Botanic Origin, Various Physicochemical and Antioxidant Properties of Honey Samples From Giresun, Turkey

Giresun-Türkiye'den Toplanan Bal Örneklerinin Bitkisel Kökeni, Fizikokimyasal ve Antioksidan Özellikleri

Research Article

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ABSTRACT

The aim of the present study is to evaluate the botanic origin, various pysicochemical parameters (pH, moisture, hydroxymethylfurfural (HMF), fructose/glucose ratio) and antioxidant properties of the four honey samples colected from Giresun-Black Sea Region of Turkey. According to the mellisopalynological analyis, two of the investigated honey samples were determined as *Castanea sativa* honey and the other two samples were determined as multifloral honeys.The pH value of honey samples were found between 3.11 and 4.44. The moisture ot the investigted samples were varied between 11.59-14.13%. The fructose contents of the samples were found between 36.58 and 43.42 g/100 g and glucose values as 24.84-33.55 g/100 g. HMF values were 1.6±1.0 mg/kg for *Castanea sativa* honeys and 0.65±0.66 mg/kg for multifloral honeys. Total phenol contents were high for *Castanea sativa* honeys compare to two multifloral honeys but total flavonoid content is found as highest in sample 3 (multifloral honey).

Key Words

Antioxidant properties, botanic origin, pysicochemical parameters, total phenolic content, total flavanoid content.

ÖZET

Bu çalışmanın amacı Türkiye'nin Giresun-Karadeniz Bölgesi'nden toplanan dört bal örneğinin bitkisel kökeni, fizikokimyasal parametreleri (pH, nem, hidroksimetilfurfural, früktoz/glukoz oranı) ve antioksidan özelliklerinin değerlendirilmesidir. Palinolojik analizlere göre, incelenen iki örnek *Castanea sativa* balı diğer ikisi de multifloral bal olarak tanımlanmıştır. Bal örneklerinin pH değerleri 3.11 ve 4.4 aralığında bulunmuştur. Nem değerleri ise %11.59-14.13 aralığındadır. Örneklerin früktoz içerikleri 36.58 ve 43.42 g/100 g aralığında ve glukoz içerikleri ise 24.84-33.55 g/100 g aralığında bulunmuştur. *Castanea sativa* ballarının HMF değerleri 1.6±1.0 mg/kg, multifloral balların ise 0.65±0.66 mg/kg olarak bulunmuştur. *Castanea sativa* ballarının toplam fenol miktarları multifloral ballara oranla yüksek çıkmıştır. Toplam flavonoid miktarları ise 3 numaralı örnekte en yüksek oranda bulunmuştur.

Anahtar Kelimeler

Antioksidan özellik, botanik köken, fizikokimyasal parametre, toplam fenolik içerik, toplam flavanoid içerik.

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INTRODUCTION

Honey is a supersaturated solution of sugars, of which fructose and glucose are the main contributors, with phenolic compounds, minerals, proteins, free amino acids, enzymes, and vitamins acting as minor components [1,2].

Honey is widely consumed by people of the world. It contains numerous health promoting flavonoids and phenolic acids that have proven benefits against disease [2]. Its characteristic is depending mainly on the floral source and also other external factors, including seasonal and environmental factors as well as processing [1, 3-5]. The physicochemical parameters of honey are defined by the EC Directive 2001/110 [6]. The certain parameters are moisture content, electrical conductivity, ash content, reducing and non-reducing sugars, free acidity, diastase activity and hydroxymethylfurfural (HMF) and antioxidant content. In this study, we aimed to determined quality of honeys from Giresun regions so we studied botanic origin, physicochemical parameters, phenol and flavonoid contents, free radical scavening and hydrogen peroxide activity.

MATERIAL AND METHODS

Melissopalynological analysis

Preparation of honey sample for pollen identification was performed according to the method described by Louveaux et al [7]. Besides the determination of botanical origin, the total pollen number (TPN) of all samples were calculated according to Moar [8]. Distribution (%) of the honey samples according to Maurizio's classes [9] , Group I (<20000) pollen grains per 10 g honey), Group II (20000-100000 pollen grains per 10 g honey), Group III (100.000–500.000 grains per 10 g honey), Group IV (500.000 –1.000.000 grains per 10 g honey), Group V (>1.000.000 grains per 10 g honey).

