



Colour and vitamin C equivalent antioxidant capacity of honey from different origins in Turkey

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ABSTRACT

The nutritional content, antioxidant capacity and sensory characteristic such as colour vary related to botanical and geographical origins. The study was aim to investigate the spectrophotometric classification of honey colour by Pfund scale and the vitamin C equivalent antioxidant capacity (VCEAC) expressed as l-ascorbic acid equivalent by MTT assay. Eight honey samples originating from different geographical areas of Turkey were supplied and coded. The colour of the samples was determined as extra white (blossom-multifloral and citrus), white (blossom-multifloral and lavender), amber (thyme-Astragalus and honeydews) and dark amber (chestnut). The highest VCEAC was statistically measured in the honeydews and citrus honey, as the lowest was in the blossom and chestnut honey. And, there was no significant interaction between the colour score and the VCEAC of the honey. In conclusion, the honey from different botanical and geographical origins in Turkey showed the antioxidant capacity independently of their colour. The results needed further studies to investigate the floral diversity and phytochemical contents which influence the colour and antioxidant capacity of honey.

INTRODUCTION

Honey is the most consumed apicultural product for nutraceutical and apitherapy purposes worldwide. The nutritional content, antioxidant capacity and sensory such as the colour of honey vary by geographical origin, flora and botanical origin. Especially, the colour and geographical origin generally influence the consumers' preferences and also the sale price of honey (FERREIRA et al., 2009; BODOR; BENEDEK, 2021; PU, 2022). The colour of honey is usually determined by some visual method comparing a standard scale. "Pfund scale" is the most commonly used technique that classifies the colour of honey from water white to dark amber (0-140 mm Pfund) (BODOR; BENEDEK, 2021).

In addition to sensory characteristics, the antioxidant capacity of honey is directly related to phenolic, polyphenol, and flavonoid substances and vitamins such as vitamins C and E in honey which is synthesized by plants. The amounts of these antioxidant compounds vary by the botanical origin of honey which determines its nutritional and sensory characteristics of honey (FERREIRA et al., 2009; MARTINELLO; MUTINELLI, 2021; PUŚCION-JAKUBIK et al., 2022). Vitamin C equivalent is commonly used to determine the total antioxidant capacity level of foods and natural product extracts. The method of Vitamin C Equivalent Antioxidant Capacity (VCEAC) is based on the colorimetric [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay reading by a spectrophotometer. The MTT reagent reacts with the reducing reagents and the antioxidants in the foods or products. And, a dark purple

formazan salt is formed as the enzymatic reduction product of MTT (MURAINA et al., 2009; LIU; NAIR, 2010).

The present study was aim to investigate the colour characteristics and antioxidant capacity of honey originating from various botanical and geographical origins in Turkey by using Pfund scale and MTT assays and the interaction between these parameters.

MATERIAL AND METHOD

Honey samples

In this study 8 honey samples were analysed from different geographical and botanical sources: two blossom, two honeydews, one citrus, one thyme-Astragalus, one lavender and one chestnut. All samples were collected from retail markets and originated from different geographical areas of Turkey (Figure 1). Detailed information and the abbreviation and codes of honey sample types were given. The samples were stored in dark room conditions until analysis.

Determination of honey colour by Pfund scale

For the determination of colour using the Pfund scale, five grams of each sample was dissolved into 10 ml of ultra-distilled water and vortexed well. The samples were heated up to 50 °C in the water bath to dissolve the sugar crystals. After incubation, 100 µl of each sample was added to each well of the microplate in six replicates. The microplate was read at 600 nm to measure the absorbance (nm). The results were evaluated



concerning the colour scale (BODOR; BENEDEK, 2021). The Pfund score of all honey was calculated with the equation 1.

$$\text{mm Pfund} = -38.70 + 371.39 \times \text{Absorbance (nm)} \quad (\text{Eq. 1})$$

Vitamin C Equivalent Antioxidant Capacity (VCEAC)

