Physicochemical profile and antioxidant activity of mint honey from Ha Giang Province, Viet Nam

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Abstract

Monofloral honey samples (*Mentha longifolia* L.) from Ha Giang, Viet Nam, were chosen for this study. The samples were analyzed by their physicochemical properties, including total phenolic content (TPC), total flavonoid content (TFC), and total carotenoid content (TCC). The levels of hydroxymethylfurfural (HMF) (33.40-37.51) mg/kg and free acid (13.67-24.17) meq/kg in mint honey were measured. Moreover, the high levels of TPC (45.15-70.44) mg GAE/100 g, TFC (3.04-5.04) mg GE/100 g, and TCC (12.54-17.01) mg β-carotene/kg content in mint honey contributed to its antioxidant activity, as indicated by IC_{50} of 37.99-50.89 mg/mL. The findings of this study emphasize the significance of mint honey as a health-promoting substance.

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1 Introduction

Honey is widely consumed worldwide for its delicious taste and high nutritional value, which includes antioxidant, antimicrobial, and anti-inflammatory properties. It contains various nutrients and bioactive compounds, including proteins, amino acids, vitamins, minerals, flavonoids, phenolic acids, and enzymes. Quality assessments of honey based on physicochemical parameters mainly depend on the floral source and seasonal, environmental, processing, manipulation, packaging, and storage conditions. In addition, phenolic compounds, ascorbic acid, flavonoids, and carotenoid derivatives have been suggested as critical elements responsible for their antioxidant potential.

Phenolic compounds found in honey are secondary metabolites derived from nectar. They are a diverse group of chemicals mainly belonging to flavonoids and phenolic acids. The concentration of polyphenols and flavonoids in honey depends on the geographical and botanical origin of the nectar. It varies between 56 mg/kg to 500 mg/kg and 0.6 mg/kg to 6.4 mg/kg of honey, respectively [1]. They have been used for honey quality assessment and marker identification to characterize honey's botanical origin. Additionally, some floral pigments, such as carotenoids, chlorophylls, and anthocyanins, also contribute as markers of honey origin. It has been reported that honey pigment has a positive relationship with its antioxidant potential [2].

Many analytical methods have been used to assess the antioxidant potential of honey, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) (free radical scavenging activity), FRAP (ferric reducing/antioxidant power), ORAC (oxygen radical absorbance capacity), AEAC (ascorbic acid content), and TEAC (Trolox equivalent antioxidant activity) [10]. Free radical scavenging ability with the half-maximal inhibitory concentration (IC_{50}) was reported in eucalyptus (31.24 mg/mL), longan (31.20 mg/mL), ginger (2.77 mg/mL), and chamomile (62.2 mg/mL) honey [3, 4]. These findings

indicated a correlation between total phenolic content (TPC) and total flavonoid content (TFC) in exhibiting antioxidant activity.

Vietnam's topographic and climatic conditions support various fruit trees, producing rich and diverse honey. However, there is limited scientific data on the quality of honey, especially honey's characteristics in each locality. Therefore, mint (*Mentha longifolia* L.) honey was chosen to evaluate the physicochemical profile and antioxidant activity for reliable quality judgment.

2 Materials and Methods

2.1 Materials

Mint honey samples (M1-M3) represented the number of production batches of the beekeeping farm; the M4 sample was purchased from the market) were collected Viet Nam. Each sample type (M1-M4) includes three different types of honey samples. Samples were stored at 20 °C in a dark room (in dark glass bottles) for a maximum of 4 months until analysis.

2.2 Chemicals

The chemicals, including 2,2-diphenyl-1-picrylhydrazyl (99 %), gallic acid (99 %), folin reagent (99 %), and DNS reagent (99 %), were purchased from Sigma-Aldrich. Other chemicals were of analytical grade.

2.3 Physicochemical Analysis

Physicochemical parameters, including moisture, HMF (5-Hydroxymethylfurfural) content, sugar, protein, free acidity, and minerals, were analyzed following the previously published methods. Water content was determined by a digital refractometer (RM 40, Switzerland). Free acidity was measured by alkalimetric titration (AOAC, 2000). The HMF content was determined using the HPLC method with UV detection (Agilent Technologies 1290, USA). Total sugar was determined through the phenol-sulfuric acid method [5]. Protein content was analyzed using Kjeldahl digestion as described in the AOAC International 979.09 method.

