UDC: 638.162:547.56(497.7)"2022/2024"
638.162:547.972.2(497.7)"2022/2024"
Professional paper

TOTAL PHENOLIC AND FLAVONOID CONTENT IN MACEDONIAN POLYFLORAL HONEY

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Abstract

Honey is increasingly acknowledged not only as a natural sweetening agent but also as a functional food, owing to its antioxidant properties, primarily derived from its phenolic and flavonoid compounds. This study provides a comparative preliminary assessment of six honey samples collected between 2022 and 2024 from various regions of North Macedonia, directly sourced from local beekeepers. The total phenolic content (TPC) was determined using the Folin–Ciocalteu method, while the total flavonoid content (TFC) was assessed through an aluminium chloride colorimetric assay. All analyses were conducted at the Faculty of Biotechnical Sciences, Bitola. The results demonstrated notable variability among samples. The meadow honey collected in 2024 exhibited the highest flavonoid concentration (257 ppm QE/100 g), whereas the lowest TFC was recorded in forest honey from the same year (3.7 ppm QE/100 g). Statistically significant differences were observed in TPC values among meadow honeys ($p < 0.005$) and in TFC values ($p < 0.001$). A moderate positive correlation between TPC and TFC ($r = 0.73$) further suggests a meaningful association between these two classes of bioactive compounds. These findings highlight the influence of botanical origin, harvest period, and regional factors on the bioactive composition of honey. The study supports the importance of continued characterization of Macedonian honey varieties, particularly in the context of their potential nutraceutical applications.

Keywords: phenolic compounds, flavonoids, geographical origin, polyfloral honey.

1. Introduction

Honey is a natural product highly appreciated for its nutritional value, pleasant taste, and health-promoting properties. Besides being a source of natural sugars, it also contains compounds that contribute to various biological activities, particularly antioxidant and therapeutic effects. These beneficial effects are mostly linked to the presence of phenolic acids and flavonoids, which are known for their ability to reduce oxidative stress by neutralizing free radicals (Beretta et al., 2005; Estevinho et al., 2008).

The content and composition of phenolic compounds in honey are strongly influenced by factors such as the type of plants bees collect nectar from, the region where honey is produced, seasonal changes, and local beekeeping practices (da Silva et al., 2016). While numerous studies from different European countries have reported variations in phenolic and flavonoid content, such data for honey from North Macedonia are still limited. Research from surrounding Balkan countries has shown that the floral and geographical origin significantly affect honey quality. For example, Bodó et al. (2021) examined flavonoid profiles in Croatian polyfloral honeys, and Đorđević et al. (2015) analyzed Serbian unifloral honeys, focusing on their phenolic and physicochemical properties.

Today, honey is widely recognized as a functional food, not just because of its natural sugars, but also due to its high levels of bioactive compounds. Phenolics and flavonoids in honey are responsible for many of its health-related functions, including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects (Bogdanov et al., 2008; Al-Mamary et al., 2002). The

https://doi.org/10.62792/ut.jftn.v8.i15-16.p3199

concentration of these compounds often depends on environmental factors like altitude, temperature, and the variety of plants in the region, all of which vary significantly across the Balkans.

For instance, studies in Greece showed that darker honey types such as chestnut honey contain higher amounts of phenolic compounds than lighter varieties (Stagos et al., 2018). Similar results were reported by Nešović et al. (2020), who found that the botanical origin of honey from Montenegro significantly influenced its phenolic content. Kečkeš et al. (2013) also reported differences in flavonoid levels between polyfloral and unifloral honeys from Serbia and pointed out the link between these compounds and honey's biological activity.

Although beekeeping is well established in North Macedonia, and the country has diverse flora and favorable natural conditions, there are very few published studies on the phenolic profile of Macedonian honey. A recent study by Petrova et al. (2024) marks one of the first steps in this area.

Therefore, this study aims to:

- Analyze and compare the total phenolic content (TPC) and total flavonoid content (TFC) in different honey types (forest vs. meadow) collected from various regions in North Macedonia, and determine if there are statistically significant differences between them ($p < 0.05$);
- Evaluate the correlation between TPC and TFC using standard laboratory methods.

