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Research Article

Reduction of Turbidity and Cream Formatıon in Ultrasound-Assisted Turkish Green Tea Extracts: Application of Tannase Enzyme

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ABSTRACT
This study was conducted to determine the composition of green tea extracts obtained by using ultrasound-assisted extraction technique and to determine the changes in color, turbidity and tea cream formation with tannase enzyme supplementation. Green tea extracts obtained at a brewing temperature of 70 °C with different tea:water ratios (1:100; 5:100 and 10:100 m/v) and brewing durations (5, 10 and 20 minutes) were supplemented with tannase enzyme. The turbidity (NTU) values of green tea extracts varied between 3.50-24.0, tea cream quantities varied between 1.35-3.98 g/100g green tea for control samples and between 0.42-2.00 g/100g green tea for tannase enzyme-supplemented samples. Tea:water ratios and brewing durations had significant effects on tea cream formation of the samples. The results showed that the amounts of tannase enzyme-supplemented tea cream samples decreased more than the control samples under all application settings, and there were significant differences between the amounts of enzyme-supplemented and unsupplemented tea cream samples.
Keywords: Green tea, NTU, color, sensory analyses, catechins, tannase

Ultrason Destekli Türk Yeşil Çay Ekstraktlarında Bulanıklığın ve Krema Oluşumunun Azaltılması: Tannaz Enzimi Uygulaması

ÖZ
Bu çalışma, ultrason destekli ekstraksiyon tekniği kullanılarak elde edilen yeşil çay ekstraktlarının kompozisyonunun belirlenmesi ve tannaz enzimi ilavesi ile renk, bulanıklık ve çay kreması oluşumundaki değişikliklerin belirlenmesi amacıyla yapılmıştır. 70 °C demleme sıcaklığında, farklı çay:su oranlarında (1:100; 5:100 ve 10:100) ve demleme sürelerinde (5, 10 ve 20 dakika) elde edilen yeşil çay ekstraktlarına tannaz enzimi ilave edilmiştir. Yeşil çay ekstraktlarının bulanıklık (NTU) değerleri 3,50-24,0; çay kreması miktarları kontrol örnekleri için 1,35-3,98 g/100g yeşil çay ve tannaz enzimi ilave edilmiş örnekler için 0,42-2,00 g/100g yeşil çay arasında değişmiştir. Çay:su oranları ve demleme sürelerinin örneklerin çay kreması oluşumu üzerinde önemli etkileri olmuştur. Sonuçlar, tüm uygulamalarda tannaz enzimi ilaveli örneklerin çay kreması miktarlarının kontrol örneklerine göre daha fazla azaldığını ve enzim katkılı ve katkısız çay kreması örneklerinin miktarları arasında önemli farklılıklar olduğunu göstermiştir.
Anahtar Kelimeler: Yeşil çay, NTU, renk, duyusal analizler, kateşinler, tannaz

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Reduction Of Turbidity and Cream Formatıon in Ultrasound-Assisted Turkish Green Tea Extracts: Application of Tannase Enzyme

Introduction

The first stage in making a beverage from raw tea leaves is extraction. Green tea extracts' anti-oxidant capabilities, volatile organic compound profile and quantity, and sensory attributes have all been found to be strongly impacted by the extraction process and extraction conditions (Liang et al., 2021; Uzuner, 2022; Ashraf et al., 2023). It is widely accepted that the traditional extraction procedures involve dissolving and the use of solvents for solid-liquid extraction processes. The traditional extraction techniques include maceration, heat-reflux process, hydro-distillation, and Soxhlet extraction. Nevertheless, a number of disadvantages have made traditional methods less popular in recent years, including the need for toxic solvents, excessive time and energy requirements, poor extraction efficiency, and high extraction temperatures that limit their use (Chen et al., 2016; Koina et al., 2023).

In addition to significantly improving the extraction yield, the application of innovative technologies under moderate processing conditions can reduce contamination and structural alterations of polyphenols associated with epimerization and oxidative oligomerization processes. These technologies also raise the target secondary metabolites' mass transfer coefficient and the rate of solvent permeability in plant cells. (Raghunath et al., 2023). As an alternative to traditional extraction, ultrasound-assisted extraction uses moderate temperatures, which prevent volatile chemicals from evaporating away from the environment and heat-induced degradation of tea components (Hu et al., 2019; Uzuner, 2022; Heydari et al., 2023).

