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Total Phenolic Content in Monofloral Honey Varieties from Beni Mellal-Khenifra, Central Morocco: Variability and Determinants

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ABSTRACT

Honey is valued not only for its taste and nutritional properties but also for its health benefits, primarily due to its phenolic compound content. Environmental and biological factors influences this content. This study investigates the total polyphenol content of monofloral honey varieties from the Beni Mellal-Khenifra region and its relationship with botanical origin, geographic location, color, and harvesting period. The results showed highly significant differences in total phenolic content among honey samples with varying botanical and geographical origins ($p < 0.0001$). The study revealed a strong correlation ($r = 0.985^*$, $p<0.05$) between total phenolic content and the location of honey collection. Honey from lower altitudes, such as citrus and anis honey, showed lower total polyphenol content levels (13.53 ± 0.004 and 19.10 ± 0.004 mg EAG/100g, respectively), while honey from higher altitudes, such as euphorbia and carob honey, exhibited higher total phenolic content levels (30.61 ± 0.009 and 28.62 ± 0.003 mg EAG/100g, respectively). Additionally, a highly significant correlation was observed between total phenolic content and honey color ($r = 0.996^{**}$, $p<0.01$), indicating that darker honey contains higher phenolic levels. Conversely, no significant association was found between total phenolic content and harvesting period ($r = 0.486$, $p>0.05$). These results highlight the impact of environmental factors on honey's phenolic composition and underscore the importance of considering geographic and botanical influences in honey quality assessment.

Keywords: Monofloral Honey, Total Polyphenol Content, Beni Mellal- Khenifra region.

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INTRODUCTION

For centuries, honey has served as both a source of nutrition and a natural remedy for numerous health issues. Traditionally, it has been used to treat sore throats, digestive problems, and skin infections, benefiting from its antibacterial, anti-inflammatory, and antioxidant properties.

In a previous study we conducted in the Beni Mellal-Khenifra region, 82% of respondents reported using honey therapeutically for 42 different health symptoms, with respiratory conditions, skin disorders, and gastrointestinal issues being the most commonly mentioned [1].

Honey's health benefits are mainly due to its content of phenolic compounds, which are considered the main group of secondary metabolites in plants [2]. Their presence in honey is due to the bees' process of mixing their bodily fluids with flower nectars or plant secretions, which are composed of water, sugars, proteins, and phenolic compounds [3]. Studies utilizing High-Performance Liquid Chromatography (HPLC) for the analysis of various honey extracts have identified 27 phenolic compounds, predominantly comprising 13 flavonoids and 14 phenolic acids [4]. Biesaga and Pyrzynska (2009) have reported that all the honey samples that they assessed contained traces of similar phenolic compounds but in different concentrations [5]. The variability in phenolic compounds in honey has been linked to several factors, including its botanical and geographical origin, the harvesting period, and its color [6, 7].

Nationally, the beekeeping sector benefits from government support under the Green Morocco Plan (PMV), aiming to increase annual honey production to 16,000 tons [8]. Regionally, the Beni Mellal-Khenifra region ranks third in honey production nationwide, achieving an annual yield of 274 tons as recorded in 2017 [9]. This study aimed to analyze the Total Polyphenol Content (TPC) in monofloral honey varieties from the Beni Mellal-Khenifra region and to examine their associations with botanical origin, geographical location, harvesting period, and color.

MATERIALS AND METHODS

Honey samples

This study focused on four honey samples from four different sites (Tadla, Tagzirte, El Ksiba and Souk Sebt) in the Beni Mellal region, specifically citrus, euphorbia, carob, and anis honey. Honey samples were collected between March and October 2021 (Table 1), filtered through sterile gauze to remove debris and stored in sterile, airtight glass containers at 4°C in the dark until use [10].

Table1. Botanical-geographical origins and harvesting period of the honey samples studied

Honey samples	Botanical origins	Harvesting site	Geographical Coordinates			Harvesting period
			Latitude (N)	Longitude (W)	Altitude (m a.s.l)	
Citrus honey	*Citrus*	Tadla	30°15'11,0919"	6°35'11,5458"	548	Mars 2021
Euphorbia honey	*Euphorbia*	Tagzirt	32°26'51,6951"	6°10'6,64932"	737	Juillet 2021
Carob honey	*Ceratonia siliqua*	El Ksiba	32°35'16,3644"	6°3'49,15152"	765	Octobre 2021
Anis Honey	*Pimpinella anisum*	Souk Sebt	32°15'31,1281"	6°49'29,3458"	443	Octobre 2021

m a.s.l: meters above sea level

Color analysis

The color intensity of the honey samples was measured according to the Pfund classification [7]. Briefly, the honey samples were diluted to 50% (w/v) with distilled water, homogenized, and centrifuged at 3200 rpm for five minutes. Absorbance was then measured at 635 nm using a spectrophotometer. The color intensity was determined using the Pfund scale, following the conversion of absorbance values into millimeters Pfund using the formula: mm Pfund = $-38.70 + 371.39 \times Abs$. The Pfund scale is ranges from < 9 for water white color to > 114 for dark amber color.

