

Effects of ultrasound application on antioxidative properties and mineral contents of black and green tea infusions prepared with different waters

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Abstract

The objective of this study was to investigate the effects of different durations of ultrasound application and various waters on antioxidative properties and mineral contents of black and green tea infusions. Different waters (pure, drinking and mineral water) were added to tea samples for preparation of tea infusions and then ultrasound was applied at different durations (0, 10 and 20 minutes). After ultrasound application, samples were kept at room temperature to complete a total of 2 hours. Color characteristics, total phenolic, total flavonoids, mineral contents and antioxidant activities of prepared tea infusions were determined. Ultrasound application significantly ($p < 0.05$) increased total phenolic and total flavonoid contents and antioxidant activities of tea infusions. The black tea infusion which was prepared with mineral water and ultrasonicated 20 min (U2-MW) had the highest total phenolic contents. The antioxidant activities of 20 min ultrasonicated green tea infusions increased by 22.71% compared to the control group.

Keywords: Antioxidant activity, black tea, green tea, natural antioxidant, ultrasonication

1. Introduction

Tea (*Camellia sinensis* L.) is one of the most popular drinks in the world. According to tea processing method, non-fermented green and white teas, partially fermented oolong teas and fermented black teas are divided into three types [1]. Tea has been associated with beneficial effects on any pathology including oxidative stress on neurodegenerative and cardiovascular diseases, diabetes, obesity and various diseases [2,3]. These effects are most likely due to a wide range of bioactive compounds, such as flavonoids, polyphenols, caffeine or theanine [3,4]. It has been reported that tea is a rich source of flavanols and flavonols [5]. Catechins in the tea are often called polyphenols. It has been reported that tea polyphenols have a great medical and health benefits and that they are a powerful source of antioxidants [6].

There are many extraction methods to obtain specific molecules from plants. The infusion method with water can be used safely for the removal of bioactive molecules from tea. In addition, the extraction of catechins depends on time and temperature [3]. Damiani et al. [7] reported that tea leaves extract made with water for 2 h at room temperature produced infusions rich in antioxidant activity.

It has been reported that the use of ultrasound in the recovery of desired compounds is an effective and efficient extraction method in terms of reduced yields and higher yields with extraction time [8,9]. The application of ultrasound is based on mechanical effects to obtain components from plants. These mechanical effects are the movements of cavitation bubbles formed by ultrasonic waves. The collapse of these bubbles destroys the cell walls of the plant. Thus, the compounds to be extracted

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are obtained faster and easier [10-12]. It has been reported that ultrasound application can be used to increase the extraction efficiency [13].

In the literature, although there are several studies on the ultrasound assisted extraction, there have been no studies yet on the effect of tea infusions prepared with different water types. Therefore, the main purpose of this study is to improve the extraction efficiency (total phenolic, total flavonoid content and antioxidant activity) by combination of ultrasound applications and different waters for black and green tea infusions.

2. Materials and Method

2.1. Materials

Black and green tea samples were obtained from a company (Çaykur, Rize, Turkey) in Rize province. Drinking water (Saka, İstanbul, Turkey) and mineral water (Beypazarı, Ankara, Turkey) were supplied from a local market in Konya.

2.2. Preparation of tea infusions

Tea infusions were prepared by adding 100 mL of water (pure, drinking and mineral water) at room temperature to 2 g of tea and leaving the infusions to stand at room temperature (20-25 °C) for 2 h, mixing manually every 30 min. Infusions were then filtered through Whatman filter paper (16 µm) [7].

2.3. Ultrasound application

Tea infusions were placed in 250 mL beakers for ultrasonic extraction with probe. Ultrasound application (Dr. Hielscher, UP400S model, GmbH, Teltow, Germany) was applied using the following parameters: frequency of 24 kHz, power density of 400 W/cm² and amplitude level of 90 %. Ultrasound system has the probe which has vibrating horn diameter of 40 mm. The probe was directly immersed in the tea infusions for ultrasound application [14].

