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Ultrasound-Assisted Turkish Black Tea Extracts: Effect of Tannase Enzyme Supplementation on Amount of Tea Cream and Catechins

Esra ESİN YÜCEL[1*] Cemal KAYA[1]

[1]Department of Food Engineering, Tokat Gaziosmanpasa University, 60250 Tokat
***Corresponding author's email:** esinyasemin@yahoo.com

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Abstract: In this study, it was aimed to determine the changes in the amount of functional components of black tea extracts obtained by ultrasound assisted extraction and treated with tannase enzyme. Therefore, Turkish black tea extracts were supplemented with tannase enzyme by using an ultrasound-assisted extraction technique with different infusion temperatures (50 and 70°C), times (5, 10, 20 minutes), and tea: water ratios (1:100, 5:100, 10:100). Total phenolic, tea cream and catechin analyses were performed on the extracts. The amount of tea cream ranged between 0.56-1.25 g/100g black tea in the tannase-supplemented samples and 1.22-2.36 g/100g black tea in the control sample. It was also observed that the amount of cream obtained by ultrasonic extraction decreased by 38.89-59.11% with the tannase enzyme application.

Keywords: Catechin, ultrasound, phenolics, tannase, tea cream, tea polyphenols

Ultrason Destekli Türk Siyah Çay Ekstraktları: Tannaz Enzim Uygulamasının Çay Kreması ve Kateşin Miktarına Etkisi

Öz: Araştırmada, ultrason destekli ekstraksiyonla elde edilen ve tannaz enzimiyle muamele edilen siyah çay ekstraktlarının fonksiyonel bileşenlerinin miktarında meydana gelen değişmelerin belirlenmesi amaçlanmıştır. Bu amaçla, ultrason destekli ekstraksiyon tekniği kullanılarak farklı demleme süre (5, 10 ve 20 dakika), çay:su oranları (1:100; 5:100; 10:100) ve sıcaklık (50 ve 70°C) uygulanarak elde edilen Türk siyah çayı ekstraktlarına, tannaz enzimi ilavesi yapılmıştır. Ekstraktlarda çay kreması, toplam fenolik madde ve kateşin miktarlarının tespit edilmesine yönelik analizler gerçekleştirilmiştir. Çay kreması miktarı, tannaz enzimi uygulanmış örneklerde 0.56-1.25 g/100g siyah çay aralığında değişirken, kontrol örneklerinde 1.22-2.36 g/100g siyah çay aralığında değişmiştir. Ultrason destekli ekstraksiyonla elde edilen ekstraktlardaki krema miktarının tannaz enzim uygulaması ile %38.89-59.11 oranında azaldığı görülmüştür.

Anahtar kelimeler: Kateşin, ultrason, fenolikler, tannaz, çay kreması, çay polifenolleri

1. Introduction

Tea consumption habits are altering rapidly, and many new tea beverages are produced as an alternative to traditionally brewed tea. One of these products is ready-to-drink (RTD) tea, which has been produced and consumed globally for years (Liang et al., 2022). Commercial production of RTD tea comprises several processes like hot water extraction, selection and blending of tea leaves, aroma recovery, filtration, concentration, cream precipitation, drying, aggregation, and aromatization (Someswararao & Srivastav, 2012). Tea cream formation is a common problem faced in RTD tea production. It occurs just as a strong, hot tea infusion cools off (Guo et al., 2021). Even though tea cream formation is not believed to be a reason for deterioration, both consumers and producers do not prefer it. Since tea cream formation induces the loss of transparency, color, and taste, which in turn affects the physical and sensory properties and biological activities of tea beverages and diminishes the preferences of consumers (Dalpathadu et al., 2022; Wang et al., 2020).

Tannase (Tannin Acylhydrolase), an extracellular enzyme from the hydrolase class, is responsible for breaking down the ester bonds of hydrolyzable tannins like ethyl gallate, tannic acid, n-propyl gallate, isoamyl gallate, and methyl gallate as well as gallic acid esters. Acting on the ester bonds in tannic acid, tannase hydrolyzes it into glucose and gallic acid (Ristinmaa et al., 2022). Tannase enzyme is commonly utilized for ready-to-drink tea production to enhance tea products' color and

sensory qualities, to increase the antioxidant properties by facilitating the tea catechins biotransformation, and to prevent cream formation (Aharwar & Parihar, 2021).

