

HMF, DIASTASE NUMBER AND TOTAL PHENOLIC CONTENT AS KEY INDICATORS OF ALBANIAN HONEY QUALITY

Xhulieta HAMITI

Department of Chemistry, Faculty of Natural Sciences, University of
Tirana, Albania

Gjyliza SHALLARI

Canadian Institute of Technology, Faculty of Engineering, Tirana,
Albania

Adelaida ANDONI

Department of Chemistry, Faculty of Natural Sciences, University of
Tirana, Albania

Corresponding author: xhulieta.hamiti@fshn.edu.al

ABSTRACT

This study examines the physicochemical properties of 29 honey samples from different Albanian beekeeping regions, analyzing parameters essential for evaluating honey quality, including humidity, ash content, pH, free acidity, refractive index, electrical conductivity, *hydroxymethylfurfural* (HMF), diastase number, and TPC (total phenolic content). The measured parameters yield mean values for humidity at 16.05%, ash content at 1.34%, and TPC at 135.88 mg GAE/kg, among others. One-way ANOVA results indicated significant regional differences in humidity ($p = 0.002$), free acidity ($p = 0.001$), electrical conductivity ($p = 0.004$), HMF ($p < 0.001$), ash content ($p = 0.023$), TPC ($p = 0.013$), and diastase number ($p = 0.042$). Correlation analysis revealed significant associations, particularly between HMF and diastase number ($r = -0.56, p < 0.01$) and between TPC and diastase number ($r = 0.45, p < 0.05$). Regression analysis identified humidity and HMF as negative factors influencing phenolic content, while ash content and diastase number showed positive relationships with it. These findings underscore the importance of closely monitoring physicochemical

properties to enhance both the marketability and health benefits of honey products.

Keywords: physicochemical properties, HMF, DN, TPC, honey, Albania.

1. INTRODUCTION

Honey, a natural sweetener derived from floral nectar through the efforts of bees, is prized not only for its nutritional and medicinal benefits but also for its unique sensory qualities. Although, honey is predominantly consumed worldwide for its sweetness, its health benefits have recently garnered attention. Honey quality is influenced by various factors, including the floral source, environmental conditions, and processing methods. Among quality indicators, *hydroxymethylfurfural* (HMF) and diastase number (DN) are critical parameters. HMF, a compound formed during heat treatment or extended storage, signals honey degradation and mishandling, as documented in (Bogdanov *et al.*, 2002; Chen *et al.* 2017). High HMF values indicate lower quality and may serve as a warning for consumers. In contrast, DN reflects the enzymatic activity in honey and serves as an indicator of freshness and quality; a higher DN value suggests fresher, higher-quality honey (Codex *Alimentarius* 2001; Almeida-Muradian *et al.*, 2005). Additionally, the Total Phenolic Content (TPC)—comprising flavonoids, phenolic acids, and other polyphenols—supports honey's biological properties, such as its anti-inflammatory, antimicrobial, and cardioprotective effects. Generally, a higher TPC correlates with greater antioxidant potential, quality, and health benefits, though this depends on honey type and processing conditions.

Honey quality is inherently complex, shaped by organoleptic, physicochemical, and microbiological characteristics. Among these, physicochemical parameters are the most universally standardized, with Codex *Alimentarius* 12-1981 specifying ranges for optimal quality. These parameters include moisture content, pollen type, electrical conductivity, pH, free acidity, HMF content, diastase activity, and total phenolic content (TPC). Recognized methodologies, such as those from Codex *Alimentarius* (2001), AOAC 980.23-1983, AOAC 958.09-1977(2010) and Harmonized Methods of the International Honey Commission (2009), ensure accurate measurement of these parameters in accordance with international food quality standards. Studies, including those by Silva *et*

al. (2016), highlight HMF, diastase number (DN), and sugar content as particularly critical in honey quality control.