The assay was adapted from the studies of Muraina et al. (2009) and Liu et al. (2010). Briefly, vitamin C (l-ascorbic acid, HPLC grade) was two-fold diluted as 600, 300, 150, 75 and 37.5 μmol with ultra-distilled water. The honey samples were diluted with ultra-distilled water (1:9 w/v). 380 μl MTT (1 mg/ml) and 20 μl of each vitamin C dilution and the samples were mixed in an Eppendorf tube and incubated at 37 $^{\circ}\text{C}$ for 4 h. After incubation, 400 μl DMSO was added to all tubes and mixed well to solve the blue formazan salt formed during the incubation. 100 μl of each mixture was added to the U-bottom 96-well microplate in six replicates. The microplate was read at 570 nm (Absorbance 96, Byonoy, Germany). The standard curve was generated with optic density (OD, nm) values of six vitamin C dilutions by linear regression analysis (vitamin C equivalence = $m \times \text{OD} + b$, R^2). The VCEAC of the samples were calculated concerning the standard curve.

Data Analysis

The Vitamin C Equivalent of the samples was calculated by the vitamin C standard curve generated with the linear regression between the dilutions of l-ascorbic acid and their optic density values (nm) in Office Excel 2016 (Microsoft,

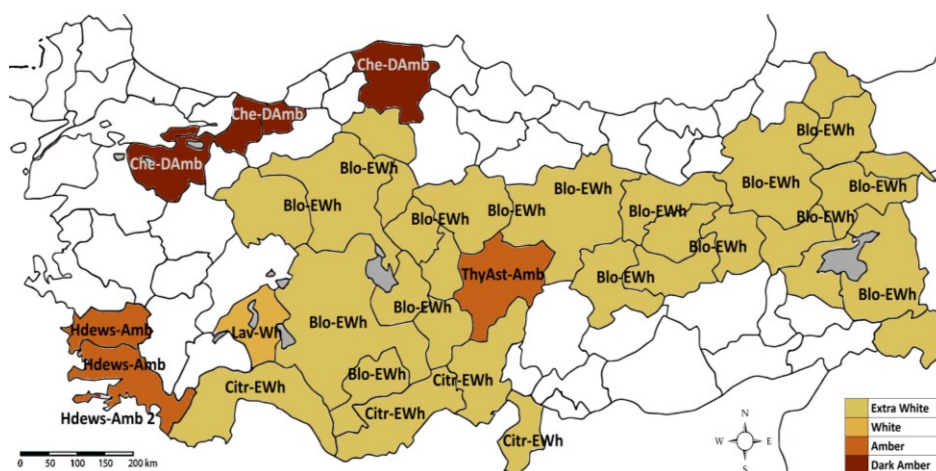


Figure 1. The geographical origins of honey samples (8 samples) in Turkey

USA). Statistical analysis (one-way ANOVA, Spearman correlation and linear regression) was conducted with SPSS version 15 software (IBM, USA).

RESULTS AND DISCUSSION

The colours of honey samples on Pfund scale

The absorbance (nm) of the samples was measured in the range from 0.135 to 0.474 nm. Their colour scores on Pfund scale were calculated as from 11.43 to 137.33. The results indicated the colours of eight samples: extra white for two samples (2-Blo-EWh and 7-Citr-EWh), white for two samples (1-Blo-Wh and 6-Lav-Wh), amber for three samples (3-ThyAst-Amb, 4 and 5-Hdews-Amb) and dark amber for one sample (8-Che-DAmb) (Table 1). The highest Pfund score was 137.33 as dark amber in the chestnut honey sample (8-Ches-DAmb) originating from the northern cities of Turkey. The lowest Pfund scores were 11.43 and 13.39 as extra white in the multifloral blossom honey sample (2-Blo-EWh) and the citrus honey (7-Citr-EWh) originating from the Middle, Eastern and Mediterranean coast of Anatolia respectively (Table 1).

Table 1. Pfund scale, colours and code of honey samples of Turkey (8 samples).

Pfund Standard		Honey Samples						
Honey colour	Pfund value	No	Botanical Origin	Geographical Origin	Abs. ₆₀₀ (nm)	mm Pfund	Colour	Code
Water white	<8	1	Blossom (multiflora)	No information	0.178	27.4	White	Blo-Wh
Extra white	8-17	2	Blossom (multiflora)	Middle Anatolia, Eastern Anatolia	0.135	11.43	Extra White	Blo-EWh
White	17-34	3	Thyme-Astragalus	Kayseri	0.336	86.08	Amber	ThyAst-Amb
Extra light amber	34-50	4	Honeydews	Marmaris	0.34	87.57	Amber	Hdews-Amb
Light amber	50-85	5	Honeydews	Muğla, Aydın	0.37	98.71	Amber	Hdews-Amb 2
Amber	85-114	6	Lavender	Isparta	0.185	30	White	Lav-Wh
Dark amber	>114	7	Citrus	Mediterranean Coast	0.14	13.29	Extra White	Citr-EWh
		8	Chestnut	Düzce, Bursa, Yalova, Sakarya, Kastamonu	0.474	137.33	Dark Amber	Che-DAmb