Bioactive components' classical extraction from seeds or plants depends upon the solvent combination, heat and/or mixing under suitable conditions. Ultrasound-assisted extraction (UAE) is as efficient as other high-temperature and long-term extractions, and its most important advantage is that it significantly shortens the extraction time (Xia et al., 2006). The effectiveness of ultrasonic extraction is explained through the simultaneous increase in hydration during sonication and facilitating mass transfer from the material to the solvent in the degradation process. This situation increases mass transfer due to vortex formation and internal diffusion with the mechanical effect of ultrasound and allows more solvent penetration into the sample. In fruit juice and wine production, the efficiency of the enzyme used in ultrasound application increases, the amount of enzyme used decreases, and the amount of extracted functional components (such as phenolic substances) increase (Singla & Sit, 2021; Serna-Jiménez et al., 2022).

Türkiye comes fifth on the list of primary tea-producer countries after India, Sri Lanka, Kenya, and China in world tea production (Mangla et al., 2022). To our knowledge, little is known about Turkish tea composition and tea cream formation. Hence, the present study provided a significant opportunity to advance our understanding of how ultrasonic-assisted extraction and tannase enzyme affects tea cream formation. In addition, this study contributes to research on ultrasonic-assisted extraction and the tannase enzyme's effects on the chemical features of Turkish black tea infusions.

2. Materials and Methods

2.1. Material

Black tea samples as raw material [5 (BOP2: Broken Orange Pekoe] used in the experiments were purchased from the Güneysu-Ulucami Tea Factory Directorate of Çay-Kur Company, Türkiye. As the solvent, distilled water was utilized. Tannase enzyme was supplied from Kikkoman, Japan (Tannase-KTFH, 60554, activity: 500 U/g or higher, optimum temperature: 40°C, optimum pH: 5.0-5.5). To determine individual catechins content of black tea extracts, GA (Sigma), EGC (Fluka), and EGCG (Merck) were used.

2.2. Methods

2.2.1. Ultrasonic-assisted extraction

The extraction of black tea leaves was carried out with distilled water at three different tea: water ratios (1:100, 5:100, 10:100) at two different infusion temperatures (50 and 70°C) for three different times (5, 10, and 20 minutes) by using Ultrasonic water bath (Elmasonic S 100H, 37 KHz frequency, Ultrasonic power effective of 600 (W)). The preparation of the extracts were described previously (Ateş et al., 2022).

2.2.2. Determination of tea cream

The tea cream amount in black tea extracts was detected in accordance with Nagalakshmi et al.'s (1984) directions.

2.2.3. Determination of total phenolic content

Black tea extracts' total phenolic content was determined using Folin-Ciocalteu's phenol reagent following the reference method of ISO 14502-1 (Anonymous, 2005). Results were explained in gallic acid equivalent (g GAE/100 g dry black tea).

2.2.4. Determination of catechin composition of black tea extracts with HPLC

To identify the individual catechins (EGC, GA, and EGCG,) an HPLC device (Perkin Elmer Series-200) was utilized (Modified from Liang et al., 2002). Operational conditions were described previously (Ateş et al., 2022).

2.2.5. Statistical analysis

Statistical Package for the Social Sciences (SPSS) software (Version 17.0) was utilized for the analysis. All experiments were conducted in triplicate; means and standard deviations (SD) were used to explain the results. ANOVA with Duncan's test was used to compare data, which indicates a statistical significance ($p<0.05$).