Honey production in Albania is a vital part of the agricultural sector, with country's diverse flora imparting unique characteristics to regional honey varieties. Studies (Dajçari and Barçuni, 2019; Dajçari *et al.*, 2020; Zekaj *et al.*, 2021) have focused on the physicochemical properties of Albanian honey, underscoring the impact of geographical and environmental factors on its quality. For example, Dajçari *et al.* (2020) analysed several quality parameters of Albanian honey, revealing significant variations tied to floral source and region. Key physicochemical parameters such as HMF and diastase number (DN), are widely recognized in assessing honey quality against international standards. Additionally, several studies have linked phenolic compounds in honey to its antioxidant properties, contributing notable health benefits (Märghitaş *et al.*, 2009; Muthusamy *et al.*, 2015; Ferretti *et al.*, 2017; Mărgăoanu *et al.*, 2020).

The objective of this study is to analyze the HMF and DN levels in honey samples collected from various Albanian regions, along with other quality parameters such as phenolic content (TPC), pH, and electrical conductivity. This research contributes to the existing literature on honey quality and provides insights into the distinctive characteristics of Albanian honey.

2. MATERIALS AND METHODS

Sampling procedure

A total of 29 honey samples were collected from experienced local beekeepers across various regions of Albania between August and September 2022, with one additional sample randomly selected from the market. All samples were stored under suitable conditions until analysis, which was conducted in accordance with ISO 12824:2016. Upon arrival at the laboratory, the physicochemical properties of the honey were analyzed following the "Harmonized Methods of the International Honey Commission" (Bogdanov 2002). To ensure reliability, two subsamples were tested per honey sample.

Physicochemical analysis methods

For botanical classification, each sample's floral origin was identified using melissopalynological methods (Louveaux *et al.*, 1978). Microscopic

analysis confirmed the monofloral or polyfloral status of each sample, as indicated by the producers.

The pH, total free acidity, moisture content, electrical conductivity, and *hydromethylfurfural* (HMF) were analyzed according to Council Directive 2001/110/EC 2001. The White's method was applied for the spectrophotometrical determination of HMF content (White Jr. 1979), while free acidity was measured by titrating a 10 g honey sample dissolved in 75 mL of CO₂-free distilled water with 0.1 M NaOH until a pH of 8.3 was reached, with results reported in meq/kg. Each parameter was measured in triplicate. Moisture content was determined using a refractometer.

Electrical conductivity measurements were taken using a digital Abbe Carl Zeiss refractometer. After a six-minute equilibration, readings were taken from a 20% (w/v) honey solution prepared with distilled water and reported in mS/cm. Ash content was determined by weighing the residue after heating the sample to no more than 600°C.

HMF concentration was determined by UV absorbance at 284 nm. To minimize interference, absorbance of the honey solution was measured before and after bisulfite addition, with background correction at 336 nm to calculate HMF levels. The HMF content and diastase activity were quantified using a validated method, employing certified reference material FAPAS T2848QC with a certified value of 31.9 ± 6.01 .

The limits of detection (LOD) and quantification (LOQ) were calculated using the standard deviation of the intercept from the calibration curve, yielding values of 2.2 mg/kg and 6.8 mg/kg, respectively. In alignment with Regulation (EC) No 110/2001 and Codex *Alimentarius* Standard 12 (1981), the LOD was set at less than 1/10 of the maximum allowable HMF level of 40 mg/kg, and the LOQ at less than 1/5 of this threshold.

Diastase activity was determined using a standard starch solution that develops an iodine-blue color within a defined intensity range. Under standardized conditions, the enzyme in the honey sample acts on the starch, and the reduction in blue color is measured at set intervals. The time required to reach an absorbance of 0.235 is calculated through a regression equation, plotting absorbance against time. Based on Schade's original research and published by Codex *Alimentarius* (2001), this method can present challenges in achieving precise and accurate measurements for diastase activity. Therefore, the precision, accuracy, and uncertainty of the method were evaluated using the certified reference material FAPAS T2848QC. Instrumental limits of detection (LOD) and quantitation (LOQ)

were also calculated, with target LODs and LOQs set at less than one-tenth and one-fifth, respectively, of the maximum level outlined in Council Directive 2001/110/EC. 2001, for effective composition assessment of honey samples.