Visual characteristics are important for food acceptance. Of them, turbidity, or cloudiness, is frequently thought to be a particularly significant element influencing the quality of many foods, including drinks. The primary factors influencing turbidity are the colloidal size entities in the liquid phase, the particle size distribution, and the differential in refractive index between the particles and the medium (Collado-Fernandez et al., 2000). Light scattered by particles in suspension or solution is measured as turbidity. In a lab setting, a nephelometer (turbidimeter) is often used to detect turbidity. Light emitted by turbidity particles falling on a surface is measured by a turbidimeter. This means that when turbidity rises, light entering the liquid is unable to continue on its path and will instead spread (deviate) to the side. Nephelometric Turbidity Unit, or NTU, is the turbidimeter's measuring unit. (Cemeroğlu B., 2004). Measuring turbidity is an effective way to look at how astringent substances that combine with proteins, such polyphenols, interact with them. Additionally, turbidity measurement would offer a highly efficient method for locating additional astringent components in tea. (Wen et al., 2022). The instability of natural and additive-free Ready-to-drink (RTD) cold tea that results in the development of haze and tea cream formation is one of the primary problems faced in its manufacture. It causes complexed substances to discolor and precipitate, affecting the color, flavor, and visual appeal (Chandiniet al., 2013; Yu & He, 2022). When evaluating tea sensory assessment, turbidity is a downside for certain, but not all, green teas. It is sometimes associated with the unfavorable term “dull.” (Wang et al., 2004). In addition to having an unpleasant look, the apparent hazes and precipitates affect the flavor and color of tea drinks (Guo et al., 2021). Tsai (1987) defined the 0–50 NTU range as "Crystal Clear, Transparent, Clear," the 50–100 NTU range as "Clear," and the 101–200 NTU range as "cloudy" for teas that are ready to consume. To our knowledge, there is no study about Turkish green tea cream formation. This study was conducted to assess the color and turbidity values of green tea extracts obtained through ultrasound technique and the changes in tea cream quantities. With the scope of this investigation conducted at brewing temperature of 70 °C varied brewing durations (5, 10 and 20 minutes) tannase enzyme was applied to green tea extracts prepared using different tea:water ratios (1:100; 5:100; and 10:100 m/v).

Materials and Methods

Material

The green tea, used as the raw material of the study, was supplied from Rize Karaali Tea (Turkey) factory. Distilled water was used as the solvent. Tannase enzyme used in this study (activity=500 U/g or higher; optimum pH range 5.0-5.5 and temperature 40 °C) was obtained from Kikkoman Company, Japan.

Method

Green tea extraction process

The extract from green tea was obtained using an Elma Sonic-S100H brand ultrasonic water bath with an ultrasonic power effective of 600 (W) and a constant 37 KHz frequency. Various brewing times (5, 10, and 20 minutes) and tea:water ratios (1:100, 5:100, and 10:100 m/v) were employed during the extraction process at a brewing temperature of 70°C. The specified ratios of tea to water (1:100, 5:100, and 10:100 m/v) were followed while weighing and filling beakers with tea samples. Next, 70 °C distilled brewing water was added, and a glass stirrer was used to agitate the mixture for 30 seconds. In an ultrasonic water bath, mixtures were allowed to steep for five, ten, or twenty minutes. Samples were filtered at the end of the specified brewing times using double-layer simple filter sheets. 1.25 U/g of the enzyme solution and 40 ml of equal amounts of the final extract, First Extract (F.E.), were added to centrifuge tubes. The samples that were supplemented with TANNASE enzyme were immediately put in a water bath at 40°C, which is the ideal working temperature for the enzyme, and left there for an hour. To inactivate the enzymes, samples were incubated for two hours at 2°C in a water bath after being pre-cooled for five minutes. After the appropriate amounts of time, sample tubes were centrifuged (Nuve, NF 800R) for 20 minutes at -2°C and 9000 rpm. Next, the clear and creamy parts were taken apart. We got clear green tea extracts and ran the following analysis on them. Concurrently, the tannase-free samples (called Control (CNTRL) samples) were prepared and subjected to the same assays. The study was carried out in two replicates.

Analyses

The composition of green tea extracts was determined by Cemeroğlu (2010), including total dry matter content, color parameters (L a b) (Minolta color meter device, CR-400), and turbidity measurements (turbidimeter (La Matte 2200, 2020 we, USA; the findings were represented in NTU). The amount of cream formed in green tea extracts was determined as specified by Nagalashmi et al. (1984). Results were expressed in g cream/100g tea. The sensory evaluation of green tea extracts was performed by a panel of 10 panelists in terms of brew color, aroma, astringency, fullness and general properties over 5 points (Sinija and Mishra, 2011).