3. Results and Discussion

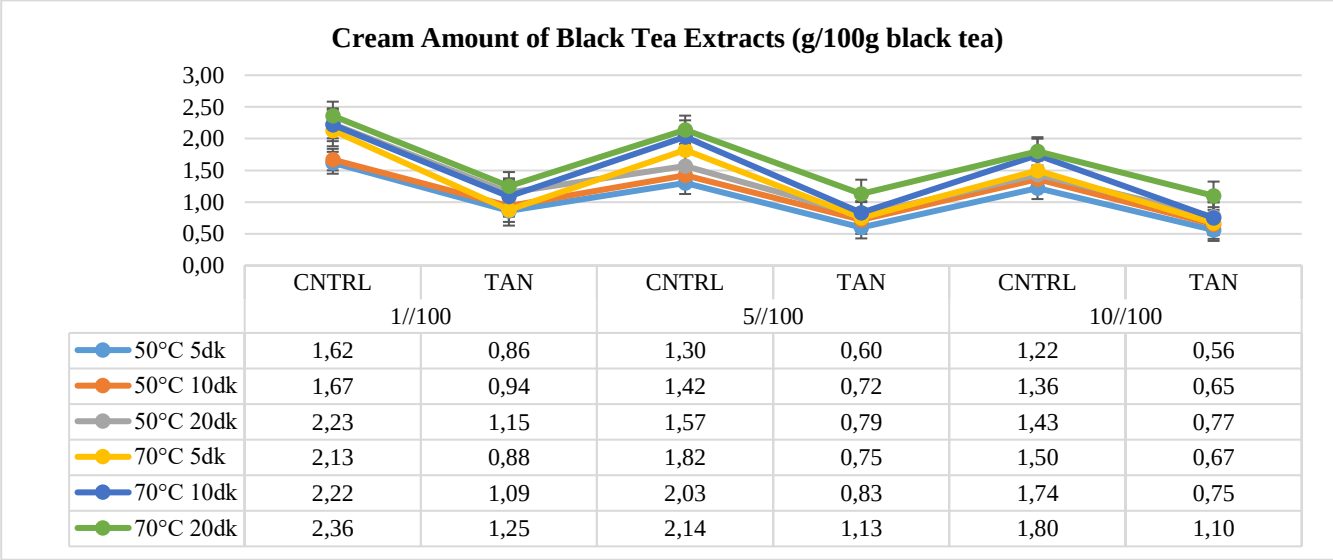
3.1. Cream amount of extracts

Figure 1 presents the cream amount of black tea extracts collected using different tea: water ratios, infusion times and temperatures, and tannase enzyme supplementation. The amount of tea cream in the samples obtained by ultrasonic extraction ranged between 1.22-2.36 g/100g black tea in the control samples and 0.51-1.25 g/100g in the tannase-supplemented samples.

Tea: water ratios, infusion time and temperatures, had a significant ($p<0.05$) impact on the amount of tea cream formed in the samples, statistically. The addition of enzymes also caused a reduction in the tea cream amount.

As the brewing time and temperature increased, the cream amount of the extracts increased, which was significant ($p<0.05$), statistically. In all application conditions in ultrasonic extraction, cream quantities of black tea extracts supplemented with tannase enzyme decreased more compared to control samples.

Similarly, Chandini et al. (2011) searched the effect of enzyme supplementation on tea cream formation to increase extraction quality using CTC-black tea. In the centrifuged (5600 g and 20 minutes) samples, the amount of cream was determined as 0.085±0.02% in the control samples, and 0.067±0.031%, 0.052±0.041% for 10 and 20 U/g-black tea in the samples with tannase enzyme, respectively. After the same samples were stored at 5°C for one week, the amount of cream was found to be 0.265±0.021% for the control sample, 0.079±0.011% and 0.065±0.032%, respectively, for 10 and 20 U/g-black tea in the tannase-supplemented samples.



	CNTRL	TAN	CNTRL	TAN	CNTRL	TAN
	1//100		5//100		10//100	
50°C 5dk	1,62	0,86	1,30	0,60	1,22	0,56
50°C 10dk	1,67	0,94	1,42	0,72	1,36	0,65
50°C 20dk	2,23	1,15	1,57	0,79	1,43	0,77
70°C 5dk	2,13	0,88	1,82	0,75	1,50	0,67
70°C 10dk	2,22	1,09	2,03	0,83	1,74	0,75
70°C 20dk	2,36	1,25	2,14	1,13	1,80	1,10

Figure 1. Cream Amount of Black Tea Extracts (g/100g black tea)
Şekil 1. *Siyah çay ekstraktlarının krema miktarı (g/100g siyah çay)*

Govindarajan et al. (2021) examined the tea cream by treating it with different tannase enzyme concentrations (0.05–0.2%). A decline in the cream formation up to 80.2% (0.091 g) in Kangra orthodox tea and 84.25% (0.124 g) in CTC was observed after treating the tannase enzyme at a concentration of 0.1%. Moreover, they found that in 100 ml of the reaction medium 0.390 and 0.520 g of tea cream formation occurred respectively. Together with the previously reported findings, the findings of this research indicated that the tannase enzyme could reduce black tea cream amounts.

