric

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analysis of the samples was performed. It was established that absorbance at 280 nm (A^{280}) exhibits an insufficiently strong correlation with total polyphenol content due to possible interference from non-phenolic components, and therefore it is not recommended for express analysis. In contrast, a strong correlation was found between optical density at 450 nm (A^{450}) and flavonoid content, which allows this parameter to be used for rapid assessment of the antioxidant potential of honey. The obtained data underscore the high competitiveness of Ukrainian honey on the international market.

Key words: *honey, polyphenols, flavonoids, spectrophotometry.*

ВИЗНАЧЕННЯ ПОЛІФЕНОЛЬНИХ СПОЛУК У ЗРАЗКАХ МЕДУ РІЗНОГО БОТАНІЧНОГО ПОХОДЖЕННЯ

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Проведено дослідження по кількісному визначенню біологічно активних компонентів українського меду, зокрема поліфенолів та флавоноїдів, які зумовлюють його антиоксидантні, антибактеріальні та протизапальні властивості. У роботі проаналізовано 13 зразків меду врожаю 2023–2024 років із різних регіонів України, що включали монофлорні (соняшниковий, липовий, ріпаковий, акацієвий), поліфлорні (різнотрав'я) та купажовані сорти. Основним методом кількісного аналізу загального вмісту поліфенолів обрано колориметричний метод Фоліна–Чокальтеу (вимірювання при 750 нм), а для визначення флавоноїдів – фотометричну реакцію з $AlCl_3$ у лужному середовищі (вимірювання при 510 нм). Встановлено, що загальний вміст фенольних речовин у вітчизняних зразках становить від 108 до 770 мг GAE/кг. Найвищі показники характерні для меду з різнотрав'я, тоді як мінімальні значення зафіксовано в акацієвому та липовому медах. Для монофлорних та поліфлорних сортів не виявлено прямої залежності показників вмісту поліфенолів від їхнього ботанічного походження. Вміст флавоноїдів у досліджуваних зразках становить від 3 до 28 мг/кг. Для швидкої наближеної оцінки загального вмісту поліфенолів у зразках проведено їх спектрофотометричне дослідження. Встановлено, що світлопоглинання при 280 нм (A^{280}) має недостатньо сильну кореляцію із загальним вмістом поліфенолів через можливий вплив нефенольних компонентів, тому воно не рекомендоване для експрес-аналізу. Натомість виявлено сильну кореляцію між оптичною густиною при 450 нм (A^{450}) та вмістом флавоноїдів, що дозволяє використовувати цей показник для оперативної оцінки антиоксидантного потенціалу меду. Отримані дані підкреслюють високу конкурентоспроможність українського меду на міжнародному ринку.

Ключові слова: *мед, поліфеноли, флаваноїди, спектрофотометрія.*

Introduction

Honey, consumed by humans since ancient times, is one of the most valuable dietary components. It is a unique natural product made by bees from flower nectar or plant secretions, yielding nectar or honeydew honey. Although the therapeutic potential of honey is still being investigated, numerous researchers confirm its exceptional biological value (Samarghandian et al., 2017; Hanif et al., 2025). Honey is known to possess antibacterial, anti-inflammatory, antioxidant, antidiabetic, and antitumor effects, as well as to regulate digestion and cardiac function. These properties are attributable to the complex chemical composition of honey, which includes volatile and non-volatile compounds: phenolic substances, amino acids, enzymes, essential oils, etc. (Bobis et al., 2020; Kiran & Riyaz, 2026). In recent years, a substantial body of literature has emerged describing the mechanisms of action of individual honey compounds at the molecular level. The study by Wilczyńska & Żak (2024) (Wilczyńska & Żak, 2024), recommends dividing these compounds into two main groups: those with antimicrobial activity and those with antioxidant activity. The antioxidant properties of honey are primarily attributable to the presence of polyphenols, represented by flavonoids, phenolic acids, and their derivatives. The content of these compounds depends primarily on the botanical origin (nectar source), which determines the type and concentration of active substances (Mustafa et al., 2025). Other key factors include: geographical origin and climatic conditions, harvest time, processing methods, and storage conditions (Kędzierska-Matysek et al., 2021; Nyarko et al., 2023). Furthermore, a number of studies (Ibrahimi & Hajdari, 2020; Mendoza Bacilio et al., 2022) confirm a direct correlation between phenolic compound content and honey color intensity –

typically, darker varieties exhibit higher antioxidant activity.