Statistical Analyses

The factorial experimental design was followed while analyzing the variance of the experimental data. Duncan's multiple range test was used to compare significant means at the 0.05 significance level.

Results And Discussion

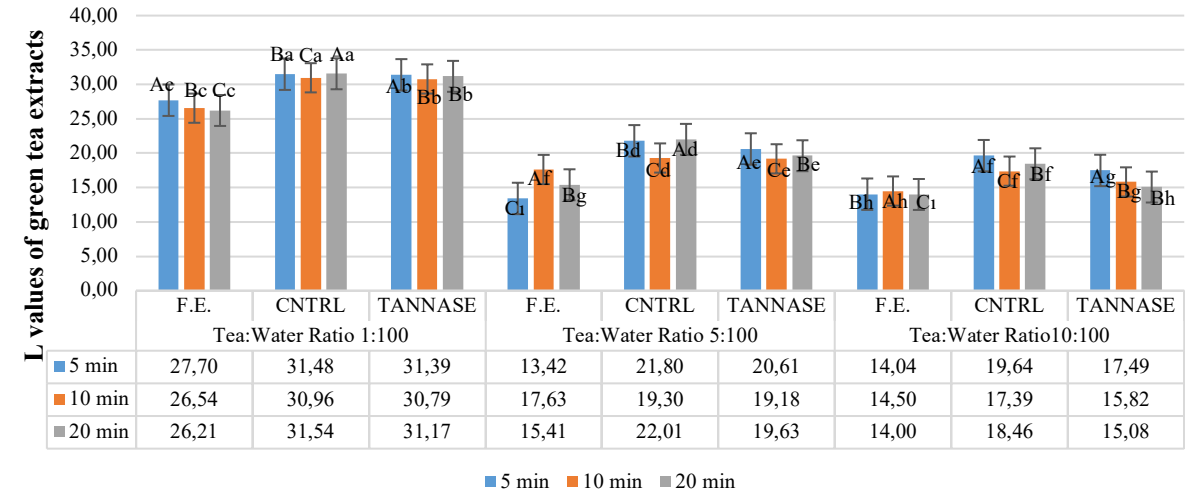
Characteristics Of Green Tea

The average dry matter content of green tea, used as raw material in the study, was 94.25%, moisture content was determined as 5.75%. Ilgaz et al. (2006) conducted a study to determine quality parameters of the 1st flash Çaykur green tea varieties and reported moisture contents as between 2.2-5% and indicated that low-moisture teas were roasted aromatic green teas. It was stated that moisture ratio of stored teas should not exceed 6% and the moisture values detected in green teas of foreign-origin were between 5.6-9.4% and this could be due to improper storage conditions. Nas et al. (1988) conducted a study on teas stored in different environments and reported average moisture content as 7.20%. It was indicated that moisture value of teas should be at most 6%, otherwise the deterioration will accelerate due to the growth of microorganisms and mold and the quality of tea will decrease over time. It was also reported that a significant part of the aroma substances could be lost as a result of non-enzymatic browning of teas with high moisture content.

Color Parameters Of Green Tea Extracts

L values of extracts

The changes in L values of green tea extracts obtained by applying varied tea/water ratios and brewing durations are given in Figure 1. As can be seen from the figure, L values of green tea samples varied between 13.42-27.70 in the first extracts, and in the samples with and without enzyme ranged between 15.08-31.39 and 17.39-31.54 respectively.



	F.E.	CNTRL	TANNASE	F.E.	CNTRL	TANNASE	F.E.	CNTRL	TANNASE
	Tea:Water Ratio 1:100			Tea:Water Ratio 5:100			Tea:Water Ratio10:100		
5 min	27,70	31,48	31,39	13,42	21,80	20,61	14,04	19,64	17,49
10 min	26,54	30,96	30,79	17,63	19,30	19,18	14,50	17,39	15,82
20 min	26,21	31,54	31,17	15,41	22,01	19,63	14,00	18,46	15,08

Figure 1. L values of green tea extracts

Supplementation of tannase enzyme into green tea extracts obtained by applying different tea/water ratios and brewing durations increased L values of the samples as compared to initial extracts, but the L values were lower than the control samples. The L values of the samples with and without enzyme supplementation differed significantly (p~<0.05).

Wang et al. (2006) used 28 different green teas and reported Hunter L value at 1:50 concentration after 5 minutes brewing as 91.97±6.20. Lu et al. (2009) extracted 1 g of ground green tea with 100 ml of boiled water at 85 °C for 20 minutes and reported L values of control samples as 97.2±5.1; L values of 2 g/l tannase-supplemented samples as 96.2±8.1 Deka et al. (2024) reported that the L value ranged between 63.65-76.35 in green tea infusions (2 g tea; 150 ml boiling water, 3 min) obtained from 5 different varieties.

