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ORIGINAL ARTICLE

Effects of Thermal Treatment, Ultrasonication, and Sunlight Exposure on Antioxidant Properties of Honey

Isıl İşlem, Ultrasonikasyon ve Güneş Işığına Maruz Kalmanın Balın Antioksidan Özelliklerine Etkileri

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ABSTRACT

Objectives: This study aimed to determine effects of controlled heating, ultrasonication, and sunlight on antioxidant capacity, total phenolic content (TPC), and total flavonoid content (TFC) of honey.

Materials and Methods: Honey was subjected to thermal treatment (for 5-20 min at 30-80˚C), ultrasonication (for 5-20 min at 37 kHz frequency), and sunlight (for 1-10 days), and the impact of these treatments on antioxidant capacity, TPC, and flavonoid contents was evaluated. One-Way ANOVA, followed by Tukey's post-hoc test, was performed to compare the differences between experimental results.

Results: Antioxidant quality of samples heated at 60˚C and 80˚C were negatively affected when compared to untreated samples ($p<0.05$); however, there were no statistically significant differences between untreated samples and samples heated at 30˚C and 45˚C. On the other hand, ultrasonication of honey samples for 60 min enhanced the antioxidant properties when compared to untreated samples ($p<0.05$). In addition, while exposure to sunlight for 10 days decreased the TPC, the TFC and antioxidant capacity began to decrease after 6 days ($p<0.05$).

Conclusion: The results suggest that producers and consumers should consider the adverse effects of sunlight and temperature on antioxidative quality of honey. Additionally, ultrasonication technique has the advantage of preserving the antioxidant properties of honey.

Key words: Honey, temperature, ultrasonication, sunlight, antioxidative quality

ÖZ

Amaç: Bu çalışma, kontrollü ısıtma, ultrasonikasyon ve güneş ışığının balın antioksidan kapasitesi, toplam fenolik içeriği (TPC) ve toplam flavonoid içeriği (TFC) üzerindeki etkilerini belirlemeyi amaçlamıştır.

Gereç ve Yöntemler: Bal, ısıl işleme (30-80˚C'de 5-20 dakika), ultrasonikasyona (37 kHz frekansında 5-20 dakika) ve güneş ışığına (1-10 gün) tabi tutulmuş ve bu uygulamaların antioksidan kapasite, TPC ve flavonoid içerikleri değerlendirilmiştir. Deneysel sonuçlar arasındaki farkları karşılaştırmak için tek yönlü ANOVA ve ardından Tukey'nin post-hoc testi yapılmıştır.

Bulgular: 60˚C ve 80˚C'de ısıtılan numunelerin antioksidan kalitesi, işlem görmemiş numunelere göre olumsuz etkilenmiştir ($p<0,05$); bununla birlikte, işlenmemiş numuneler ile 30˚C ve 45˚C'de ısıtılan numuneler arasında istatistiksel olarak anlamlı bir fark belirlenmemiştir. Diğer taraftan, bal örneklerinin 60 dakika ultrasonikasyonu, işlem görmemiş örneklere kıyasla antioksidan özelliklerini arttırmıştır ($p<0,05$). Ayrıca 10 gün güneş ışığına maruz kalma TPC'yi azaltırken, 6 gün sonra TFC ve antioksidan kapasite azalmaya başlamıştır ($p<0,05$).

Sonuç: Sonuçlar, üreticilerin ve tüketicilerin, güneş ışığının ve sıcaklığın balın antioksidan kalitesi üzerindeki olumsuz etkilerini göz önünde bulundurmaları gerektiğini göstermektedir. Ek olarak, ultrasonikasyon tekniği balın antioksidan özelliklerini koruma avantajına sahiptir.

Anahtar kelimeler: Bal, sıcaklık, ultrasonikasyon, güneş ışığı, antioksidan kalite

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INTRODUCTION

Honey is a natural product generated by honeybees; it has great market potential due to its health benefits in humans. Honey is a well-known source of enzymatic and non-enzymatic antioxidants, such as glucose oxidase, catalase, phenolics, flavonoids, vitamins, proteins, and Maillard reaction products.[1] Phenolics are the main compounds in honey, which contribute significantly to the antioxidant properties of honey.[2] However, the antioxidant activity varies, depending on the floral source, season, and environmental factors.[3]

Honey has a unique combination of components, a characteristic that makes it a valuable diet for consumers. However, raw honey is not normally commercialized, as further treatment is needed for large scale marketing.[4,5] Controlled heating is one of the important steps in processing honey. It can demolish the bacteria yeast that cause undesirable fermentation during storage of the product; it also facilitates liquefaction, so as to obtain a fluidy and non-crystallized product.[4-6] Besides thermal treatments, ultrasonication has been also used as an alternative to promote the marketability of honey.[7]

Honey is inevitably exposed to sunlight from the period of production to consumption. Sunlight exposure increases ultraviolet (UV) radiation. It is well-known that UV radiation adversely affects the quality of foods,[8] but there is no information on the effects of natural UV radiation on the antioxidant properties of honey.