Spectrophotometry is one of the most accessible and widespread methods for quantitative determination of certain honey components. In particular, the determination of total polyphenol content is predominantly based on the Folin–Ciocalteu colorimetric method (Gośliński et al., 2021). This method employs the eponymous reagent, which consists of phosphotungstic acid $H_3PW_{12}O_{40}$ and phosphomolybdic acid $H_3PMo_{12}O_{40}$ heteropolyacids. The reduction of these heteropolyacids by reducing agents in an alkaline medium yields a blue coloration measured at 750 nm. However, since this assay is based on a redox reaction, compounds other than phenols, such as reducing sugars and ascorbic acid, may also react with the heteropolyacids (Truchado et al., 2008). Standard solutions of gallic acid are used for calibration curve construction; therefore, the quantitative phenol content values obtained are expressed in gallic acid equivalents (mg GAE/kg). Depending on the aforementioned factors, the polyphenol content in Polish honey ranged from 71.7 to 202.6 mg GAE/kg (Pyrzynska & Biesaga, 2009), while in Croatian honey this parameter was 56–500 mg GAE/kg (Kenjerić et al., 2007). Meanwhile, the average total polyphenol content in Ethiopian honey samples varied from 170.3 to 420.4 mg GAE/kg (Yayinie et al., 2022). A study of honey from Colorado (USA) recorded total phenolic content levels of 816–1057 mg GAE/kg (Nyarko et al., 2023).

Photometry can also be applied for the determination of flavonoids (a subclass of polyphenols); the method for their determination in honey is described by Paula et al. (2024). The principle of the photometric determination of flavonoids involves the nitrosation of ring B of flavonoids followed by a reaction with $AlCl_3$ in an alkaline medium to form a colored product. The absorbance is measured at 510 nm. A calibration curve is constructed using a standard catechin solution, and accordingly, the results are expressed in mg catechin equivalents per kg of honey. Compared with the total phenolic content, the flavonoid content in honey is lower. For example, for Brazilian honey (Al-Farsi et al., 2018), the total phenolic content determined using the Folin–Ciocalteu reagent ranged from 250 to 548 mg GAE/kg, while the total flavonoid content determined using aluminum chloride was 9 to 48.6 mg catechin/kg of honey. For quantitative determination of the comprehensive phenolic profile of honey, high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) are employed, most commonly in combination with diode array detection (DAD) and mass spectrometry (MS). In the study by Badjah Hadj Ahmed et al. (2014), carbon nanotubes were proposed as a solid-phase extraction adsorbent for the simultaneous determination of phenolic acid, flavonoid, and other phenol content in various honey samples by HPLC. According to the studies by (Akbari et al., 2020; Soares et al., 2017), the phenolic profile of honey can be used as a «fingerprint» for determining its geographical origin and detecting adulteration. However, a significant limitation of these methods is the high cost of equipment and reagents, the need for a large number of analytical standards of phenolic compounds, and complex sample preparation.

The high export potential of Ukrainian honey (3rd place in the world ranking for 2024) imposes stringent certification requirements. Despite the fact that the current DSTU 4497:2005 standard regulates basic organoleptic and physicochemical characteristics, it does not account for the concentration of secondary metabolites, in particular polyphenols. Therefore, studying the polyphenolic composition of domestically produced honey is a relevant and strategically important issue that will enhance the competitiveness of Ukrainian products on the international food market.

Materials and Methods

The objects of investigation were 13 honey samples harvested in 2023–2024, selected based on the principle of diversity in botanical and geographical origin. The sample set included monofloral varieties (sunflower, linden, rapeseed, black locust), polyfloral wildflower honey, and two blended samples (No. 4 and No. 6). The detailed characteristics of the samples are presented in Table 1.

All reagents used in the analysis were of analytical grade. Standard solutions of 1.0 g/L catechin and 1.0 g/L gallic acid were prepared by dissolving an accurately weighed amount of the substance in ethanol. The Folin–Ciocalteu reagent was prepared according to the standard procedure (Pérez et al., 2023). Into a 1000 mL round-bottom flask containing 350 mL of distilled water, sodium tungstate weighing (50.00 ± 0.01) g and sodium molybdate weighing (12.25 ± 0.01) g were introduced. To the mixture, 25 mL of concentrated orthophosphoric acid and 50 mL of concentrated hydrochloric acid were added.