Physicochemical Characterization of Honey pH

A pH meter (Ohaus, Starter 3100, USA) was used to measure the pH of a 10% (w/v) solution of honey prepared in distilled water.

Moisture

Moisture analyses were done by a portable refractometer and determines as % ratio.

Sugar Analysis by High Performance Liquid Chromatography

The sugar analysis were done by High Performance Liquid Chromatography (HPLC) with RID dedector and by using a carbohydrate column [10].

Determination of HMF by High Performance Liquid Chromatography (HPLC)

The samples analysed by High Performance Liquid chromatography with UV detector and with C18-reversed phase column.

Antioxidant Analyses

Determination of total flavonoid contents (TFC)

The total flavonoids contents of the extracts were determined according to the colorimetric method described by Chung et al [11] with minor modifications. Sample solutions (0.5 mL) were added to a tube containing 1.5 mL of absolute ethanol. To the mixture was added subsequently $AlCl_3.6H_2O$ solution (0.1 mL, 10.0%) and potassium acetate (0.1 mL, 1.0 $mol.L^{1}$). Distilled water was added to bring the total volume to 5.0 mL and the absorbance was read after 30 min at 415 nm (Optizen Pop UV/Vis Single Beam). Total flavonoids contents were expressed as microgram of catechin equivalent that was obtained from standard graph (R^2=0.9979).

Determination of Total Phenolic Contents (TPC)

Total phenolic contents of samples were analyzed by the Folin & Ciocalteu's phenol reagent (Folin C) colorimetric method described by Slinkard and Singleton [12]. Sample solutions (0.5 mL) was mixed with 7.0 mL of distilled water and subsequently with Folin C reagent (0.5 mL). After 3 min, Na_2CO_3 solution (3.0 mL, 2.0%) was added into the mixture. The color developed for 1 hour and the absorbance was measured at 760 nm in a spectrophotometer (Optizen Pop UV/Vis Single Beam). Gallic acid was used as the standard, and total phenolic content was expressed as microgram of gallic acid equivalent by using an equation that was obtained from standard gallic acid graph (R^2= 0.9995).

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity

The DPPH radical scavenging activities were studied by following a previous report with slowly modified [13]. Serially diluted samples (3.0 mL) at the different concentrations (10-100 μg/mL) were added to DPPH solutions (1.0 mL, 0.2 mM) in ethanol. The mixtures were shaken forcefully and allowed to sit at room temperature for 30 min. Then, absorbance was recorded at 517 nm by using a spectrophotometer (Optizen Pop UV/Vis Single Beam) and the results were expressed as SC_{50} (μg/mL) by linear regression analysis and represent mean of the data.

Determination of Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity was assayed according to the medhod described by Ruch et al [14]. Briefly, the samples were dissolved in 0.04 M phosphate buffer (pH = 7.4) and 3.4 mL of the samples were mixed with 0.6 mL of 40 mM H_2O_2 solution (prepared in the same buffer). The absorbance of the mixture was measured at 230 nm versus blind sample after 10 min with UV/VIS spectrophotometer (Optizen, Korea). Phosphate buffer without hydrogen peroxide used as blank. The decrease in absorbance value showed the high level of hydrogen peroxide scavenging activity designation. The results were expressed as SC_{50} values (μg/mL).

Statistical Analysis

Experimental results were given as mean ± S.D. of the three paralel measurements. P values of < 0.05 were regarded as significant and P values of < 0.01 were regarded as very significant.

RESULTS AND DISCUSSION

Four honey samples were tested in this study in order to assess their floral origin, some physicochemical characterization (pH, moisture, fructose and glucose content, F/G hydroxmethylfurfural content) and various antioxidant properties.

Melissopalynological analysis

Honey sample 2 and 4 are unifloral because they contained a predominant pollen-type (frequency 45%) and are classed as *Castanea sativa* honey. Other honey samples (1 and 3) are multifloral because no dominant pollen was found. The pollen analysis showed the main botanical species for the investigated samples are *C. sativa* and Fabaceae species. Table 1 shows pollens belong to taxa and percentage distribution. Honey samples classified with TPN 10 values. Sample 1 and 3 were found in Group 1, Sample 2 and 4 were found in Group 2.