3.2. Phenolic compounds of extracts

Total phenolic compounds of black tea extracts obtained by different tea: water ratios, infusion temperatures and times, and tannase enzyme supplementation are given in Figure 2. Total phenolics of samples extracted using the ultrasonic method with different extraction conditions varied between 1.67-3.69 g GAE/100 g dry black tea in the first extracts, and the samples with and without enzyme ranged between 1.84-3.89 g GAE/100 g dry black tea and 1.67-3.85 g GAE/100 g, respectively. The total quantity of phenolic substances in the black tea extracts increased when the infusion temperature and time rose, and the differences between these amounts were significant ($p<0.05$). It was determined that with the increase in the tea ratio in the extraction process, there was a decrease in the total amount of phenolic substances at all infusion temperatures and times, and these quantitative differences were significant ($p<0.05$). It was also discovered that with the increase of the tea: water ratio in the extraction process, in other words, with the decrease in the solvent (water) ratio, the transfer of phenolic substances to the extract decreases, and the yield of extract decreases in absolute terms. In all conditions, by comparison with the

control samples, there was an increase in total phenolics of enzyme-supplemented samples, which was found to be significant ($p<0.05$).

Rusaczonek et al. (2010) indicated that the total amount of phenolic substances in black tea after 5 minutes of infusion with water at boiling temperature at a 1:100 tea: water ratio was 112-151 mg GAE/g. Hanay (2011), using a tea: water ratio of 2.83:250 in Turkish black tea, declared that after 20 minutes of infusion at 90°C, the total phenolic content of the extract was 38.37±0.75 mg/g.

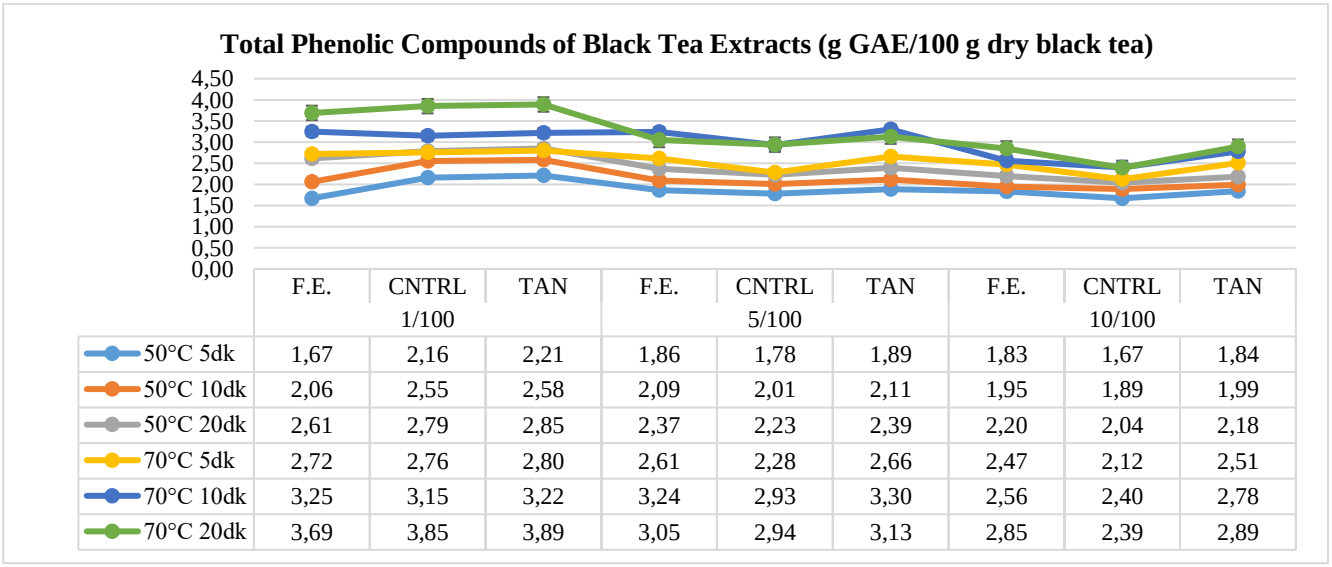
It was noticed that the amounts of total phenolic substance found in the current study are lower than the amounts discovered by other researchers. The differences in the amounts are believed to be related to the distinctions in tea type, harvest season, geographical origin, process, and extraction conditions.