2.4 Determination of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Content of Carotenoids

TPC was determined by a spectrophotometric method using the Folin-Ciocalteu reagent. The result was expressed as mg gallic acid equivalents (GAE)/100 g [6]. TFC was detected by mixing honey with a solution of $AlCl_3$, and the absorbance was measured at 415 nm. Quercetin was used as the standard, and the results expressed in mg of quercetin equivalents (mg QE/100 g) [7].

2.5 Determination of antioxidant capacity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of honey was measured using the method of Brand-Williams et al. (1995). The diluted extract (0.1 mL) was mixed with 3.9 mL of 60 µM methanol DPPH solution. The decrease of absorbance at 515 nm was measured after 30 min of incubation against a methanol blank [8]. DPPH free radical scavenging activity (DPPH %) was calculated using equation (1). The results are recorded based on the IC_{50} value, the concentration at which the sample can reduce 50 % of DPPH free radicals.

$$DPPH\ (\%) = \frac{ABS_C - ABS_T}{ABS_C} \times 100 \quad (1)$$

Abs_C: Absorbance of the control sample

Abs_T: Absorbance of the specimen

2.6 Statistical Analysis

All experiments were conducted thrice; the results were reported as the mean ± standard deviation. One-way analysis of variance (ANOVA) was used to determine differences between means using Minitab 16 software (Minitab Co., State College, PA, USA). p-values < 0.05 were considered statistically significant.

3 Results and Discussion

3.1 Physicochemical Parameters

Table 1 presents the physicochemical attributes of mint honey, such as moisture, free acidity, HMF, protein, sugars, and minerals. The honey's moisture content of M1 and M4 samples exceeded the maximum acceptable limit of 20 % set by the International Quality Regulations [9]. The free acidity and HMF content meet the regulatory requirements of the Codex Alimentarius Commission (HMF content < 40 mg/kg and free acidity < 50 meq/kg).

Moisture plays a critical role in assessing the quality of honey because excess water in honey can promote yeast fermentation, producing ethyl alcohol and carbon dioxide. This means that moisture is an essential factor in determining the stability of honey. The moisture content of honey can vary depending on factors such as the source of the honey, time of harvest, environmental conditions, and the actions of the beekeeper. Vietnamese honey typically contains moisture in the range of (10.78-23.96) % [10], while the moisture

content of (17.0-20.0) % was obtained for honey from Thailand [11].

HMF is essential in indicating the purity and freshness of honey. It is a substance formed through the Maillard reaction, which occurs when acidic honey is heated. In our study, a low HMF content indicates that the honey has not been exposed to high heat or poor storage conditions. In the study of Pham et al. (2022), the HMF content of Vietnamese honey ranged from 12.37 mg/kg (rubber honey) to 58.58 mg/kg (lychee honey) [10]. According to the Codex Alimentarius Commission (2001), certain types of botanical honey from tropical countries have a higher maximum HMF content of 80 mg/kg.

Table 1 Physicochemical parameters of mint honey

Parameters	M1	M2	M3	M4
Moisture (%)	18.15 ± 0.62^{c}	19.50 ± 0.31^{b}	22.38 ± 0.11^{b}	24.50 ± 0.14^{a}
Free acidity (meq/kg)	13.67 ± 2.08^{c}	23.67 ± 0.29^{a}	17.33 ± 0.76^{b}	24.17 ± 2.91^{a}
HMF (mg/kg)	36.13 ± 0.58^{b}	33.40 ± 0.80^{c}	37.51 ± 0.28^{a}	33.89 ± 0.72^{c}
Protein (%)	0.12 ± 0.02^{c}	0.18 ± 0.01^{b}	0.10 ± 0.01^{c}	0.26 ± 0.05^{a}
Sugar (%)	82.96 ± 3.50^{a}	81.11 ± 2.50^{a}	79.29 ± 3.15^{ab}	76.97 ± 1.61^{b}
Ash (%)	0.28 ± 0.01^{c}	0.54 ± 0.02^{a}	0.35 ± 0.01^{b}	0.22 ± 0.02^{d}

Data are expressed as mean values ± standard deviation from three samples of each honey type.