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method as described in (Gülçin *et al.*, 2020). A 0.2 mL aliquot of honey stock solution was diluted with 3.16 mL distilled water, followed by 0.2 mL of Folin-Ciocalteu reagent and through mixing. After three minutes, 0.8 mL of 7.5% Na₂CO₃ was added, and the mixture was incubated at room temperature in the dark for 2 hours with intermittent shaking. Absorbance was measured at 760 nm, and a standard curve using gallic acid was used to calculate the total phenolic content, expressed in mg gallic acid equivalent (GAE) per gram of honey.

Statistical analysis

Data analysis was conducted using SPSS software, version 26. All physicochemical parameters were subjected to descriptive statistics to summarize the data distribution into means and standard deviations. Additionally, Pearson's correlation coefficients were calculated to assess the correlations among HMF, DN, TPC, and other physicochemical parameters by following the methodological approach described in (Field 2013). Furthermore, regression analysis was performed to explore the impact of major physicochemical variables on the levels of HMF and DN, aiming to better understand their predictive relationships. This comprehensive statistical methodology allows for a detailed analysis of the variables influencing honey quality.

3. RESULTS AND DISCUSSION

Melissopalynological analysis

Palynological analysis is essential for differentiating honey produced in distinct geographical and climatic regions. The frequency distribution of pollen from various plant species identified in the honey samples is in Table 1 reported. The melissopalynological characteristics confirmed the floral origin of the majority of the samples. Out of 16 samples, all 16 were classified as multifloral, containing a mixture of pollen from different plant species, with frequencies categorized as (S), (r), and (i). Twelve samples were identified as typical monofloral honey, with a dominant frequency

(D) of *Castanea sativa* pollen, which reached approximately 77%. Two samples exhibited a significant presence of *Arbutus unedo* pollen, up to 72%, while two others showed a high frequency of *Erica* pollen at 84%. In this study, monofloral honey was confirmed by a high pollen frequency (> 45%), which facilitated the identification of the specific plant species.

Table 1. Botanical and geographic origin of the honey

No. samples	Honey type	Taxon	Pollen frequency
16	Polyfloral	<i>Quercus(s)</i> , <i>Castanea (r)</i> , <i>Trifolium(r)</i>	(S), (s), (r)
9	Monofloral	<i>Castanea Sativa (D)</i> , <i>Allium (s)</i> , <i>Arbutus (r)</i>	77%
2	Monofloral	<i>Arbutus Unedo (D)</i> , <i>Helianthus Galega</i>	72%
2	Monofloral	<i>Erica (D)</i> , <i>Arbutus (s)</i> <i>Crataegus,</i>	84%
D: > 45% pollen; S: 16-45% pollen; s: 3-15% pollen; r: 1-3% pollen; i: >1% pollen			

Physicochemical Properties of Honey Samples

The mean of physicochemical characteristics of the analyzed honey samples are given in Table 2. This analytical outline includes humidity, ash content, pH, free acidity, electrical conductivity, HMF, diastase number, and total phenolic content, all elements important for honey quality evaluation.

Table 2. Summary of Physicochemical Properties of Honey Samples

Parameter	Mean \pm SD	Range	Q1 (25th Percentile)	Q2 (Median)	Q3 (75th Percentile)
Humidity (%)	16.05 \pm 2.54	13.06 - 22.2	14.2	15.8	17.6
Ash (%)	0.55 \pm 1.11	0.05 - 0.64	0.8	1.2	1.92
pH	4.34 \pm 0.26	3.84 - 5.20	4.1	4.3	4.6
Free Acidity(me/kg)	29.93 \pm 10.73	14.45 - 49.5	20	28	37.5

EC ($\mu\text{S/cm}$)	682.9 ± 290.2	134.9 - 1430	490	674.5	950
HMF (mg/kg)	18.25 \pm 22.4	0.06 - 88.32	5	10.5	25
DN (Schade)	7.43 \pm 7.41	3.00 - 41.10	4	6	10.5
TPC (mgGAE/kg)	135.88 \pm 50.76	70.6 - 268.5	100	135	171.25

The average humidity of the honey samples was 16.05% (SD = 2.54), with values ranging from 13.06% to 22.20%. The first quartile (Q1) value of 14.20%, indicates that 25% of the samples had moisture levels lower than 16.05%. The ideal moisture content for honey is typically below 20%. Twelve samples had moisture levels below 16%, suggesting a longer shelf life and lower risk of fermentation. Only one sample, a monofloral *Arbutus*, exceeded 20% threshold, with a value of 22.2%, making it more prone to fermentation (Bogdanov *et al.*, 2002; Dajçari *et al.*, 2020).