2.4. Experimental design

In this study, tea infusions were prepared with pure (PW), drinking (DW) and mineral (MW) water. Black and green tea infusions without ultrasound application were kept at room temperature for 2 hours (U0). 10 minutes ultrasound was applied to tea infusion for the group of U1 and then they were kept for 110 minutes. For the group of U2, 20 minutes ultrasound was applied and then tea infusions were left at room temperature for 100 minutes (U2). After tea infusions were prepared,

they were filtered and analyses were carried out on the filtrates. The experimental design applied in this study is shown in Table 1.

2.5. Total phenolic content (TPC)

Total phenolic contents of the tea infusions were determined following the Folin-Ciocalteu method [15]. 2.5 mL of Folin-Ciocalteu solution (diluted 10 times with distilled water) was added to 0.5 mL of the tea infusions and they were kept for 0.5-2 minutes. Then, 2 mL of Na₂CO₃ (7.5%) solution was added. This mixture was incubated at 50 °C in a water bath for 5 min. It was then cooled to room temperature and read at the spectrophotometer (HITACHI U-1800) at a wavelength of 760 nm. The results were given as gallic acid equivalents (GAE) per g of 100 mL tea infusion.

2.6. Total flavonoid content (TFC)

The total flavonoid contents of tea infusions were measured using a colorimetric assay according to the method of Chang et al. [16]. 0.5 mL of tea infusion was transferred in glass tubes and 2.5 mL of pure water and 150 µL of 5% NaNO₂ solution were added, respectively. After mixing, 10% AlCl₃ (300 µL), 1 mL of 1M NaOH and 550 µL of distilled water were added and mixed. It was left for 5 min at room temperature. The absorbance of the solution was measured at 510 nm using the spectrophotometer (HITACHI U-1800). The results were expressed as mg Catechin Equivalents (CE) / 100 mL infusion using the linear regression value obtained from the catechin calibration curve.

2.7. Antioxidant activity

Antioxidant activities of tea infusions were determined according to the method based on inhibition of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical used by Li et al. [17]. The results were given as %.

2.8. Mineral analysis

Mineral contents of infusions and water were determined by injecting directly into ICP-AES device (Varian-Vista, Australia). Minerals are determined using the Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) and the application conditions are as follows: 0.7-1.5 kw RF Power, 10.5-15 L/m plasma gas flow rate (Ar), 1.5 L/m auxiliary gas flow rate (Ar), 5-12 mm detection height [18].

Table 1. Experimental design of the study

Duration of ultrasound application (minutes)	Water treatment	Black tea infusion samples	Green tea infusion samples
0 (U0)	Pure water (PW)	U0-PW	U0-PW
	Drinking water (DW)	U0-DW	U0-DW
	Mineral water (MW)	U0-MW	U0-MW
10 (U1)	Pure water (PW)	U1-PW	U1-PW
	Drinking water (DW)	U1-DW	U1-DW
	Mineral water (MW)	U1-MW	U1-MW
20 (U2)	Pure water (PW)	U2-PW	U2-PW
	Drinking water (DW)	U2-DW	U2-DW
	Mineral water (MW)	U2-MW	U2-MW

2.9. Color

The color intensities of the samples (CR-400 Minolta Co, Osaka, Japan) are determined using the colorimeter device [19]. L^* ($L^*= 0$ black (darkness), $L^*= 100$ white (lightness)), a^* ($a^*=+60$ red, $a^*=-60$ green) and b^* ($b^*=+60$ yellow, $b^*=-60$ blue) values are based on the criteria given by CIELab (International Commission on Illumination) based on three-dimensional color measurement.

2.10. Statistical analysis

Collected data were analysed by two-ways analysis of variance (ANOVA) using MINITAB for Windows, Release 16.0. Factors included the duration of ultrasound (0, 10, 20 min), the type of tea (black and green tea infusions) and their interaction on the studied parameters such as total phenolic, flavonoid, antioxidant and color properties. Each parameter was analyzed in

triplicate samples with two replications. Tukey test was used for significant differences.