The a values of extracts

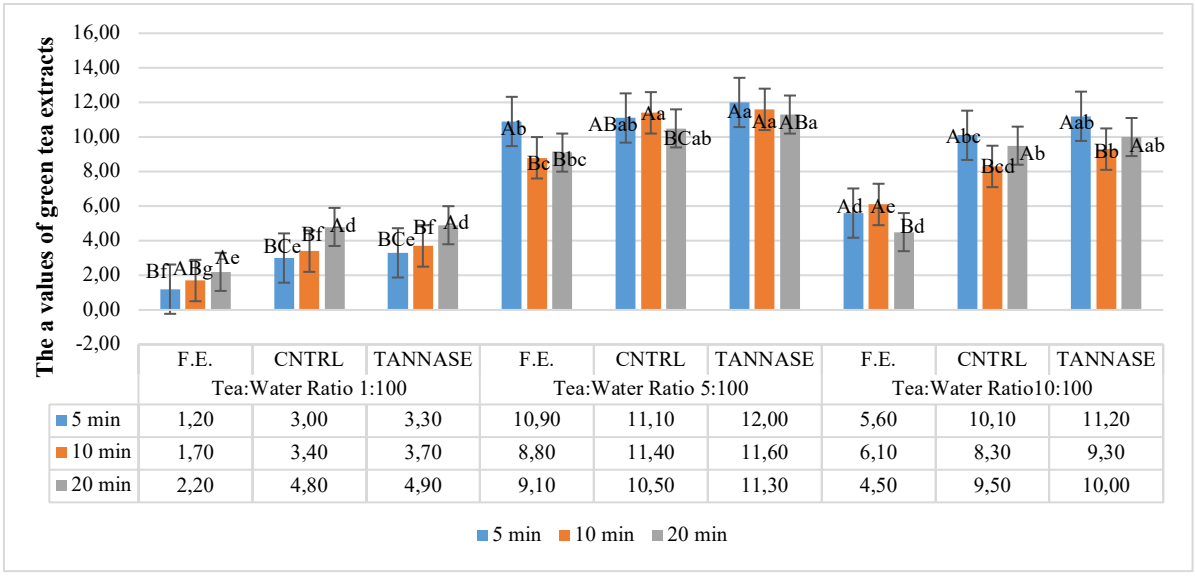
The changes in a values of green tea extracts obtained by applying different tea/water ratios and brewing durations are presented in Figure 2. As can be seen from the figure, a values of green tea samples varied between 1.20-10.90 in the first extracts, and in the samples with and without enzyme ranged between 3.30-12.00 and 3.00-11.40 respectively.

When the ratio of tea to water and the brewing duration were changed to create green tea extracts, the sample a values were improved by adding tannase enzyme. The differences between the sample a values obtained with and without the enzyme supplementation were determined to be statistically significant (p<0.05). Wang et al. (2006) used 28 different green teas and reported average Hunter a value at 1:50 concentration after 5 minutes brewing as -0.70±0.69. Lu et al. (2009) extracted 1 g of ground green tea with 100 ml of boiled water at 85 °C for 20 minutes and reported a values of control samples as -1.1±0.1; a values of 2 g/l tannase-supplemented samples as 1.4±0.1. Deka et al. (2024) reported that the a value ranged between -2.85 to -0.79 in green tea infusions (2 g tea; 150 ml boiling water, 3 min) obtained from 5 different varieties.

The b values of extracts

The changes in b values of green tea extracts obtained by applying different tea/water ratios and brewing durations are presented in Figure 3. As can be seen from the figure, b values of the green tea samples varied between 9.19-27.69 in the first extracts, and in the samples with and without enzyme ranged between 18.95-27.69 and 16.90-26.98 respectively. The results showed that b values of the samples were significantly impacted by both tea:water ratios and brewing times ($p<0.05$); b values rose as the ratio of tea to water increased and fell as the length of brewing increased. The highest b value (27.69) was obtained from tannase-supplemented samples with 10:100 tea:water ratio and 10 minutes of brewing duration.