3.3. Catechin amounts of black tea extracts

The most remarkable components of tea are phenolic compounds. The major tea polyphenols are catechins that constitute about 75-80% of the soluble solid fraction (Lu et al., 2009). The main tea catechins are catechin (C), gallocatechin (GC), epicatechingallate (ECG), epicatechin (EC), epigallocatechin (EGC), and epigallocatechingallate (EGCG), and EGCG accounts for more than 50% of phenolic compounds (Li et al., 2017). Catechins provide color, aroma, and taste in tea production. Thus, the astringency and bitterness of tea are due to catechins (Yang et al., 2007).

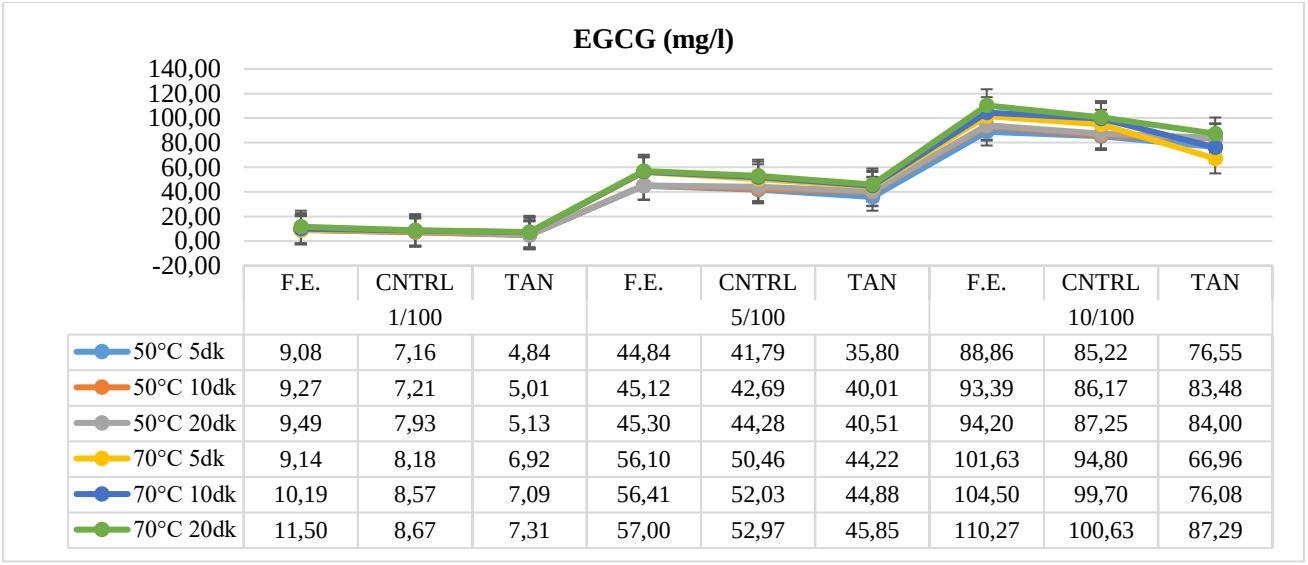
When infusion time is lengthened, chemical changes in the catechins may occur, and catechins' epi-forms can be changed to non-epi forms, called epimerization. The epicatechins existing in tea, which are EC, ECG, EGC, and, EGCG, are called cis-type. They can be transformed into their epimers which are non-epicatechins such as C, CG, GC and GCG. Epimerization, which can also occur at high temperatures, can be reversed. According to Wang et al. (2020), temperature rise resulted in the decline of catechin concentrations while their isomers increased. A diminishing trend in total catechins with the temperature rise demonstrated catechin degradation. Also, it was claimed that tea catechins could be changed to their epimers throughout the brewing, production, and storage processes of conventionally tea infusions and canned tea drinks (Saklar et al., 2015).

Figure 3 presents EGCG values of black tea extracts detected by different tea: water ratios, infusion temperatures and times, and tannase enzyme supplementation. The amount of EGCG in the samples found out by the ultrasonic extraction was 9.08-110.27 mg/l in the first extracts, 4.84-87.29 mg/l and 7.16-100.63 mg/l range in the tannase-supplemented and control samples, respectively.