3. Result and Discussion

3.1. Total phenolic content

Table 2 shows that total phenolics contents of black and green tea infusions are significantly different ($p<0.01$). There was a significant increase in ultrasound treated samples compared to the control for contents of total phenols. The total phenolic contents of black tea infusion of pure, drinking and mineral waters were found to be 61.31, 65.72, 69.67 mg GAE/100 mL, respectively. The green tea infusions prepared with pure, drinking and mineral water contained 89.77, 87.76 and 81.44 mg GAE/100 mL phenolic substances, respectively. The total phenolic content of black tea was lower than green tea. Similarly, Zuo et al. [20] stated that the total phenolic content of black and oolong tea was lower than that of green tea by the effect of fermentation.

Table 2. The content of total phenolic (TPC), total flavonoid (TFC) and antioxidant activity (DPPH) in black and green tea infusion (mean \pm standard error)

Treatments	Black tea infusions			Green tea infusions		
	TPC	TFC	DPPH	TPC	TFC	DPPH
Water types (A)						
Pure water (PW)	61.31 \pm 12.07 ^c	38.94 \pm 7.02 ^a	46.25 \pm 1.75 ^a	89.77 \pm 2.96 ^a	59.77 \pm 2.88 ^b	51.07 \pm 5.14 ^a
Drinking water (DW)	65.72 \pm 2.19 ^b	36.39 \pm 4.94 ^b	45.37 \pm 4.25 ^a	87.76 \pm 4.33 ^b	60.96 \pm 2.02 ^a	47.73 \pm 3.08 ^b
Mineral water(MW)	69.67 \pm 18.60 ^a	32.09 \pm 5.80 ^c	35.56 \pm 2.35 ^b	81.44 \pm 2.77 ^c	60.47 \pm 1.42 ^{ab}	35.89 \pm 4.46 ^c
Significance	**	**	**	**	**	**
Ultrasound durations (B)						
U0 (Control)	57.62 \pm 5.61 ^b	28.74 \pm 2.65 ^c	39.32 \pm 5.24 ^c	83.33 \pm 3.90 ^c	58.54 \pm 0.82 ^c	40.69 \pm 7.47 ^c
U1	59.56 \pm 4.60 ^b	37.07 \pm 4.23 ^b	42.65 \pm 5.80 ^b	85.35 \pm 3.92 ^b	59.82 \pm 1.38 ^b	44.06 \pm 6.71 ^b
U2	79.53 \pm 11.66 ^a	41.61 \pm 3.01 ^a	45.21 \pm 5.40 ^a	90.28 \pm 4.40 ^a	62.85 \pm 0.97 ^a	49.93 \pm 7.44 ^a
Significance	**	**	**	**	**	**
AxB	**	**	**	ns	**	*

Means within a column with different letters are significantly different **($p<0.01$), *($p<0.05$).

U0: 0 min ultrasonication; U1: 10 min ultrasonication; U2: 20 min ultrasonication

Units: TPC (mg GAE/100 mL), TFC (mg CE/100 mL), DPPH (%)

Figure 1 shows the interaction of different waters and ultrasound applications on total phenolic and flavonoid contents of black tea infusions. As the time of ultrasound application increased, the total phenolic content of the samples increased. Wang et al. [21] indicated that the amount of total phenolics substance in kiwi juice increased as the ultrasonication time increased. Aadil et al. [22] also determined that 90 min ultrasonication increased the total phenolic content of grape juice compared to the control group. Golmohamadi et al. [23] reported that as the time and frequency of sonication increased, the total phenolic content of raspberry puree increased. They explained that the heat generated during ultrasound application caused the increase of the transfer of phenolic substance to the solution. On the other hand, it has been reported that the phenolic contents may increase as a result of the breakdown of tea tissues by ultrasound application [24,25]. In this study, 20 minutes ultrasound application to black and green tea infusions caused an increase in the phenolic content in this study. While 20 minutes ultrasound infusion of black tea contains 79.53 mg GAE/100 mL total phenolics substance, green tea has 90.28 mg GAE/100 mL phenolics substance content.

3.2. Total flavonoid content

Total flavonoid contents of samples are shown in Table 2. During ultrasound application, mechanical

waves cause surface erosion and particle fragmentation. Thus, more bioactive substances are released and the total amount of flavonoid has increased [9]. In this study, a significant increase was determined in the content of total flavonoid ($p < 0.05$). The tea infusions treated with 20 min ultrasonication had higher total flavonoid contents compared to control and 10 min ultrasonicated tea infusions. As the time of ultrasound application increased, the flavonoid content of tea infusions increased ($p < 0.05$).