Wang et al. (2006) used 28 different green teas and reported average Hunter b value at 1:50 concentration after 5 minutes brewing as 8.95±2.52. Lu et al. (2009) extracted 1 g of ground green tea with 100 ml of boiled water



	F.E.	CNTRL	TANNASE	F.E.	CNTRL	TANNASE	F.E.	CNTRL	TANNASE
	Tea:Water Ratio 1:100			Tea:Water Ratio 5:100			Tea:Water Ratio10:100		
5 min	1,20	3,00	3,30	10,90	11,10	12,00	5,60	10,10	11,20
10 min	1,70	3,40	3,70	8,80	11,40	11,60	6,10	8,30	9,30
20 min	2,20	4,80	4,90	9,10	10,50	11,30	4,50	9,50	10,00

Figure 2. The a values of green tea extracts

at 85 °C for 20 minutes and reported a values of control samples as 23.20±1.10; a values of 2 g/l tannase-supplemented samples as 24.20±3.10. Deka et al. (2024) reported that the b value ranged between 8.93 to 38.66 in green tea infusions (2 g tea; 150 ml boiling water, 3 min) obtained from 5 different varieties.

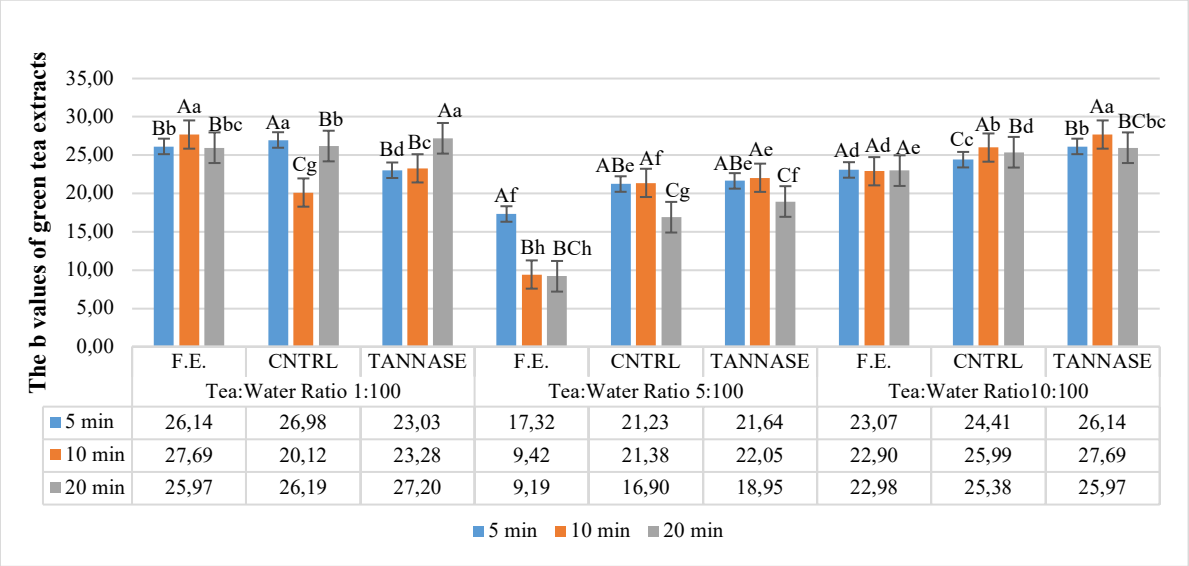


Figure 3. The b values of green tea extract

Turbidity (NTU) Values Of Extracts

The changes in turbidity (NTU) values of green tea extracts obtained by applying different tea/water ratio and brewing durations are presented in Figure 4. As observed in the graph, the green tea samples' turbidity (NTU) values ranged from 7.20 to24.00 in the initial extracts, and in the samples with and without enzyme, they ranged from 3.50 to 6.88 and 4.48 to 11.13, respectively, under all circumstances. This decrease was determined to be significant ($p<0.05$). It was determined that tannase-supplemented samples were clearer. The results indicated that there was a substantial ($p>0.05$) rise in the turbidity (NTU) values of the extracts with increasing tea:water ratio and brewing time. It was demonstrated that brewing periods and tea:water ratios affected the NTU values of the enzyme-supplemented samples, which declined relative to the control samples.

Lu et al. (2009) extracted 1 g of ground green tea with 100 ml of boiled water at 85 °C for 20 minutes and reported NTU values of control samples as 17.10± 3.20; NTU values of 2 g/l tannase-supplemented samples as tea:water ratios and brewing durations had significant effects on tea cream quantities of the samples ($p<0.05$). Enzyme supplementation provided a significant ($p<0.05$) reduction in tea cream quantities. With longer brewing times came higher amounts of tea cream, and it was discovered that there were significant differences between the extracts' cream contents ($p<0.05$). The extracts' cream quantities, however, were found to decrease as the tea ratio rose, and these differences were shown to be statistically significant ($p<0.05$) for all extract cream quantities. The cream amounts of the green tea extracts treated with tannase enzyme were observed to decrease more than those of the control samples under all application settings during ultrasonic extraction. Moreover, differences between the cream quantities of samples with and without enzyme supplementation were found to be statistically significant ($p<0.05$).