Given the health benefits and demand for high quality honey, the preservation and enhancement its antioxidant properties during processing and storage are considerably important. Therefore, this study aimed to determine the antioxidant capacity, total phenolic content (TPC), and total flavonoid content (TFC) of honey, following its exposure to controlled heating, ultrasonication, and sunlight.

MATERIALS AND METHODS

Materials

Three bottles of same brands of honey were purchased from a common chain market in Turkey. The honey brand chosen for this study is well-known in Turkey. The brand officially declared that they own British Retail Consortium certificate and that all chemical and physical analysis were performed to assure the authenticity of the honey.

Methods

Thermal treatment

Samples were separately subjected to thermal processing in a water bath for 5, 10, 15, and 20 min at 30°C, 45°C, 60°C, and 80°C. Afterward, antioxidant capacity, TPC, and TFC of the samples were determined at room temperature.

Ultrasonication

Sonication of the samples was performed at 37 kHz frequency for 5, 15, 30, and 60 min using an ultrasonic cleaning bath.

Exposure to sunlight

Samples were placed outdoor during daytime (average maximum temperature: 26.0°C) and night time (average minimum temperature: 15.5°C) in May for 1, 3, 6, and 10 days.

Analysis of antioxidant capacity

Cupric reducing antioxidant capacity (CUPRAC)

CUPRAC was determined according to the method described by Apak et al.[9] In brief, 1 g of processed honey sample was dissolved in 2.5 mL of distilled water. Then, 0.1 mL of the solution was mixed with 0.75 mL of $CuCl_2$ (10 mM), 0.75 mL of neocuproine (7.5 mM), 0.75 mL of CH_3COONH_4 buffer (1M, pH: 7.0), and 0.75 mL of distilled water. Absorbance was measured at 450 nm after 30 min. Trolox was used as a reference standard. Results were expressed as µmol Trolox equivalent (TE) per one gram (µmol TE/g).

Trolox equivalent antioxidant capacity (TEAC)

TEAC was determined according to the method described by on Re et al.[10] In brief, 0.1 mL of honey solution (1 g/2.5 mL) was mixed with 2 mL of $ABTS^{\cdot+}$ solution. After 15 min, absorbance was measured at 734 nm. A standard curve was constructed using Trolox and the results were expressed as µmol TE/g.

TPC and TFC

TPC was determined according to the method described by Fu et al.[11] In brief, 0.1 mL of honey solution (1 g/2.5 mL) was mixed with 1.0 mL of 1:10 diluted Folin-Ciocalteu reagent. 1.0 mL of saturated sodium carbonate solution was added after 4 min. This mixture was incubated for 2 h at room temperature. The absorbance of mixture was measured at 760 nm after incubation. Gallic acid (GA) was used as standard to produce the calibration curve. The results were expressed as mg of gallic acid equivalent (GAE) per 100 g.

TFC was determined according to the method described by Meda et al.[12] In brief, 1.5 mL of 2% aluminum trichloride in methanol was mixed with the same volume of honey solution (1 g/2.5 mL). After 10 min, absorbance was measured at 415 nm. A standard curve was constructed using quercetin and the results were expressed as mg of quercetin equivalent (QE) per 100 g (mg QE/100g).

Statistical analysis

Statistical analysis was done using GraphPad Prism 5 (Prism 5 for Windows Version 5.03, GraphPad Software, Inc) and Microsoft Excel. All experiments were conducted in triplicate. One-Way ANOVA was performed and significant differences between means were determined by Tukey's post-hoc test at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

Effect of thermal treatment

Table 1 shows the antioxidant capacity, TPC, and TFC of honey before and after heat treatment. For untreated samples, CUPRAC, TEAC, TPC, and TFC were found to be 2.75, 1.14 (µmol TE/g),

27.75 (mg GAE/100g), and 6.76 (mg QE/100g), respectively. For samples heated at 30°C and 45°C for 5 min, the highest TPC and TFC values, respectively, as compared to the rest, were obtained. In the cases of CUPRAC and TEAC, the highest results were obtained for untreated samples. On the contrary, antioxidant capacity, TPC, and TFC of samples decreased with the increase of treatment temperature. To ascertain whether these differences are statistically significant, One-Way ANOVA, followed by Tukey's post-hoc test, was applied to the data. As seen in Table 1, statistical differences between the untreated samples and samples subjected to 60°C (in CUPRAC and TFC assays) and 80°C heating (in all assays) were significant. Also, findings revealed that the process time and the treatment temperature affected the antioxidant capacity, TPC, and TFC of the samples.