The findings of this research will help clarify the antioxidant potential of Macedonian honey, contributing valuable data for future studies on honey quality and its possible use in functional foods and nutraceuticals.

2. Materials and Methods

2.1. Honey Samples: This study included a total of six honey samples, comprising forest and meadow honeys, collected from various regions across North Macedonia. The sampling was conducted during the period 2022–2024, and the samples were obtained directly from local beekeepers to ensure authenticity and traceability to the production site.

2.2. Determination of Total Phenolic Content (TPC): The total phenolic content in the honey samples was quantified using the Folin–Ciocalteu spectrophotometric method, following the procedure described by Singleton and Rossi (1965), with minor adjustments. For each sample, 0.2 mL of diluted honey extract was mixed with 0.8 mL of Folin–Ciocalteu reagent. After a 5-minute reaction period, 1 mL of 7.5% sodium carbonate solution and 1 mL of 95% ethanol were added. The mixture was then incubated in the dark at room temperature for 50 minutes. Absorbance was measured at 725 nm using a UV-Vis spectrophotometer. The results were expressed as mg gallic acid equivalents (GAE) per liter, based on a standard calibration curve prepared with gallic acid.

2.3. Determination of Total Flavonoid Content (TFC): Total flavonoid content was analyzed using the aluminum chloride ($AlCl_3$) colorimetric method, as outlined by Park et al. (2013). A 1% ethanol extract of each honey sample was reacted with 10% $AlCl_3$ solution and left to incubate in the dark for 50 minutes. The absorbance of the resulting complex was recorded at 400 nm. Quercetin was used as a reference standard, and the flavonoid concentration was expressed in mg quercetin equivalents (QE) per liter.

2.4. Statistical Analysis: All measurements were conducted in triplicate to ensure accuracy and reproducibility. Descriptive statistics, including mean values and standard deviations (SD), were calculated for each parameter. The Pearson correlation coefficient was applied to evaluate

the relationship between TPC and TFC values. To determine significant differences between honey types, a one-way analysis of variance (ANOVA) was performed, with significance thresholds set at $p < 0.05$ and $p < 0.01$.

3. Results and Discussion

3.1. Total Phenolic and Flavonoid Content in Honey Samples: The phytochemical evaluation of six honey samples (three forest and three meadow), collected from different regions of North Macedonia between 2022 and 2024, revealed notable differences in total phenolic content (TPC) and total flavonoid content (TFC) (Table 1). In general, forest honey samples showed higher concentrations of phenolic compounds, with TPC values ranging from 278.0 to 298.0 ppm GAE/100 g. However, their flavonoid levels were lower, ranging from 3.7 to 5.5 ppm QE/100 g, which is consistent with the chemical characteristics of forest-origin honeys, such as chestnut honey, known for their rich phenolic profile and moderate flavonoid content.
On the other hand, meadow honeys demonstrated moderate TPC values, measured between 186.7 and 236.3 ppm GAE/100 g, but exhibited significantly higher and more variable flavonoid content, ranging from 31.4 to 257.0 ppm QE/100 g. The highest TFC values were observed in samples collected in 2024, indicating possible year-to-year variability. Interestingly, marked differences in flavonoid content were detected even among meadow honeys collected in different years and localities (2023 vs. 2024), despite their classification as the same honey type. These variations may be attributed to environmental factors, including seasonal changes, floral diversity, and climatic conditions during the nectar flow period. Such findings align with earlier reports suggesting that botanical origin and ecological conditions significantly influence the phytochemical composition of honey.