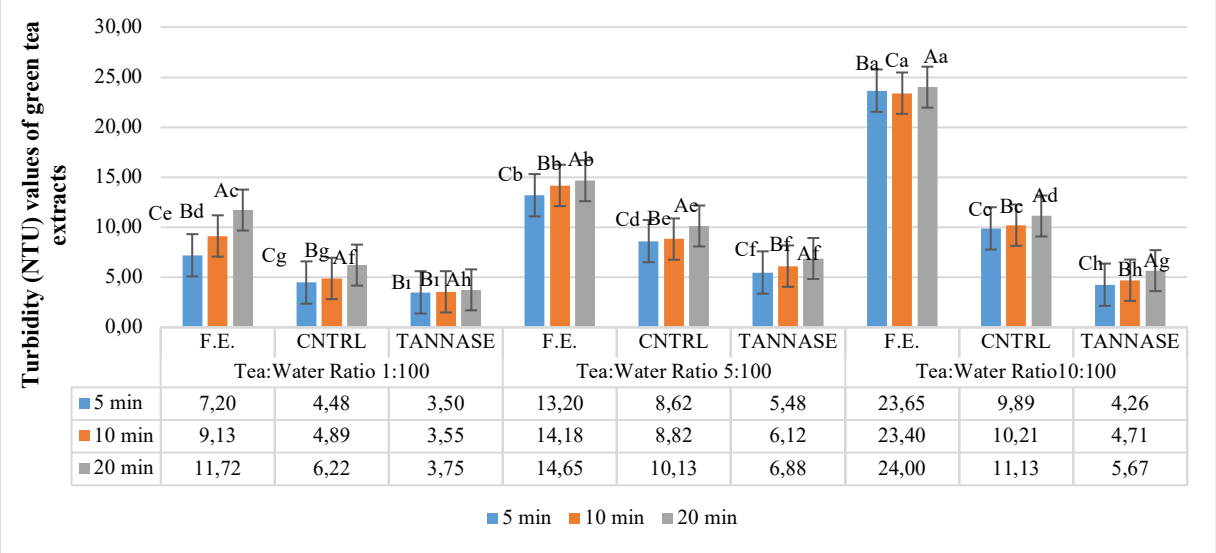


Figure 4. Turbidity (NTU) values of green tea extracts

Hong et al. (2014) examined the change in physical stability of green tea extracts treated with tannase enzyme, extracted green tea at 50g/l concentration at 80°C for 20 minutes and treated extracts with 5% tannase enzyme and reported that enzyme supplementation caused a decrease in the amounts of EGCG (epigallocatechin gallate) and ECG (epicatechin gallate) and an increase in the amounts of epigallocatechin (EGC), epicatechin (EC) and GA (gallic acid) due to its activity to catalyze the hydrolysis of ester and depside bonds of gallic acid esters like EGCG. Lu et al. (2009) extracted 1 g of ground green tea with 100 ml of boiled water at 85 °C for 20 minutes and treated resultant extracts with 2 g/l tannase enzyme and stored enzyme-treated samples at 4 °C for 4 weeks and reported cream quantity was 0,2g/100g for enzyme-treated samples and 0.9g/100g for the control samples. Xia et al. (2006) obtained extracts from green teas by both conventional extraction (3g tea with 300 ml of water for 15 min at 85 °C) and ultrasonic extraction method (3g of tea with 300 ml of water at 60 °C for 40 min) and reported that