The total flavonoid contents of black tea infusions prepared with pure, drinking and mineral water were found as 38.94, 36.39 and 32.09 mg CE/100 mL, respectively. The green tea infusions prepared with pure, drinking and mineral water had 59.77, 60.96 and 60.47 mg CE/100 mL total flavonoid contents, respectively. Since galloylated proanthocyanidins are dissolved during the fermentation of black tea, green tea contains more flavonoids than black tea [26-28]. Similarly, Muthumani and Kumar [29] stated that with the increase of fermentation time, the amount of flavonoids decreased. The interaction between the duration of ultrasound application and different waters is shown in Figure 1. The black tea infusion group of U2-PW had the highest flavonoid content, while the group of U0-MW had the lowest total flavonoid content.

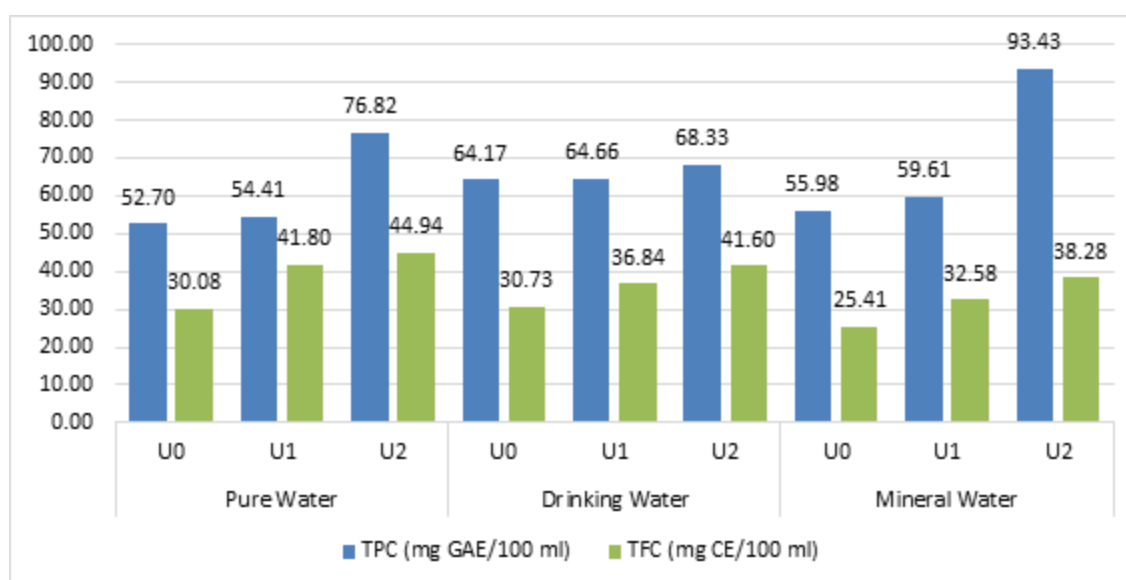


Figure 1. Effect of interaction of different waters and ultrasound applications on total phenolics and flavonoids contents of black tea infusions (U0: 0 min ultrasonication; U1: 10 min ultrasonication; U2: 20 min ultrasonication)