Physicochemical Characterization of Honey pH

The pH of honey samples varied between 3.22 and 4.44. These results are similar to those obtained by Giorgi et al [15] for honey samples from Valcamonica valley of the Lombardia region. Atanassova et al [16] found pH 5.65 for *C. sativa* honey from Bulgaria. Fallico et al [17] found pH 5.9 Sicilian *C.sativa* honey, our *Castanea* honey samples are more asidic. Our results were lower than the pH range 4.50-5.64 declared by Ünal and Küplülü [18], Derebasi et al [19] for Turkish honey. Our honey samples are more asidic. The pH values of honey are of great importance during extraction and storage, since acidity can influence the texture, stability, and shelf life of honey [20].

Moisture

Moisture is one of the most important factor to be considered as a quality parameter of honey. The moisture content in the investigated honey samples was found to be 11.59-14.13% (Table 2), which are within the limit (≤20%) recommended by the international quality regulations (2001). These findings similar to those obtained by reported by Ünal and Küplülü [18]. Derebasi et al [19], for Turkish honey (12.08-25).

Sugar Content

Honey consists of a mixture of sugars, mostly glucose and fructose [21]. The results of the sugar analysis of all the four honey samples showed Table 2. The fructose contents of the samples varied between 36.58 and 43.42 g/100 g. The glucose contents of the samples were within a range of 24.84 to 33.55 g/100 g. These findings are in agreement with those reported by Buba et al [22] and Ciappini et al who [23], found similar range of the fructose and glucose contents for the samples.

Table 1. Pollen types recovered from the honey samples and their frequency (%).

Samples	*Apiaceae*	*Asteraceae*	*C.sativa*	*Fabaceae*	*Lamiaceae*	*Rosaceae*	*Rhododendron*	*Taraxacum*	*Undefinied*
1	5.88	2.94	23.53	32.35	5.88	17.65	11.76		
2			65.48	19.05	0.6	0.6	14.29		
3			44.29	26.43	0.71	6.43	18.57	3.57	
4		1.16	96.53	1.16		0.58			0.58

Table 2. The results of the analysis of physicochemical parameters.

Samples	pH	Moisture (%)	Fructose (g/100 g)	Glucose (g/100 g)	F/G (g/100 g)	Total F+G	HMF (mg/kg)
1	3.22	16.2	43.42	31.53	1.34	74.95	2.31
2	4.44	17.4	40.9	33.55	1.21	74.45	1.11
3	4.08	16.5	36.58	24.84	1.47	61.42	0.18
4	4.34	15.7	39.51	26.07	1.5	65.58	0.89

F + G levels in this study are ranged between 61.42 and 74.95. Fructose and glucose are the dominant sugar types in honeys, which although no limits have been fixed for their individual values, their sum (Fructose+glucose) has been fixed at a value of ≥ 60 g/100 g as one of the requirements of the international standard for honey established by Codex Alimentarius Commission. The total content of glucose and fructose is over 60 g/100 g of honey for all samples (Table 2).

Can et al [24] were found glucose contents 19.35±3.00 g/100 g, fructose contents 38.44±2.72 g/100 g ranged between in 7 chestnut honeys. They found 7 multifloral honey's glucose contents average 25.07± 6.59 g/100 g, fructose contents average 32.35±5.65 g/100 g. The fructose/glucose ratio were within the range of 1.21 to 1.5 [24]. We found in this study that the fructose contents average are 41.47±2.8 g/100 g, glucose contents average 28.8±3.86 g/100 g for chestnut honey, fructose contents average 38.74±3.05 g/100 g, glucose contents average 29.19±6.16 g/100 g for multifloral honeys. Our results are a bit higher than these results.

Honey's Sugar Composition Depends on the Floral and Region Origin [25,26]. F/G ratio are being used for the prediction of honey crystallization. Honey crystallization is slower when the F/G ratio is more than 1.3 and it is faster when the ratio is below 1.0 [27]. According to these findings the crystalization will be slower for all investigated samples.