Honey is rich in natural antioxidants, such as enzymes, vitamins, phenolic acids, and flavonoids.[13] However, these compounds may undergo several irreversible changes during thermal treatments.[1] Escriche et al.[4] evaluated the effect of industrial heat treatment on the phenolic compounds of Spanish honeys. According to their results, a significant decrease in the concentration of some phenolic compounds in these honeys was observed after the thermal treatment. Kowalski[14] investigated the impact of heating at 90°C for 60 min on antioxidant properties of honey through TPC and $ABTS^{\cdot+}$ assays. It was observed that there was a significant decrease in the antioxidant properties of honeydew honey after processing. Chaikham and Prangthip[15] reported that TPC, TFC, and antioxidant capacity (measured by FRAP and DPPH assays) of longan-flower honey diminished after heating at 100°C for 5 min.

Finally, heating honey at high temperatures could degrade their antioxidant compounds content,[16] and this could explain why the antioxidant capacity, TPC, and TFC of the treated honey samples decreased when compared to those of the untreated samples.

Effect of ultrasonication

Results of the impact of ultrasonication on antioxidant properties of honey are shown in Table 2. The values of treated samples increased with increase of the treatment time as compared to the values of the untreated samples. However, statistical

Table 1. Effects of thermal treatment on antioxidant capacity, TPC, and TFC of honey

	Method			
	CUPRAC*	**TEAC***	**TPC****	**TFC*****
Untreated sample	2.75±0.10	1.14±0.02	27.75±0.57	6.76±0.06
30°C temperature				
5 min	2.66±0.05	1.04±0.03	28.21±0.07	6.70±0.07
10 min	2.62±0.08	1.04±0.02	27.77±1.25	6.78±0.14
15 min	2.67±0.15	1.07±0.07	27.98±0.69	6.75±0.08
20 min	2.69±0.11	1.08±0.02	26.59±0.79	6.72±0.14
45°C temperature				
5 min	2.71±0.07	1.10±0.02	27.73±0.38	6.79±0.24
10 min	2.68±0.10	1.08±0.01	27.94±0.85	6.69±0.18
15 min	2.69±0.12	1.07±0.06	27.59±1.08	6.57±0.11
20 min	2.73±0.13	1.08±0.03	27.82±0.78	6.50±0.05
60°C temperature				
5 min	2.47±0.07	1.06±0.01	27.60±0.45	6.50±0.04
10 min	2.41±0.11[a]	1.03±0.02	27.12±0.31	6.17±0.12[d]
15 min	2.38±0.15[a]	1.02±0.04	26.86±0.28	5.79±0.15[d]
20 min	2.30±0.01[a]	1.03±0.06	26.23±0.66	5.62±0.11[d]
80°C temperature				
5 min	2.32±0.10[a]	1.02±0.01	27.12±0.18	6.30±0.14[d]
10 min	2.32±0.12[a]	0.96±0.01[b]	27.05±0.74	6.01±0.16[d]
15 min	2.22±0.07[a]	0.96±0.01[b]	24.90±1.03[c]	5.69±0.18[d]
20 min	2.19±0.02[a]	0.96±0.04[b]	24.45±0.70[c]	5.74±0.12[d]

[a, b, c, d]The rows that do not share the same superscripts are significantly different from each other in Tukey's post-hoc test ($p<0.05$). *µmol TE/g, **mg GAE/100g, ***mg QE/100g, CUPRAC: Cupric reducing antioxidant capacity, TEAC: Trolox equivalent antioxidant capacity, TPC: Total phenolic content, TFC: Total flavonoid content, GAE: Gallic acid equivalent, QE: Quercetin equivalent

differences between the samples subjected to ultrasonication for 60 min, and the untreated samples in terms of TPC, TFC, and CUPRAC were observed. In the case of TEAC assay, there were no significant differences between the treated and untreated samples. The differences between the antioxidant capacity assays CUPRAC and TEAC were due to the difference in the two assays.[17,18]

Ultrasonication is an alternative and innovative technology to obtain fluidy and non-crystallized products. It is more effective to preserve the nutritional l values of honey by this method rather than thermal treatments.[7,15] However, there are limited data on the impact of ultrasonication on antioxidant properties of honey. Similar to the current assay, Chaikham and Prangthip[15] reported that the TPC, TFC, and antioxidant capacity of honey increased after processing for 20 min. Pollen is one of the important contents of honey;[13] it has multiple essential components, such as proteins, vitamins, and phenolic compounds.[19] Ultrasonication has the capability to increase the permeability of plant tissues caused by cell disruption, thereby resulting in the liberation of all the compounds present in the cell.[20] In view of the fact that a pollen is produced by plants as a male cell, existing antioxidant compounds in pollens could be released after ultrasonication, thereby causing an increase in the TPC, TFC, and antioxidant capacity of honey.