The average free acidity was 29.93 meq/kg (SD = 10.7), indicating that all samples fall within the standard requirement of less than 50 meq/kg (Buchmann *et al.*, 2018). These values suggest that samples are of good quality and freshness, as higher acidity levels often indicate improper storage.

The average electrical conductivity was 682.87 $\mu\text{S/cm}$, with values ranging from 134.90 to 1430.00 $\mu\text{S/cm}$. This wide range reflects the diverse botanical origins of the honey, potentially indicating varying mineral content and organic acids (Zekaj *et al.*, 2021).

The average HMF concentration was 18.25 mg/kg, with values ranging from 0.06 to 88.32 mg/kg. While 21 samples had HMF levels below 40 mg/kg, which is acceptable for fresh honey, 8 samples exhibited higher values, indicating poor storage or excessive heat exposure.

The average diastase number (DN) was 12.49 ± 7.41 . Diastase activity is a critical indicator of honey freshness and quality, with low values suggesting aging or overheating. However, no specific interpretations for these samples were provided (Mëhilli *et al.*, 2019).

The mean total phenolic content (TPC) was 135.88 mg GAE/kg (SD = 50.76), with values ranging from 12.46 to 268.53 mg GAE/kg. Aside from three monofloral samples with lower TPC values (33.77 mg/kg, 30.09 mg/kg, 12.46 mg/kg), the remaining samples showed moderate to high

TPC levels. Phenolic compounds are crucial for honey's antioxidant properties, contributing to its health benefits.

Overall, the honey samples display considerable variation in their physicochemical properties, likely reflecting differences in floral origin, processing, and storage conditions. Most samples appear to be of good quality based on their humidity, free acidity, and low HMF levels. However, 5 honey samples showed HMF levels exceeding 40 mg/kg and lower diastase activity, suggesting that these samples may not meet the quality standards required for further study.

Correlation Analysis

The relationships between key physicochemical parameters were analyzed using Pearson's correlation coefficients, as in **Table 3 reported**.

Table 3. Pearson Correlation Coefficients Among Physicochemical Parameters

Parameter	HMF	DN	TPC	pH	EC	Free Acidity
HMF	1.00	-0.56**	-0.32	0.21	0.15	0.38*
DN	-0.56**	1.00	0.45*	-0.25	0.30	-0.48*
TPC	-0.32	0.45*	1.00	-0.10	0.35*	-0.22
pH	0.21	-0.25	-0.10	1.00	0.05	0.10
EC	0.15	0.30	0.35*	0.05	1.00	-0.12
Free Acidity	0.38*	-0.48*	-0.22	0.10	-0.12	1.00

Note: * $p < 0.05$; ** $p < 0.01$.

Pearson's correlation coefficients analysis among the key physicochemical parameters of honey provides insights into the interrelationships affecting honey quality. These results are generally consistent with existing literature on honey quality and its determinants.

A significant negative correlation was found between HMF and DN ($r = -0.56$; $p < 0.01$). Although this is not an extremely strong correlation in our results, it indicates that higher HMF levels correspond with lower enzymatic activity (DN). However, most of our samples have low HMF levels alongside high DN values. For example, the M9 sample has an HMF value of 0.06 mg/kg and a DN value of 41.1 Schade units, reflecting high enzymatic activity despite the low HMF.

Additionally, a positive correlation was observed between total phenolic content (TPC) and DN ($r = 0.45$; $p < 0.05$), supporting the idea that phenolic compounds may enhance enzymatic activity. This finding aligns with previous studies by Mëhilli *et al.*, (2019) and Buchmann *et al.*, (2018), suggesting that higher antioxidant levels (i.e., phenolic compounds) might contribute to better honey quality. However, many of our samples with high TPC values exhibit relatively low DN values, indicating that the relationship may be more complex.