Different superscript letters (a, b, c, and d) show the statistical difference ($p < 0.05$) between the tested groups.

Sugars comprise most honey components and can be utilized to evaluate honey quality. The primary carbohydrates in floral honey are fructose and glucose, which constitute (65-80) % of the total soluble solids [12]. A study by Boussaid et al. (2018) on honey in Tunisia found that the total sugar content varies from 70.9 % to 77.86 %. Specifically, the total sugar content was 76.5 % for peppermint honey, 74.3 % for rosemary honey, 77.86 % for eucalyptus honey, 75.94 % for musk honey, and 70.9 % for orange honey [13].

The mint honey samples contain a modest amount of protein (0.10-0.26) %. A previous study disclosed that the protein content in 45 honey samples ranged from 0.1 % to 0.5 % [14]. A high protein content in honey can indicate high pollen levels, which shows that the honey is entirely created from natural, high-quality pollen. Moreover, mineral profiles in mint honey may be utilized to differentiate mint honey from various floral honey types.

3.2 Total Phenolic content (TPC), Total Flavonoid Content (TFC), Carotenoid Content And Antioxidant Activity

The antioxidant properties of mint honey samples were screened using the DPPH test for radical scavenging activity. The IC_{50} values of mint honey ranging from (37.99-50.89) mg/mL were approximately (879-1178) times higher than that of the positive control sample, ascorbic acid (IC_{50} = 0.0432 mg/mL). According to the study by Chua et al. (2011), the IC_{50} value of tualang honey was 5.8 mg/mL, and gelam was 6.68 mg/mL [15]. However, according to Kishore et al. (2011), the IC50 value of tualang honey was 48.896 mg/mL, and gelam was 15.681 mg/mL [16].

TPC and TFC of mint honey were comparable to other honey types. The TPC and TFC of coffee honey ranged from 56.077 mg QE/100 g to (67.011 ± 0.21) mg GAE/100 g and from (4.551 ± 0.140) mg QE/100 g to (8.520 ± 0.057) mg QE/100 g, respectively [17]. The carotenoid contents of mint honey ranged from (12.54 to17.01) mg β-carotene/kg, higher than the contents reported by Bueno-Costa et al. (2016), which obtained contents ranging between 0.56 mg to 6.19 mg β-carotene/kg [7]. Although mint honey contains a small amount of carotenoids, it contributes to its color and antioxidant properties.

It's reported that honey's polyphenol content correlates significantly with its color, implying that darker honey has higher phenolic compounds and enhanced antioxidant activity. The antioxidant activity of dark and light honey in Portugal was measured with an average value of (27.24 to 68.17) mg/mL, respectively [18]. These results are consistent with our data, which related that mint honey had a light amber color, measuring (31.3 ± 15.7) mm on the Pfund scale [19].

Table 2 Total polyphenol, flavonoid, and carotenoid contents and antioxidant properties of mint honey.

Sample	TPC (mg GAE/100g)	TFC (mg QE/100g)	TCC (mg β-carotene/kg)	IC_{50} (mg/mL)
M1	70.44 ± 1.73^{a}	5.04 ± 0.12^{a}	15.14 ± 0.84^{b}	37.99 ± 0.77^{d}
M2	60.19 ± 3.08^{b}	4.55 ± 0.28^{b}	17.01 ± 0.70^{a}	40.91 ± 0.39^{c}
M3	55.43 ± 2.37^{c}	3.75 ± 0.16^{c}	14.41 ± 0.47^{b}	45.15 ± 0.43^{b}
M4	45.15 ± 1.35^{d}	3.04 ± 0.24^{d}	12.54 ± 0.97^{c}	50.89 ± 0.37^{a}
Ascorbic acid	-	-	-	0.0432 ± 0.0007^{e}

Data are expressed as mean values ± standard deviation of three independent experiments.