Total Polyphenol Content (TPC) analysis

TPC was determined using the Folin-Ciocâlteu reagent method as described by Živić *et al.* (2019) with some modifications [11]. A volume of 0.2 mL of honey dissolved in distilled water was mixed with 1.5 mL of Folin-Ciocâlteu reagent (10%) diluted in distilled water. After 5 minutes, 1.5 mL of sodium carbonate (Na2CO 3 ; 7.5%) dissolved in distilled water was added to the mixture, and the final solution was incubated in the dark for 30 minutes at 25°C. Absorbance was measured at 760 nm using a spectrophotometer, and TPC was calculated based on a standard calibration curve using gallic acid, expressed as milligrams Gallic Acid Equivalent per 100 g of honey (mg GAE/100g). The standard curve was produced for gallic acid within the concentration range from 100 to 1000 mg/L (R2 =0.9998, y=0.1254x-0.1424). The TPC was reported as the mean value of triplicate assays and expressed as mg gallic acid

equivalent per 100 g of honey sample (mgGAE/100 g).

Statistical analysis

All analyses were conducted in triplicate, and results are presented as means ± standard deviations (SD). The statistical significance of differences in TPC was evaluated using analysis of variance (ANOVA). A confidence level of 95% ($p < 0.05$) was used to determine statistical significance. Pearson's correlation coefficient (r) was applied to assess correlations between the studied parameters.

RESULTS AND DISCUSSION

Table 2 presents the color intensity and TPC of the analyzed honey samples. The results reveal highly significant differences in TPC levels among the various honey samples with different botanical origins ($p<0.0001$). Euphorbia honey exhibited the highest TPC concentration, with a value of 30.61 ± 0.009 mg GAE/100g. In contrast, citrus honey showed lower levels (13.53 ± 0.004 mg GAE /100g). These findings are consistent with other studies that have indicated a strong relationship between TPC and the floral origin of honey [12-14].

Table 2. Color intensity and TPC in the analyzed honey samples

Honey samples	Pfund scale values	USDA color standard	TPC (mgGAE/100g)
Citrus honey	32.12± 2.17	White	13.53± 0.00^{d}
Euphorbia honey	157.68 ± 1.23	Dark amber	30.61 ± 0.00^{a}
Carob honey	151.22± 2.27	Dark amber	28.62 ± 0.00^{b}
Anis honey	65.36± 2.33	Light amber	19.10 ± 0.00^{c}

a, b, c, d: Different letters indicate significant differences.

Becerril-Sánchez *et al*. (2021) have documented that TPC varies depending not only on the botanical origin of the honey but also on its geographic production location [12]. Similarly, monofloral honey from China of the same floral origin, but of different geographical origin, showed significant differences in TPC values [15]. Furthermore, Neupane et al. (2015) reported significant variations in average TPC in terms of the altitude at which honey samples were collected in the Nepal Himalayas [16]. These results corroborate the conclusions of our study, where we found a statistically significant correlation between TPC and the honey harvesting site. Statistical analysis highlighted a strong correlation (r = 0.985*, $p < 0.05$) between TPC and the location of honey collection (Table 3). Citrus and anise honey, collected at lower altitudes (548 m a.s.l and 443 m a.s.l, respectively), showed the lowest phenolic concentrations, namely 13.53 ± 0.004 and 19.10 ± 0.004 mg EAG/100g, respectively. In contrast, euphorbia and carob honey, collected at higher altitudes (737 m a.s.l and 765 m a.s.l, respectively), exhibited higher levels, with values of 30.61 ± 0.009 and 28.62 ± 0.003 mg EAG/100g, respectively (Table 2). This difference can be explained by the fact that plants grown at higher altitudes synthesize secondary metabolites to protect themselves from extreme climatic conditions [12].

In our study, it is evident that honey color is linked to its TPC. We observed a highly significant correlation between TPC and the Pfund scale values (r = 0.996**, $p < 0.01$) (Table 3). Consequently, darker honey, such as euphorbia and carob honey, contained higher levels of TPC (30.61 ± 0.009 and 28.62 ± 0.003 mg EAG/100g, respectively). These findings align with research conducted in different contexts, reinforcing the understanding that honey color can be a reliable indicator of its phenolic content [6, 7, 17-19]**.**

On the other hand, our results indicated no significant association between TPC and the harvesting period (r = 0.486, $p > 0.05$) (Table 3). This result contrasts with other studies that have suggested a correlation between TPC levels and the harvesting period. For instance, Lachman et al. (2010) reported that the highest concentrations of TPC were found in honey collected at the beginning of June and July, with notably lower levels in honey collected during other months [20]. Muanda (2010) explains that this variation is due to the production of secondary metabolites, including polyphenols, by plants in spring and early summer as part of their defense mechanisms against ultraviolet radiation and to attract insects for effective pollination [21]. Furthermore, other researchers have pointed out that seasonal differences in TPC were influenced by the abundance of

flowering. Specifically, during periods of limited flowering, beekeepers may need to supplement the bees' diet with sugar syrup, which can result in reduced phenolic content and other components in the honey [22].

Table 3. Correlation matrix of the studied parameters (Pearson correlation coefficients).

		Harvesting location (Altitude)	Pfund-scal values	Harvesting period
TPC	Pearson correlation	.985*	.996**	.486
	Sig. (bilateral)	.015	.004	.514

*. The correlation is significant at the 0.05 level (bilateral).
**. The correlation is significant at the 0.01 level (bilateral).

CONCLUSION

This study highlighted significant differences in the TPC of monofloral honey varieties from the Beni Mellal-Khenifra region, with variations primarily influenced by botanical origin, geographic location, and color. These findings confirm the importance of floral and geographic origin, as well as honey color, as indicators of the phenolic quality of honey.

CONFLICTS OF INTEREST

The authors have no competing interests to declare relevant to this article's content.

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