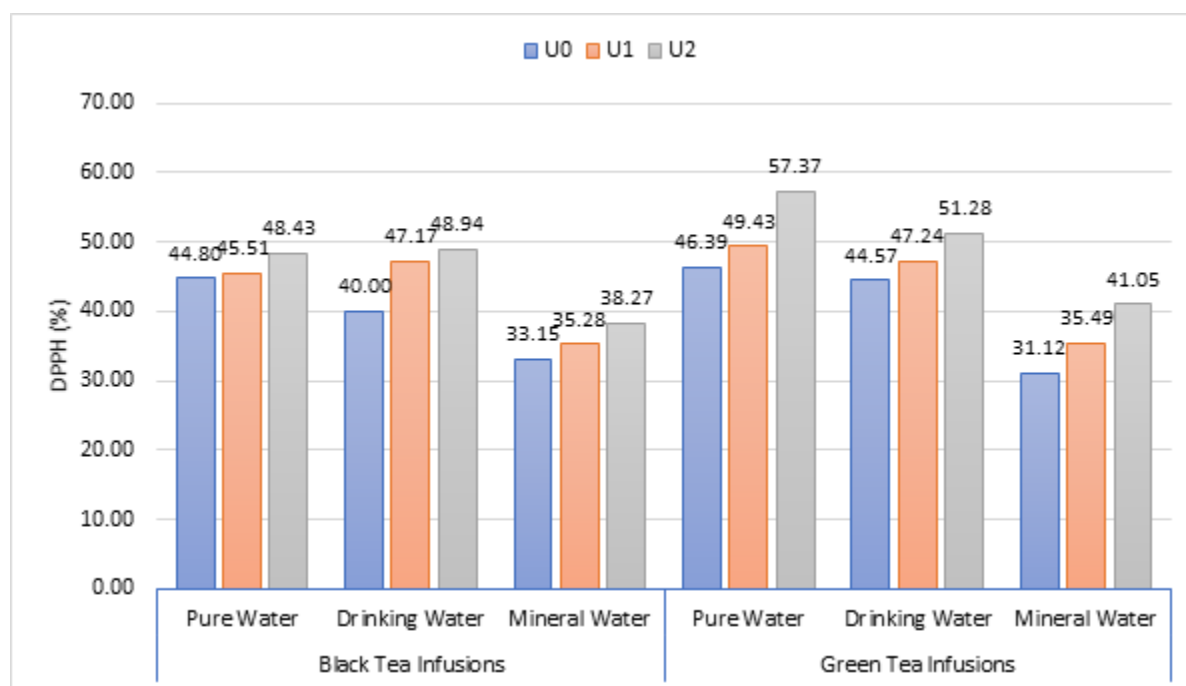


Figure 2. Effect of interaction of different waters and ultrasound applications on total antioxidant activity (%) (U0: 0 min ultrasonication; U1: 10 min ultrasonication; U2: 20 min ultrasonication)

3.3. Total antioxidant activity (DPPH)

The total antioxidant capacity of tea infusions treated with different ultrasound processing time has been shown in Table 2. Figure 2 shows the interaction of different waters and ultrasound applications on total antioxidant activity of black and green tea infusions. Ultrasound application increased the antioxidant activity of samples. The total contents of phenolic substances in tea infusions give an idea about the hydroxyl groups that provide antioxidant activity. Although black tea infusions prepared with mineral water had the highest total phenolic contents, it had the lowest antioxidant activity. This situation could be explained that all phenolic substances are extracted from extractable proteins by the Folin-Ciocalteu method used for the determination of the total phenolic content but not all of the phenolic groups have antioxidant activity [30].

The antioxidant activities of black and green tea infusions prepared with pure water were determined as 46.25% and 51.07%, respectively. Fermentation process causes decrease in antioxidant activity [31]. Therefore, the antioxidant activity of black tea, which was applied fermentation process during production, was found to be lower than green tea.

3.4. Mineral contents

Table 3 indicates the mineral contents of tea infusions and different waters which were used in this study. Tea infusions increased the mineral contents compared to waters. As expected, the mineral contents of tea infusions prepared with mineral water were found to be higher than that of pure and drinking waters. Because mineral water contains more minerals than drinking water and pure water. On the other hand, 20 min ultrasound application generally increased the mineral contents of tea infusions.

3.5. Color

Color properties of samples are given in Table 4. There was significant change in color values of tea infusions at all ultrasound durations ($p < 0.01$). The lightness (L^*) values showed that black tea infusions were the darker than green tea infusions. L^* values of black and green tea infusion decreased with ultrasound application for 20 minutes. As shown in Table 4, L^* values of black and green tea infusions without ultrasound were measured as 32.97 and 41.91, respectively. The effect of 20 min ultrasound application on L^* value was 22.11 and 36.29, respectively. As the ultrasound time increased, the lightness value decreased.