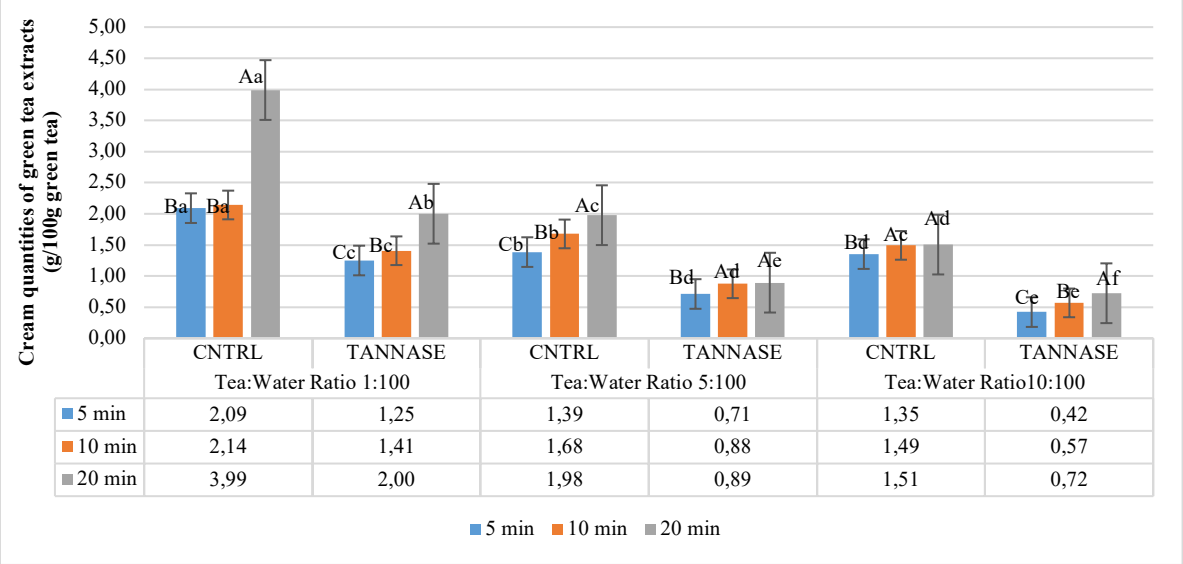


Figure 5. Cream quantities of green tea extracts (g/100 g green tea)

protein and pectin contents of the extracts obtained through ultrasonic extraction method were lower than the classical extraction method and accordingly, tea cream formation was realized at a minimum level. Xu et al. (2012) cooled and centrifuged green tea extracts obtained at 1:15 tea:water ratio at 75 °C for 15 minutes and concentrated extracts in an industrial evaporator at 50 °C under 90 kPa pressure at 7 different levels (5, 10, 20, 30, 40, 50, 60 °Brix) and reported tea cream quantities obtained at 5-60°Brix level as between 12.6±0.26 -32.2±9.23 mg/ml. Noh et al. (2014) reported that the catechins in green tea extract might inhibit the tannase enzyme, and that supplementing with more than 2% of green tea extract had an inhibitory impact on tannase activity.

Sensory Evaluation Of Extracts

The findings regarding the sensory (aroma, astringency, brew color, fullness, general evaluation) traits of green tea extracts are presented in Figure 6. In terms of aroma, present scores of ultrasonically-extracted samples varied between 1.30-3.60 for control samples and between 1.65-3.65 for enzyme-supplemented samples. In terms of astringency, present scores of ultrasonically-extracted samples varied between 1.45-3.20 for control samples and between 1.65-3.20 for enzyme-supplemented samples. In terms of brew color, present scores of ultrasonically-extracted samples varied between 1.20-4.10 for control samples and between 1.40-4.10 for enzyme-supplemented samples. In terms of fullness, present scores of ultrasonically-extracted samples varied between 1.70-3.50 for control samples and between 1.60-3.25 for enzyme-supplemented samples. In terms of general evaluation, the overall evaluation scores of the ultrasonically-extracted samples varied between 1.60-3.20 for control samples and between 1.80-3.10 for enzyme-supplemented samples.

Xia et al. (2006) obtained extracts from green teas by both conventional extraction (3g tea with 300 ml of water for 15 min at 85 °C) and ultrasonic extraction method (3g of tea with 300 ml of water at 60 °C for 40 min) and conducted sensory analyses on these extracts and stated that extracts obtained by classical method showed slight bitterness and astringency, while the extracts obtained by ultrasonic extraction method were more appreciated in terms of sensory quality because they had a fresh taste and aroma.