Abs.₆₀₀: absorbance (OD) at 600 nm

The honey colour can be differed by beekeepers' handling method, mineral content, exposure to high temperature and light, and botanical origin of honey (RATIÜ et al., 2020; ALBU et al., 2021). Many studies reported various Pfund scores for honey originating from different countries. While honey such as multiflora blossom, citrus and acacia generally had the colours of water white, white or extra white, chestnut honey had a darker colour worldwide, also in Europe and Turkey. (KIVRAK et al., 2017; RATIU et al., 2020; ALYGIZOU et al., 2021; SÁNCHEZ-MARTÍN et al., 2022).

Vitamin C Equivalent Antioxidant Capacity (VCEAC)

The vitamin C equivalence of the samples was calculated with regards to the standard curve by linear regression analysis (vitamin C equivalence, $\mu\text{mol} = 0.00006 \times \text{Abs.} + 0.0506$, $R^2 =$

0.9958) (Table 2, Figure 2). Vitamin C equivalents were determined between 412.22 and 1117.78 $\mu\text{mol}/\text{mg}$ or 725.99 and 1968.59 $\mu\text{g}/\text{mg}$ (Table 2). There was a significant difference between the honey samples ($P < 0.01$). The highest VCEAC was statistically measured in the honeydews honey (5-Hdews-Amb) and the citrus honey (7-Ctr-EWh), the lowest VCEAC was statistically determined in the chestnut honey (8-Che-DAmb) and both blossom honey (1-Blo-Wh and 2-Blo-EWh) (Table 2). There were no significant differences between the honey samples botanically originating from the honeydews (4-Hdews-Amb), the thyme-astragalus (3-ThyAst-Amb) and the lavender (6-Lav-Wh) ($P > 0.05$). Also, there was no significant correlation (β coefficient = 0.117; $P = 0.0585$) and regression ($R^2 = 0.003$, $P = 0.791$) between Pfund scores and VCEAC of the honey samples.

Table 2. The Vitamin C Equivalent Antioxidant Capacity (VCEAC) of honey samples of Turkey

Standard Curve			Honey samples			
Vit C (μmol)	OD ₅₇₀ ±SD (nm)	Linearity	No / Code	OD ₅₇₀ ±SD (nm)	Vit C $\mu\text{mol}/\text{mg}$ Honey	Vit C mg/100g Honey*
600	0.088±0.0042	$R^2 = 0.9958$ $y = 0.00006x + 0.0506$	1 Blo-Wh	0.078±0.0036	456.67±60.09	8.04±1.06c
300	0.069±0.0014		2 Blo-EWh	0.078±0.0051	462.22±85.53	8.14±1.5c
150	0.059±0.0042		3 ThyAst-Amb	0.111±0.0017	1006.67±28.87	17.73±0.51ab
75	0.054±0.0078		4 Hdews-Amb	0.111±0.0012	1012.22±19.25	17.83±0.34ab
37.5	0.054±0.0007		5 Hdews-Amb 2	0.118±0.0025	1117.78±41.94	19.69±0.74a
18.75	0.051±0.0007		6 Lav-Wh	0.105±0.0001	906.67±1.67	15.97±0.03b
18.75	0.051±0.0007		7 Ctr-EWh	0.114±0.0010	1056.67±16.67	18.61±0.29a
18.75	0.051±0.0007		8 Che-DAmb	0.075±0.0040	412.22±67.36	7.26±1.19c