Different superscript letters (a, b, c, and d) show the statistical difference ($p < 0.05$) between the tested groups.

3.3 PCA Analysis

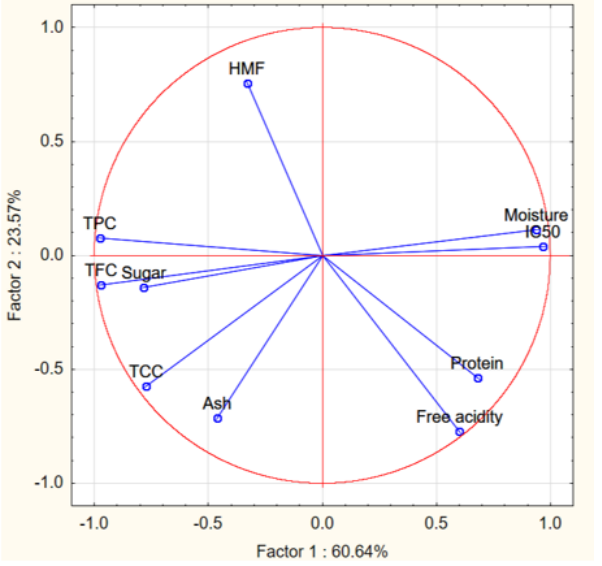

Figure 1 Principal component analysis (PCA) biplot for physicochemical parameters, including TPC, TFC, and TCC, and antioxidant activity (IC_{50}, the half-maximal inhibitory concentration) of mint honey.

Principal Component Analysis (PCA) is an analytical method to assess the interrelationships between data and their distribution. The result shows critical data structures based on physicochemical properties, phenolic compounds, and antioxidant activity (Figure 1). The total variability amounts to 84.21 %, with PC1 explaining 60.64 % and PC2 accounting for 23.57 % of the variation. The analysis revealed that moisture, IC_{50}, TPC, TFC, and sugar were closely associated with PC1, while HMF, TCC, ash, protein, and free acidity were predominant in PC2. The data points located adjacent and opposite directions to one another on the plot exhibit a positive correlation and a negative correlation, respectively. This finding demonstrates that TPC, TFC, and TCC are positively correlated to the antioxidant potency of mint honey.

4 Conclusion

The study offers insights into mint honey's primary physicochemical and antioxidant properties. Our findings also highlight the presence of essential nutrients in mint honey, which is crucial for its characterization and quality assessment. These results revealed the high quality of Vietnamese mint honey. The highest level of bioactive compounds consists of phenolic and flavonoid, followed by carotenoids which contribute to antioxidant activity. The high antioxidant activity of mint honey provides significant health benefits.

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Tính chất lý hóa và hoạt tính chống oxy hóa của mật ong bạc hà từ Hà Giang, Việt Nam

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Tóm tắt Trong nghiên cứu này, các mẫu mật ong đơn hoa (*Mentha longifolia* L.) từ Hà Giang, Việt Nam đã được chọn. Các mẫu được phân tích các tính chất lý hóa, tổng hàm lượng phenolic, tổng hàm lượng flavonoid và hàm lượng carotenoid. Mức độ hydroxymethylfurfural (HMF) (33,40-37,51) mg/kg và axit tự do (13,67-24,17) meq/kg trong mật ong bạc hà đã được ghi nhận. Hơn nữa, mức độ cao của tổng hàm lượng phenolic (TPC) (70,44 mg GAE/100 g), flavonoid (TFC) (3,04-5,04) mg GE/100 g và carotenoid (TCC) (12,54-17,01) mg β-carotene/kg trong mật ong bạc hà đã góp phần vào hoạt động chống oxy hóa của nó, thể hiện qua IC_{50} 37,99-50,89 mg/mL. Những phát hiện của nghiên cứu này nhấn mạnh tầm quan trọng của mật ong bạc hà như một chất tăng cường sức khỏe.

Từ khóa Mật ong bạc hà, hợp chất phenolic, hoạt tính chống oxy hóa, mật ong đơn hoa