Table 1. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of analysed honey samples

No.	Type of honey & Locality	Harvesting Year	TPC (ppm GAE/100 g)	TFC (ppm QE/100 g)	p-value TPC	p-value TFC
1	Forest honey – Locality 1	2022	278.0	3.7	ns	ns
2	Forest honey – Locality 2	2024	298.0	5.5	ns	ns
3	Meadow honey – Locality 3	2023	186.7	31.4	p = 0.022	p = 0.001
4	Meadow honey – Locality 4	2024	228.0	257.0	p = 0.015	p = 0.000
5	Meadow honey – Locality 5	2024	236.3	8.1	p = 0.012	p = 0.021
6	Meadow honey – Locality 6	2024	209.0	224.0	p = 0.042	p = 0.000

TPC: (significant difference, $p < 0.05$)
TFC: (significant difference, $p < 0.01$)

The results of the TPC and TFC analysis indicate clear seasonal and geographical variability, particularly in TFC values. These findings underscore the influence of botanical origin and environmental conditions on the composition of honey. According to one-way ANOVA, statistically significant differences in TPC were found between meadow honey samples from different harvesting years (2023 vs. 2024) and sampling sites ($p < 0.05$). Even more pronounced were the differences in TFC, which varied significantly between meadow honeys collected from

distinct locations ($p < 0.01$). These results demonstrate that both seasonal dynamics and regional biodiversity have a measurable impact on the bioactive composition of honey.

3.2. Correlation between TPC and TFC values: The Pearson correlation analysis indicated a moderate positive correlation between total phenolic content (TPC) and total flavonoid content (TFC) across all honey samples, with a correlation coefficient of $r = 0.73$ and a significance level of $p < 0.05$ (Table 2). This suggests that samples with higher phenolic content also tend to contain higher levels of flavonoids. Similar trends were reported in earlier studies by Đorđević et al. (2020) and Blasa et al. (2006), reinforcing the relationship between these two classes of bioactive compounds in honey.

Table 2. Correlation between total phenolic and flavonoid content in analysed honey samples

Parameter	Total Flavonoids	Total Phenolics
Total Flavonoids	1.000	0.73*
Total Phenolics	0.73*	1.000

Significant at $p < 0.05$

3.3. Environmental and Botanical Influences: The differences observed in TPC and TFC values among the forest and meadow honeys analyzed in this study can be explained by several interconnected environmental and botanical variables:

Floral origin: The specific plant species that bees forage from significantly affect the phenolic and flavonoid composition of honey. This observation is consistent with results reported by Estevinho et al. (2008) and Blasa et al. (2006).

Seasonal and geographical conditions: Environmental stress during the blooming period—such as limited water availability, high temperatures, or increased UV radiation—can enhance the synthesis of secondary metabolites, including phenolic compounds (Alvarez-Suarez et al., 2010).

Climate-related and ecological variability: Factors such as altitude, soil properties, and the diversity of local vegetation likely contributed to the observed variation in flavonoid content, particularly among meadow honey samples from different locations. Comparable results were presented by Tsavea et al. (2020) in their analysis of Greek honeys, highlighting the importance of local environmental conditions in shaping honey's phytochemical profile.

3.4. Functional Implications: The moderate positive correlation ($r = 0.73$) between phenolic and flavonoid content supports the use of these compounds as key indicators of antioxidant potential in honey. This finding aligns with previous research by Beretta et al. (2005) and Miguel et al. (2007), who associated higher polyphenol concentrations with enhanced antioxidant activity, particularly in terms of radical scavenging capacity. Therefore, monitoring TPC and TFC may be a valuable approach in assessing the nutraceutical quality of honey.

4. Conclusion

The results of this study clearly demonstrate that botanical origin, geographical location, and year of harvest significantly influence the total phenolic and flavonoid content in Macedonian honey. These factors, particularly floral diversity and regional environmental conditions, play a crucial role in determining the bioactive and functional properties of honey. The findings support the growing recognition of honey as a valuable functional food, with its antioxidant potential closely linked to its phenolic composition. To gain a more comprehensive

understanding of these relationships, future studies should include expanded geographic sampling and detailed profiling of individual phenolic and flavonoid compounds. This research provides a scientific basis for the valorization and promotion of Macedonian honey as a product with high nutritional and health-promoting value, supporting its potential in local and international markets.

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