Table 1

Characteristics of the investigated samples

Sample No.	Geographical origin	Botanical origin	Color
1	Dnipropetrovsk region	Polyfloral (wildflower)	Golden
2	Dnipropetrovsk region	Sunflower	Light yellow
3	Dnipropetrovsk region	Sunflower	Bright yellow
4	Dnipropetrovsk region	Blend (Sunflower + wildflower)	Yellow
5	Dnipropetrovsk region	Rapeseed	Yellow
6	Dnipropetrovsk region	Blend (Wildflower + rapeseed)	Yellow
7	Cherkasy region	Linden	Light yellow
8	Kyiv region	Sunflower	Light yellow
9	Kyiv region	Black locust	Pale yellow
10	Kyiv region	Polyfloral (wildflower)	Light brown
11	Kyiv region	Polyfloral (wildflower)	Amber
12	Cherkasy region	Buckwheat	Amber
13	Cherkasy region	Black locust	Pale yellow

The flask was connected to a reflux condenser and heated on a water bath for 10 hours. Subsequently, lithium sulfate weighing (75.00 ± 0.01) g and 25 mL of distilled water were added to the solution, thoroughly mixed, and 5 drops of liquid bromine were introduced. To remove residual bromine, the mixture was boiled for 15 min without a condenser under constant stirring. The resulting reagent was cooled to room temperature, filtered through filter paper into a 500 mL volumetric flask, and the volume was adjusted to the mark with distilled water. Absorbance measurements were performed on a Shimadzu UV2100 PC spectrophotometer (Shimadzu, Japan) in the UV and visible spectral ranges.

Preparation of honey samples for measurement

A 5.000 g aliquot of honey was weighed in a graduated beaker and dissolved in approximately 10 mL of deionized water. The solution was quantitatively transferred into a 25 mL volumetric flask and diluted to the mark with deionized water. The resulting sample was filtered through a 0.45 μm regenerated cellulose syringe membrane filter to eliminate turbidity.

Procedure for the determination of total polyphenol content

Into a 50 mL volumetric flask, 5 mL of the honey filtrate was introduced, 4.0 mL of the Folin–Ciocalteu reagent was added, followed by 6.0 mL of a 20% Na_2CO_3 solution, and the flask was made up to volume with distilled water. The mixture was thoroughly mixed and, after 2.5 hours, absorbance measurements were taken at a wavelength of 750 nm with an optical path length of 0.5 cm. The reference solution contained all reagents except honey. The calibration curve for total polyphenol content determination was constructed using standard gallic acid solutions in the concentration range of 1–15 mg/L, prepared as described above. The polyphenol concentration in terms of gallic acid was determined from the calibration curve equation A^{750} = (0.008 ± 0.002) + (0.0707 ± 0.0008) × C (mg/L) and recalculated to gallic acid equivalents (mg GAE/kg) taking into account the honey sample weight. The limit of detection, calculated using the 3S criterion, was 0.1 mg/kg.

Procedure for the determination of flavonoids in honey samples

Into a test tube, 2 mL of the honey filtrate was added, followed by 3 mL of distilled water and 0.5 mL of a 15% $NaNO_2$ solution; the mixture was left to stand for 6 minutes, then 0.5 mL of a 5% $Al_2(SO_4)_3$ solution was added, the mixture was stirred, left for another 6 minutes, and 4.0 mL of a 4% sodium hydroxide solution was added. The mixture was thoroughly stirred and subsequently left to stand for an additional 10 minutes at room temperature. Absorbance measurements were taken at a wavelength of 510 nm with an optical path length of 1.0 cm. The reference solution contained all reagents except honey. The calibration curve for total flavonoid content determination was constructed using standard catechin solutions in the concentration range of 0.5–10 mg/L. The flavonoid concentration in terms of catechin was determined from the calibration curve equation: A^{510} = (0.039 ± 0.006) + (0.0436 ± 0.0010) × C (mg/L) and recalculated to catechin

equivalents (mg/kg) taking into account the honey sample weight. The limit of detection, calculated using the 3S criterion, was 0.05 mg/kg.

Spectrophotometric studies in the visible and UV regions

For the analysis, honey solutions with different mass fractions were prepared and pre-filtered through a 0.45 μm membrane filter. The absorbance of 50% honey filtrates was measured in the 400–600 nm range (1.0 cm cuvette), recording values at a wavelength of 450 nm. The optical density of 20% filtrates was determined in the 250–450 nm range with the same optical path length.