Table 3. Effect of different waters and ultrasound application on mineral contents (mg/L) of tea infusions (mean \pm standard deviation)

	Samples	Ca	K	Mg	Mn	Na	P	Zn
Black Tea Infusion	U0-PW	17.2 \pm 1.9	207.0 \pm 10.1	9.2 \pm 1.3	2.0 \pm 0.3	21.9 \pm 2.2	18.6 \pm 2.8	1.3 \pm 0.2
	U1-PW	14.4 \pm 1.1	201.4 \pm 9.3	9.1 \pm 2.0	2.3 \pm 0.5	21.1 \pm 3.0	18.9 \pm 1.7	2.2 \pm 0.3
	U2-PW	14.6 \pm 1.4	209.1 \pm 10.8	11.6 \pm 1.7	3.4 \pm 1.1	12.5 \pm 4.1	20.7 \pm 3.1	7.6 \pm 0.8
	U0-DW	14.2 \pm 0.9	212.3 \pm 7.7	10.2 \pm 2.4	3.2 \pm 0.7	16.9 \pm 1.3	18.8 \pm 2.4	5.3 \pm 1.3
	U1-DW	13.5 \pm 0.5	212.1 \pm 8.1	11.6 \pm 3.1	3.8 \pm 0.6	14.2 \pm 2.4	20.0 \pm 0.7	1.5 \pm 0.5
	U2-DW	15.4 \pm 0.8	216.9 \pm 11.7	14.5 \pm 3.6	3.8 \pm 1.2	24.6 \pm 3.5	20.6 \pm 1.2	6.1 \pm 1.0
	U0-MW	28.3 \pm 1.3	289.2 \pm 16.3	26.7 \pm 2.5	5.2 \pm 0.4	140.0 \pm 3.4	17.3 \pm 2.4	1.3 \pm 0.4
	U1-MW	30.4 \pm 1.8	277.0 \pm 14.4	26.3 \pm 2.8	4.5 \pm 1.0	153.7 \pm 2.8	17.9 \pm 1.8	6.2 \pm 1.1
	U2-MW	23.2 \pm 2.0	282.9 \pm 11.9	25.1 \pm 3.0	4.5 \pm 0.8	139.8 \pm 2.7	19.2 \pm 1.3	4.0 \pm 1.0
Green Tea Infusion	U0-PW	14.4 \pm 3.3	174.1 \pm 12.3	10.3 \pm 1.2	6.0 \pm 0.9	16.0 \pm 1.7	10.5 \pm 1.2	6.5 \pm 2.1
	U1-PW	21.1 \pm 5.1	176.0 \pm 10.0	12.7 \pm 2.7	6.0 \pm 0.7	30.2 \pm 3.3	13.5 \pm 1.4	6.8 \pm 1.9
	U2-PW	23.7 \pm 4.7	174.7 \pm 8.7	14.5 \pm 4.1	6.2 \pm 1.0	38.7 \pm 3.7	13.6 \pm 0.8	3.3 \pm 1.4
	U0-DW	22.3 \pm 4.1	178.2 \pm 9.9	15.4 \pm 2.0	7.5 \pm 1.3	17.8 \pm 1.4	10.5 \pm 2.6	0.5 \pm 0.1
	U1-DW	24.9 \pm 4.3	187.0 \pm 15.4	16.3 \pm 3.3	6.1 \pm 0.7	34.2 \pm 1.9	11.4 \pm 1.3	5.7 \pm 0.5
	U2-DW	27.8 \pm 3.6	185.8 \pm 11.1	17.9 \pm 3.0	6.9 \pm 0.8	37.5 \pm 2.0	13.4 \pm 1.5	2.3 \pm 0.3
	U0-MW	40.5 \pm 2.9	239.7 \pm 15.8	28.3 \pm 1.6	9.7 \pm 1.2	153.1 \pm 8.5	9.9 \pm 3.1	1.3 \pm 0.4
	U1-MW	37.2 \pm 4.5	248.0 \pm 16.7	27.6 \pm 1.5	6.1 \pm 1.5	153.6 \pm 7.6	11.4 \pm 2.4	2.1 \pm 0.4
	U2-MW	37.8 \pm 1.8	247.5 \pm 14.4	27.2 \pm 2.5	4.7 \pm 0.4	140.1 \pm 6.0	12.7 \pm 2.3	1.7 \pm 0.3
Different waters	Pure water	1.20 \pm 0.08	1.79 \pm 0.47	0.00 \pm 0.00	0.04 \pm 0.02	3.53 \pm 0.8	0.11 \pm 0.02	0.04 \pm 0.01
	Drinking water	33.98 \pm 3.28	1.41 \pm 0.14	6.00 \pm 0.59	0.01 \pm 0.00	5.99 \pm 0.7	0.06 \pm 0.02	0.01 \pm 0.00
	Mineral water	57.82 \pm 5.55	32.55 \pm 4.88	16.79 \pm 2.26	0.05 \pm 0.02	154.76 \pm 5.2	0.04 \pm 0.01	0.19 \pm 0.03