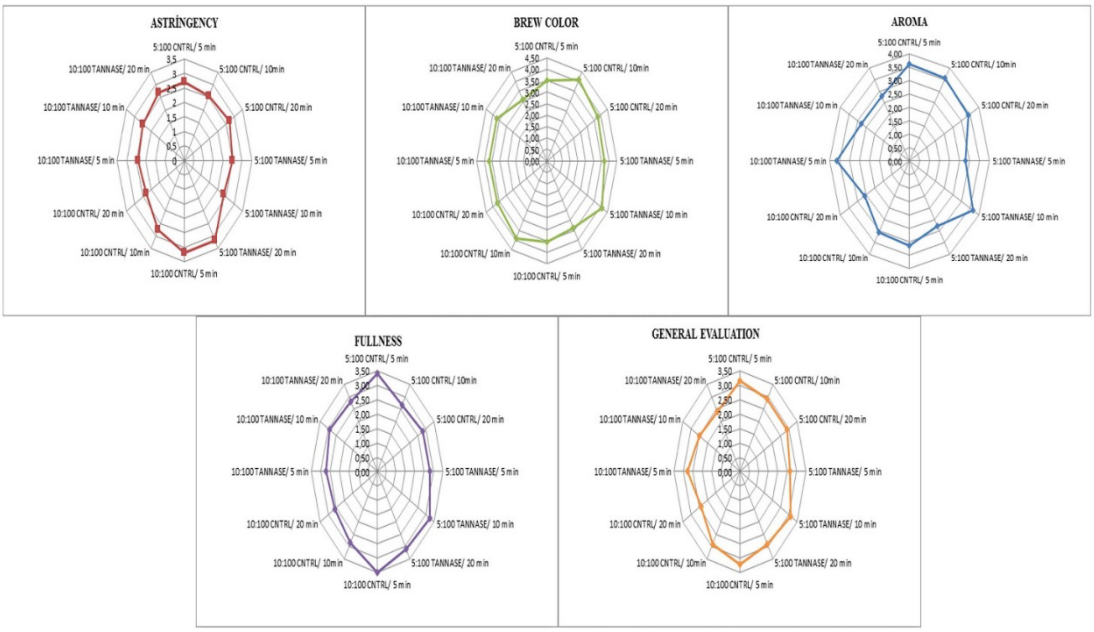


Figure 6. Scores for sensory evaluation of green tea extracts

Lu et al. (2009) examined the sensory properties of the extracts extracted from 1 g of ground green tea with 100 ml of boiled water at 85 °C for 20 minutes. It was reported that mouth feeling scores of control samples was determined as 5.00±1.20 and mouth feeling of 2 g/l tannase enzyme-supplemented samples was measured as 6.20±1.30. The taste values of the control group samples changed between 5.9±1.4; taste scores of 2 g/l tannase enzyme-supplemented samples varied between 6.20±1.30. The general acceptability values of the control samples changed between 6.80± 0.90; general acceptability scores of 2 g/l tannase enzyme-supplemented samples varied between 6.40±0.80. It was reported that sensory quality, aroma and external appearance of tannase enzyme-supplemented green teas were better than the control samples. Zhang et al. (2016) indicated that the sweet aftertaste of green tea infusion was enhanced with the decreased ratio of EGCG/epigallocatechin (EGC) and ECG/epicatechin (EC) by treating with tannase.

Conclusion

In this study, green tea samples produced in Turkey were subjected to ultrasonic extraction method at 70 °C with different tea water ratios (1:100; 5:100; 10:100) and different brewing durations (5, 10, 20 minutes). Then, to improve the extracts' stability and clarity by reducing cream formation, tannase enzyme was added as a supplement. Tea cream quantities of ultrasonic-extracted samples varied between 1.35-3.98 g/100g green tea for control group without tannase enzyme supplementation and between 0.42-2.00 g/100g green tea for enzyme-supplemented samples. In comparison to control samples, tannase-supplemented samples with a 10:100 tea:water ratio and a 5-minute brewing time showed the largest decrease in tea cream formation (68.94%). The results showed that the tannase enzyme-supplemented samples' tea cream quantities decreased more than the control samples under all application conditions. Furthermore, there was a significant difference ($p<0.05$) between the cream quantities of the enzyme-supplemented and unsupplemented samples. The samples that were extracted using ultrasonic technology had turbidity (NTU) values ranging from 3.50 to 24.00. Under all circumstances, the NTU values of the samples treated with enzymes decreased relative to the control samples; the effects of tea:water ratios

and brewing times showed a significant ($p<0.05$) drop in NTU values. It was determined that tannase-supplemented samples were clearer. Sensory assessments revealed that sensory quality, aroma and appearance of tannase enzyme-supplemented green tea extracts were more appreciated than the control samples. With the addition of tannase enzyme, astringency of tea decreased due to the decrease in the amount of EGCG, which gives astringency to green tea. With the use of ultrasonic extraction method, temperature norms were kept at low levels and thus sensory quality of green tea increased. Overall assessment of the present findings revealed that tannase enzyme supplementation together with ultrasonic-assisted extraction provided significant contributions in ensuring product stability and reducing cream formation, an important problem in production of cold teas.

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