Results and Discussion

It is known that in the absorption spectra of polyphenols, a characteristic band is present at 280 nm (Harbertson & Spayd, 2006). Although such a determination is not absolutely selective, absorbance at this wavelength can be used for a rapid approximate estimation of total polyphenol content in various matrices. The absorption spectra of the investigated honey solutions in the 250–450 nm region are shown in Fig. 1. Table 2 presents the total polyphenol content determined by the Folin–Ciocalteu method, as well as the absorbance values at 280 nm (A^{280}) for the investigated samples. According to the presented data, the total phenolic compound content in the investigated samples (determined by the Folin–Ciocalteu method) varies from 108 to 770 mg GAE/kg. The minimum value was recorded in acacia honey, while the maximum was found in wildflower honey. It is noteworthy that buckwheat honey, which tra-

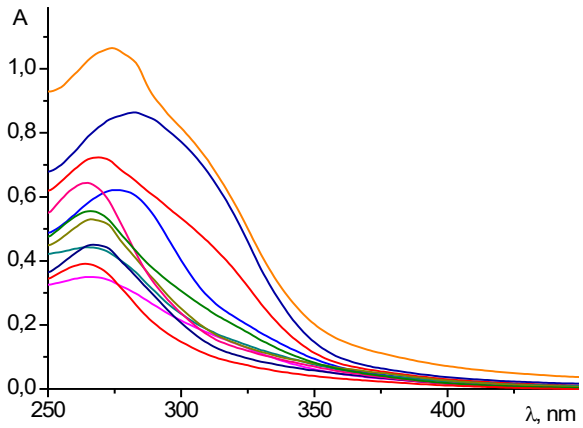

Fig. 1. Absorption spectra of the investigated solutions with a honey mass fraction of 20%, optical path length 1.0 cm (samples Nos. 7 and 12 are not shown)

Table 2

Total phenolic content in the samples and optical density of the solutions

Sample No.	**Total phenolic content, mg GAE/kg** **X ±ΔX (P=95%, n=3)**	**A^{280}**
1	185 ± 13	0,6685
2	165 ± 3	0,6158
3	244 ± 7	0,3853
4	174 ± 17	0,3114
5	332 ± 4	0,4363
6	241 ± 13	0,3779
7	146 ± 3	3,9282
8	123 ± 3	0,4743
9	165 ± 24	0,4595
10	365 ± 24	0,8611
11	770 ± 42	1,0396
12	543 ± 5	3,7441
13	108 ± 5	0,2921

ditionally leads in polyphenol content, showed a lower value of 543 mg GAE/kg. No direct correlation was found between polyphenol content and botanical origin for monofloral and polyfloral varieties.

It was established that honey absorbance at 280 nm (Fig. 2) correlates with the total polyphenol content (R = 0.7671). However, the correlation is not sufficiently strong to use the A^{280} value for estimating total polyphenol content, and for two samples (Nos. 7 and 12) anomalously high A^{280} values were observed, which may be attributed to the presence of other organic substances with strong absorption in the ultraviolet region of the spectrum. Thus, the A^{280} value is not recommended for rapid estimation of total polyphenol content.

Honey absorbance at 450 nm is commonly used to characterize honey color (Beretta et al., 2005; Moniruzzaman et al., 2013). The major contributors to absorbance at this wavelength are pigments and compounds with pronounced antioxidant activity. The results of flavonoid determination in the investigated honey samples by the method described above, as well as the A^{450} values, are presented in Table 3. The absorption spectra of the honey samples in the 350–600 nm range are shown in Fig. 3. The dependence of A^{450} on total flavonoid content is presented in Fig. 4. From the presented data, it is evident that a strong correlation exists between flavonoid content and A^{450}, and this correlation is substantially stronger than that between total polyphenol

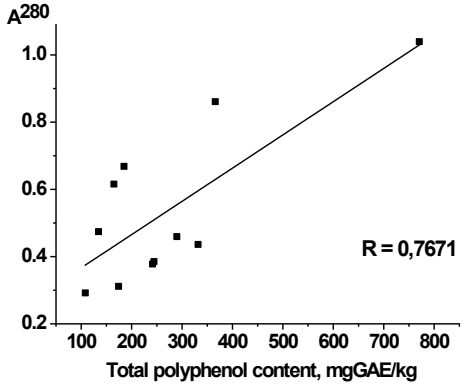

Fig. 2. Dependence of absorbance at 280 nm on the total polyphenol content (samples Nos. 7 and 12 are not shown)