Apart from the limited studies relevant to the impact of ultrasonication on antioxidant properties of honey, many studies have been conducted to examine the influence of ultrasonication in preserving the nutrional qualities of fruit juices, although its positive effect in terms of antioxidant properties have been demonstrated.[21-23]

Effect of sunlight exposure

Table 3 shows the effects of sunlight exposure on the TPC, TFC, and antioxidant capacity of honey. TPC of the samples exposed to sunlight began to change after 10 days, whereas exposure to sunlight caused changes in the TFC and TEAC of the samples after 6 days ($p<0.05$). However, CUPRAC did not change significantly in any of the samples when compared with the untreated sample. Direct sunlight exposure initiates the generation of free radicals that accelerate the degradation reactions that adversely affect the quality of foods and beverages.[8] This could explain the decrease of TPC, TFC, and antioxidant capacity of the honey. Until now, there is no study examining the influence of direct sunlight exposure on the antioxidant capacity, TPC, and TFC of honey. However, several authors report that sunlight induced quality loss of fruit products, such as pummelo (*Citrus maxima*) essential oil[24] and strawberry juice.[25]

CONCLUSION

The treatments significantly affected the antioxidant properties of honey, depending on the processing time. Thermal treatment and sunlight exposure had a negative influence on the antioxidant quality of honey. However, ultrasonication significantly increased the values of these parameters in all assays, except TEAC assay, in which the increment was statistically significant. Therefore,

Table 2. Effects of ultrasonication on antioxidant capacity, TPC, and TFC of honey

Method	Untreated sample	Ultrasonication			
		Process time (min)			
		5	15	30	60
CUPRAC*	2.75±0.10	2.76±0.04	2.86±0.25	2.99±0.14	3.22±0.06[a]
TEAC*	1.14±0.02	1.19±0.03	1.19±0.02	1.22±0.06	1.24±0.08
TPC**	27.75±0.57	28.16±0.41	29.13±0.80	29.18±1.22	30.85±0.56[c]
TFC***	6.76±0.06	6.85±0.03	6.97±0.05	7.10±0.07	7.30±0.09[d]

[a, c, d]Shows significant differences between the treated and untreated samples according to Tukey's post-hoc test ($p<0.05$). *µmol TE/g, **mg GAE/100g, ***mg QE/100g, TPC: Total phenolic content, TFC: Total flavonoid content, CUPRAC: Cupric reducing antioxidant capacity, TEAC: Trolox equivalent antioxidant capacity, GAE: Gallic acid equivalent, QE: Quercetin equivalent

Table 3. Effects of sunlight exposure on antioxidant capacity, TPC, and TFC of honey

Method	Untreated sample	Sunlight exposure			
		Process time (day)			
		1	3	6	10
CUPRAC*	2.75±0.10	2.72±0.04	2.58±0.04	2.55±0.09	2.50±0.11
TEAC*	1.14±0.02	1.11±0.04	1.11±0.05	1.01±0.02[b]	1.01±0.06[b]
TPC**	27.75±0.57	27.88±0.42	26.98±1.66	25.83±1.15	24.49±0.96[c]
TFC***	6.76±0.06	6.63±0.12	6.53±0.10	6.27±0.08[d]	6.26±0.18[d]

[b, c, d]Shows significant differences between the treated and untreated samples according to Tukey's post-hoc test ($p<0.05$). *µmol TE/g, **mg GAE/100g, ***mg QE/100g, CUPRAC: Cupric reducing antioxidant capacity, TEAC: Trolox equivalent antioxidant capacity, TPC: Total phenolic content, TFC: Total flavonoid content, GAE: Gallic acid equivalent, QE: Quercetin equivalent

ultrasonication could be an alternative technique for preserving the antioxidant properties of honey instead of industrial thermal treatment. On the other hand, it is suggested that producers and consumers should consider the negative effects of sunlight on the antioxidants properties of honey during storage, since exposure of the honey samples to sunlight resulted in a decrease in the antioxidant capacity, TPC, and TFC.

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