*Statistical significant with $P < 0.01$; OD₅₇₀: absorbance at 570 nm

The phenolic, polyphenol, flavonoid and vitamin C contents of honey mostly indicate its antioxidant capacities such as Gallic acid equivalent, ascorbic acid equivalent (AAE) and free radical-scavenging (FERREIRA et al., 2009; MARTINELLO; MUTINELLI, 2021). Many previous studies suggested that the botanical origin, nutritional and colour characteristics directly influenced the amounts of these antioxidant compounds. These studies indicated that the honey had 21.9-58.21, 17.8-165.7, 9.5-17.8 and 0.8-28.2 mg AAE/100 g honey harvested from Turkey (Rhododendron), Italy (honeydews), Spain (chestnut, honeydews, multiflora) Brazil (Meliponinae) respectively (SILICI et al., 2010; ESCUREDO et al., 2013; ATTANZIO et al., 2016; BILUCA et al., 2016, 2020). In this study, ascorbic acid equivalents were measured between 7.26 and 19.69 mg/100g honey expressed as VCEAC by MTT assay in harmony with the results of previous studies.

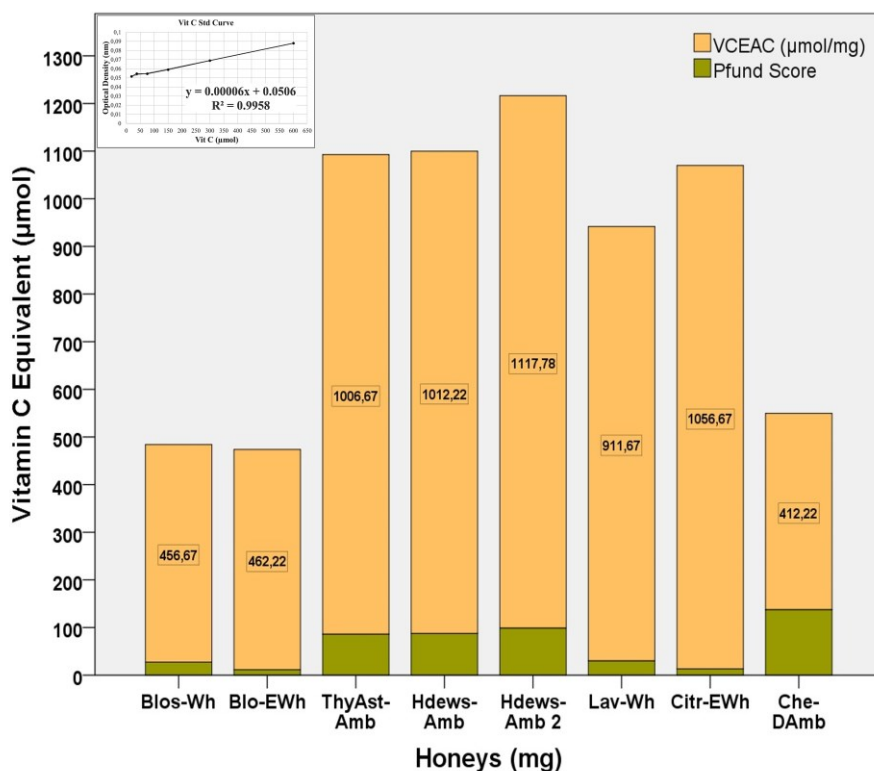


Figure 2. The vitamin C standard curve, Vitamin C Equivalent Antioxidant Capacity (VCEAC) and Pfund scores of honey samples of Turkey (8 samples).

Ferreira et al. (2009) suggested that the phenolic, flavonoids, ascorbic acid (14.5 mg/ 100g honey), beta-carotene contents, DPPH scavenging and TBARS activity increased by getting darker the colour of honey. Thus, the colourization and pigmentation in honey positively correlated with physicochemical parameters, nutritional content, phenolic compounds and flavonoids of honey (TSIGOURI et al., 2004; EL SOHAIMY et al., 2015; MALDONADO et al., 2021). Moreover, consumer preference and the price of honey are influenced by sensory characteristics (Bodor; BENEDEK, 2021; PU, 2022). But, no significant correlation and regression were determined between Pfund scores and VCEAC of the honey samples in this study.

CONCLUSION

Vitamin C equivalent could be considered as a marker of the antioxidant capacity of honey from different botanical and geographical origins. The highest antioxidant capacity was found in honeydews and citrus honey which has lighter colour than dark amber coloured chestnut honey. So, the honeys originating from Turkey also showed the vitamin C equivalent antioxidant capacity independently of their colour. It might be related and influenced the floral diversity and the phytochemical contents of honey such as phenolic, polyphenol, flavonoid and ascorbic acid.

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