Table 3

Results of honey analysis for total flavonoid content

Sample No.	Total flavonoid content, mg/kg	A^{450}
	X ±ΔX (P=95%, n=3)	
1	7,6 ± 0,7	0,1027
2	6,2 ± 1,9	0,1385
3	10,2 ± 0,4	0,1567
4	7,6 ± 1,5	0,1304
5	7,6 ± 0,8	0,1062
6	6,8 ± 1,2	0,0837
7	6,8 ± 0,4	0,0654
8	5,6 ± 0,4	0,0539
9	6,7 ± 1,2	0,0662
10	15,5 ± 0,4	0,2112
11	26,2 ± 2,4	0,3703
12	28,2 ± 1,2	0,5595
13	3,3 ± 1,2	0,0357

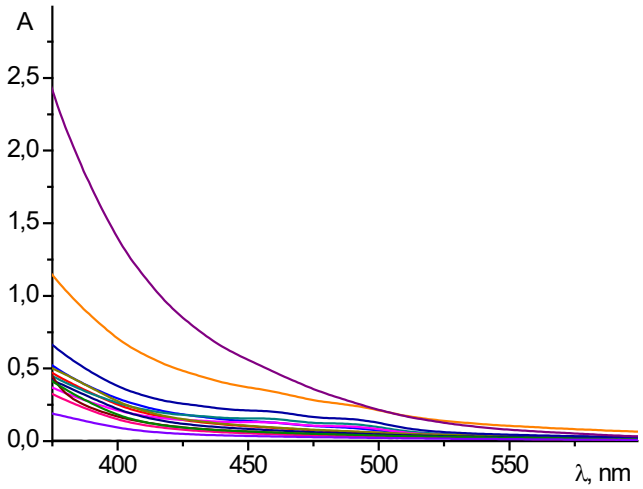

Fig. 3. Absorption spectra of the investigated solutions with a honey mass fraction of 50%, optical path length 1.0 cm

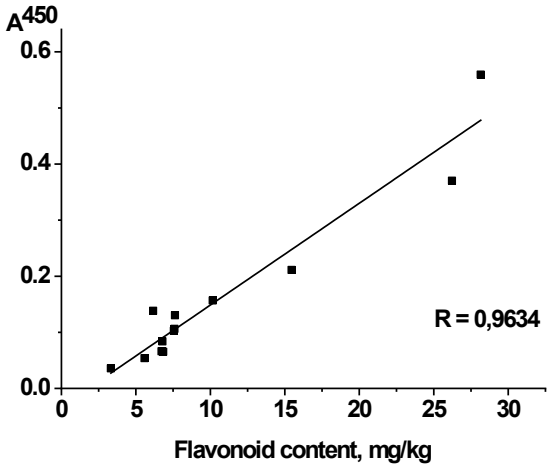

Fig. 4. Dependence of absorbance at 450 nm on flavonoid content

content and A^{280}. Therefore, the use of the A^{450} value for the overall assessment of antioxidant activity of honey is more appropriate than absorbance at 280 nm, where other non-phenolic components of honey, which exhibit weaker antioxidant activity, likely contribute to the absorption.

Conclusions

The total phenolic content in 13 Ukrainian honey samples obtained from different regions and different botanical sources was determined by the Folin–Ciocalteu method. The total phenolic content ranged from 108 to 770 mg GAE/kg. The highest polyphenol content was found in wildflower honey, while the lowest was observed in linden honey. No direct correlation was found between polyphenol content and botanical origin for monofloral and polyfloral varieties. The flavonoid content, determined photometrically by the formation of a colored complex with aluminum, ranged from 3 to 28 mg/kg.

Overall, the phenolic content in Ukrainian honeys is generally consistent with values reported for honeys from other countries, and in some cases exceeds them. It was established that honey absorbance at 280 nm, as a method for express estimation of total phenolic compound content, does not correlate sufficiently strongly with total polyphenol content and is not recommended for use. In contrast, the correlation between absorbance at 450 nm and total flavonoid content is strong and can be used for rapid assessment of the antioxidant properties of honey.

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