The correlations between the key parameters (TPC, HMF, DN, and free acidity) in our samples are not particularly strong, likely due to the complex interactions between these factors in defining honey quality. Continuous monitoring of such physicochemical properties is crucial for maintaining honey quality, ensuring its marketability, and maximizing the health benefits associated with high-quality honey.

Regression Analysis

Multiple regression analysis was performed to determine the relationships between total phenolic content (TPC) as the dependent variable and various independent variables, including humidity, ash, pH, free acidity, refractive index, electrical conductivity, HMF, and diastase number.

Table 4. Summary of Regression Analysis for Total Phenolic Content (TPC)

Variable	Coefficient (β)	Standard Err.	tValue	p-Value
Humidity (%)	-0.49	0.12	-4.08	0
Ash (%)	1.75	0.4	4.38	0
pH	2.35	1.2	1.96	0.052
Free Acidity (meq/kg)	-1.25	0.27	-4.63	0
Electrical Conductivity ($\mu\text{S/cm}$)	0.01	0.01	0.81	0.418
HMF (mg/kg)	-0.3	0.08	-3.75	0
Diastase Number (DN)	2.1	0.85	2.47	0.014

The regression model was significant: $F(8, 91) = 16.34$, $p < 0.001$, explaining 58% of the variance in TPC ($R^2 = 0.58$), meaning that 58% of

the variation in TPC levels can be attributed to the factors included in the model. The key predictors for TPC were as follows:

Humidity: Negatively influenced TPC, which agrees with Alissandrakis *et al.*, (2007), as excess moisture can dilute phenolic compounds.

Free Acidity: Similar to humidity, higher levels of free acidity were associated with lower TPC, indicating reduced honey quality, consistent with Tiwari *et al.* (2020).

HMF. A negative relationship was found between HMF levels and total phenolic content (TPC), suggesting that proper storage is essential, as increased HMF indicates honey degradation, as described by Bogdanov *et al.*, (2002).

Diastatic number. Conversely, an increase in diastase number (DN) showed a positive correlation with TPC, indicating that higher enzymatic activity is associated with elevated levels of phenolic compounds. This supports findings from previous studies, including Mēhilli *et al.*, (2019) and Zekaj *et al.*, (2021), which emphasize the significance of enzymatic activity in enhancing honey quality. In this research, various key physicochemical parameters were measured, including TPC, DN, humidity, ash content, pH, free acidity, refractive index, electrical conductivity, and HMF, followed by statistical analysis.

Descriptive statistics provided mean values and variability for these parameters. Correlation analysis revealed significant relationships between the parameters, with a notable negative correlation between HMF and DN, indicating that higher HMF levels reduce enzymatic activity.

4. CONCLUSIONS

This study aimed to assess the physicochemical properties of 29 honey samples from various botanical origins, which will be utilized in a subsequent investigation of honey's therapeutic effects, specifically its role in wound healing. Several key factors, including humidity, ash content, free acidity, *hydroxymethylfurfural* (HMF), and *diastatic number* (DN), were found to significantly influence the total phenolic content (TPC) of honey. Higher humidity negatively impacted TPC, supporting the hypothesis that excess moisture dilutes phenolic compounds. Therefore, moisture control is essential for maintaining honey quality.

A positive correlation between ash content and TPC suggests that increased mineral content may enhance the concentration of phenolic

compounds. Conversely, higher free acidity was associated with lower TPC, indicating that increased acidity may negatively affect honey quality. Additionally, elevated HMF levels were negatively correlated with TPC, reinforcing the idea that improper storage or heating, which increases HMF, degrades honey quality by reducing the concentration of phenolic compounds. The positive relationship between DN and TPC in our samples indicates that higher enzymatic activity is linked to improved phenolic compound concentration, further supporting the notion that enzymatic activity is a key indicator of honey quality. The regression model accounted for 58% of the variance in TPC, suggesting that while the included predictors (humidity, ash content, free acidity, HMF, and DN) are significant, other unexplored factors likely contribute to the remaining 42% of variability. In conclusion, moisture control, proper storage (to limit HMF), and the maintenance of enzymatic activity are critical for ensuring high phenolic content and overall honey quality. Effective monitoring of these factors is essential for the production of high-quality honey.

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