U0: 0 min ultrasonication; U1: 10 min ultrasonication; U2: 20 min ultrasonication

Table 4. Effect of different waters and ultrasound application on color properties of black and green tea infusions (mean \pm standard error)

Treatments	Black Tea Infusions			Green Tea Infusions		
	<i>L*</i>	<i>a*</i>	<i>b*</i>	<i>L*</i>	<i>a*</i>	<i>b*</i>
Water types (A)						
Pure water (PW)	31.00 \pm 7.75 ^a	13.17 \pm 3.01 ^c	27.48 \pm 7.56 ^a	50.94 \pm 5.28 ^a	-1.64 \pm 1.62 ^c	36.78 \pm 0.68 ^b
Drinking water (DW)	26.61 \pm 1.15 ^b	16.63 \pm 0.51 ^a	24.95 \pm 1.72 ^b	47.89 \pm 3.08 ^b	0.19 \pm 0.80 ^b	40.22 \pm 3.82 ^a
Mineral water(MW)	22.00 \pm 7.50 ^c	14.88 \pm 2.64 ^b	18.10 \pm 10.23 ^c	21.42 \pm 2.59 ^c	19.28 \pm 0.56 ^a	20.54 \pm 3.64 ^c
Significance	**	**	**	**	**	**
Ultrasound durations (B)						
U0 (Control)	32.97 \pm 6.30 ^a	12.39 \pm 3.01 ^c	30.99 \pm 4.92 ^a	41.91 \pm 14.20 ^a	5.36 \pm 10.97 ^b	35.22 \pm 9.77 ^a
U1	24.49 \pm 3.18 ^b	15.44 \pm 1.34 ^b	21.57 \pm 3.87 ^b	42.04 \pm 15.67 ^a	5.36 \pm 10.32 ^b	31.65 \pm 7.18 ^b
U2	22.11 \pm 6.08 ^c	16.85 \pm 0.51 ^a	17.97 \pm 8.59 ^c	36.29 \pm 14.01 ^b	7.11 \pm 9.82 ^a	30.66 \pm 11.51 ^c
Significance	**	**	**	**	**	**
AxB	**	**	**	**	**	**

U0: 0 min ultrasonication; U1: 10 min ultrasonication; U2: 20 min ultrasonication

Similarly, Aadil et al. [22] showed that the grape juice, which has been ultrasonicated for 90 minutes, has a lower *L** and *b** value than the untreated. Redness (*a**) indicated that all the black tea infusions were red while the green tea infusions were green. Türkmen [32] stated that black tea gave darker color than green tea due to fermentation.

Therefore, *a** values of black tea infusions prepared with pure water and drinking water were measured as 13.17 and 16.63 respectively, while green tea infusions were measured as -1.64 and 0.19, respectively. As the ultrasonication time increased, the *a** value of black and green tea infusions

increased. In addition, the increase in ultrasound time led to a decrease in b^* values ($p < 0.01$).

4. Conclusion

In this study, it was determined that ultrasound application significantly increased total phenolics and flavonoids, total antioxidant activity and some mineral content compared to the non-sonicated tea infusions. As a result, antioxidative quality parameters of the tea infusions were improved by means of ultrasound application. 10 min of ultrasound application increased the total antioxidant amount of black and green tea by 8.47% and 8.28%, respectively. 20 minutes of ultrasound application increased by 14.98% and 22.71%. The current study implied that ultrasound as a simple and economical technique could be used to enhance quality to tea infusions.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest.

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