HMF

HMF or 5-hydroxymethyl- 2-furaldehyde, is an organic compound obtained from sugars like fructose, cellulose, inulin and starch [28]. HMF content is widely recognized as a parameter that indicates the freshness of honey [29]. High concentrations of HMF in honey are an indicator of overheating and storage in poor conditions [30]. The European Union [6] established that the highest allowed amount of HMF in honey should be 40 ppm, with the exception of honeys of tropical origin (80 ppm). The obtained results were all lower than today legal limits (Table 2). In this study, we found HMF contents average as 1.6± 1.0 mg/kg for *C. sativa* honeys and, 0.65±0.66 mg/kg for multifloral honeys. Can et al [24] found HMF contents as 9.28±7.13 mg/kg for chestnut honey, 14.71 ±12.10 mg/kg for multifloral honeys. Our results are lower than these results. Fallico et al [17] examined four different Sicilian honeys and they determined that HMF wasn't detectable in Chestnut honeys before the heating treatment. This is supported by our own research findings. Our all honey samples have very little HMF levels so several factors influence the formation of HMF in honey: temperature and time of heating [31], storage conditions; use of metallic containers [32] and the chemical properties of honey, which are related to the floral source.

Table 3. The results of the analysis of Total Flavonoid, Total Phenol, DPPH and H_2O_2 of samples.

Samples	Total Flavonoid (mg CAE/100 g)	Total Phenol (mg GAE/100 g)	DPPH SC_{50} (μg/mL)	H_2O_2 SC_{50} (μg/mL)
1	0.29	4.48	71.92	122.48
2	0.99	7.51	39.77	83.72
3	2.49	5.49	39.77	220.46
4	1.08	8.01	19.04	22.69

Antioxidant Analyses

Total Flavonoid Content

Total flavonoid content of the honey samples varied between 0.29–2.49 mg CAE/100 g (Table 3). The highest value was determined in honey sample 3 multifloral honey but the chestnut pollen is considerably high compare to the other pollen. The sample 4 and 2 follow this sample. However, the lowest value was observed in honey sample 1. Flavonoids contents varied with the origin of honey samples and ranged from 0.93 mg CAE/100 g (Jordan Valley) to 4.6 mg CAE/100 g (Umm Alyanabea) [33]. Flavonoid content of Litchi Honey Procured from Gazipur and Tangail District, Bangladesh varied between 4.024 and 4.954 mg Catechin/100 g [34]. Our results were lower values compared with these results.

Total phenolic content

Total phenolics were highest 8.01 mg GAE/100 g in the sample 4 and lowest 4.48 mg GAE/100 g in the sample 1 (Table 3). Lachman et al [35] declared that total phenolics were ranged from 3.92 mg CAE/100 g (multifloral honey) to 16.71 mg CAE/100 g (honeydew honey). Our results are in these range. Ertürk et al [36] declared that the total phenol content of honeys from east blacksea region was found in the range of 0.058 to 0.396 mg GAE/g, which was determined using gallic acid as standard (R^2 = 0.997). Our results are similar to the results of this study. Total phenolic content of chestnut honeys (sample 2,4) are found higher compared to the multifloral honeys.

DPPH Free Radical Scavenging Activity and Hydrogen Peroxide Scavenging Activity

DPPH free radical and hydrogen peroxide scavenging activities of four honey are in the range of 19.04–71.92 and 22.69–220.46 respectively as SC_{50} values (μg/mL). While the sample 4 has highest DPPH Free Radical Scavenging Activity, sample 1 has the lowest activity (Table 3). Ertürk et al [36] declared that the honeys of East Black Sea has a value of '29.388-458.450 (mg/mL)' DPPH Free Radical Scavenging activity. Sarıkaya et al [37] determined the Free Radical Scavenging Activities of honey and pollen samples as '5.7±0.4–9.0±0.7' that collected from Zonguldak (Turkey). While The highest hydrogen peroxide removing activity was found in sample 4, the lowest activity is sample 2.

CONCLUSION

The investigated honey samples showed different levels of flavonoids and phenolics, which role as effective natural antioxidants. Among the studied honeys, *C. sativa* honey presented total phenol content and hydrogen peroxide removing activity. The important influence of the botanical origin on the pysicochemical and antioxidant properties of honey was confirmed by the variability of the studied parameters.

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