	F.E.	CNTRL 1/100	TAN	F.E.	CNTRL 5/100	TAN	F.E.	CNTRL 10/100	TAN
50°C 5dk	1,67	2,16	2,21	1,86	1,78	1,89	1,83	1,67	1,84
50°C 10dk	2,06	2,55	2,58	2,09	2,01	2,11	1,95	1,89	1,99
50°C 20dk	2,61	2,79	2,85	2,37	2,23	2,39	2,20	2,04	2,18
70°C 5dk	2,72	2,76	2,80	2,61	2,28	2,66	2,47	2,12	2,51
70°C 10dk	3,25	3,15	3,22	3,24	2,93	3,30	2,56	2,40	2,78
70°C 20dk	3,69	3,85	3,89	3,05	2,94	3,13	2,85	2,39	2,89

Figure 2. Total Phenolic Compounds of Black Tea Extracts (g GAE/100 g dry black tea)
Şekil 2. *Siyah çay ekstraktlarının toplam fenolik madde değerleri (g GAE/100 g kuru siyah çay)*



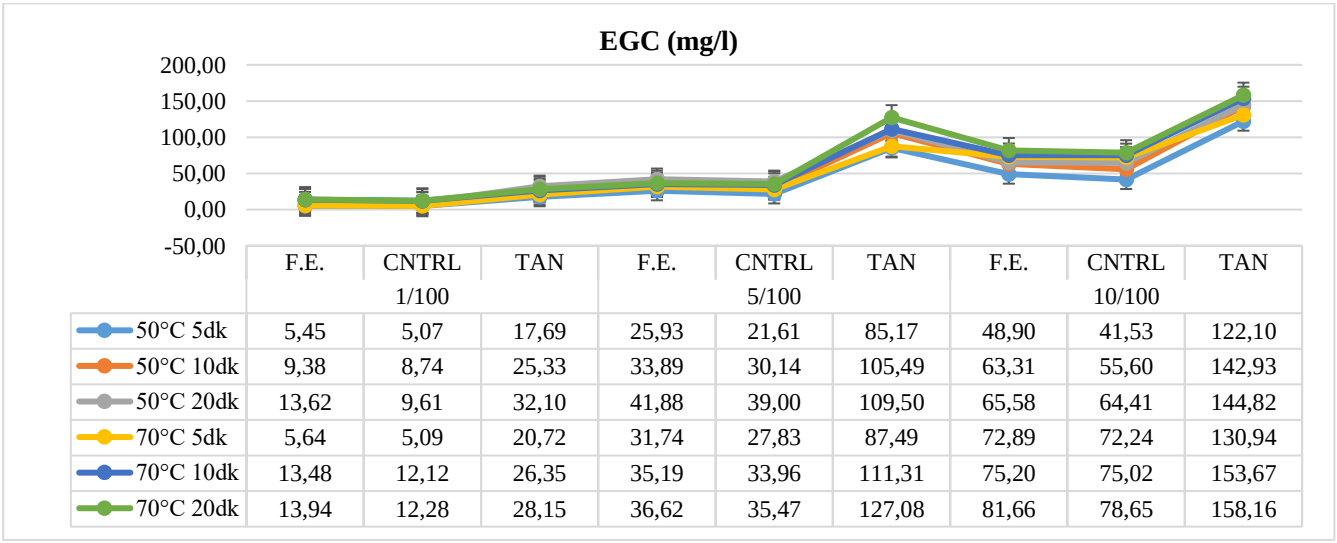
	F.E.	CNTRL 1/100	TAN	F.E.	CNTRL 5/100	TAN	F.E.	CNTRL 10/100	TAN
50°C 5dk	9,08	7,16	4,84	44,84	41,79	35,80	88,86	85,22	76,55
50°C 10dk	9,27	7,21	5,01	45,12	42,69	40,01	93,39	86,17	83,48
50°C 20dk	9,49	7,93	5,13	45,30	44,28	40,51	94,20	87,25	84,00
70°C 5dk	9,14	8,18	6,92	56,10	50,46	44,22	101,63	94,80	66,96
70°C 10dk	10,19	8,57	7,09	56,41	52,03	44,88	104,50	99,70	76,08
70°C 20dk	11,50	8,67	7,31	57,00	52,97	45,85	110,27	100,63	87,29

Figure 3. EGCG amount of black tea extracts (mg/l)
Şekil 3. *Siyah çay ekstraktlarının epigallokateşingallat (EGCG) miktarı (mg/l)*

It is apparent from this figure that the EGCG values of the extracts increased as a result of the increase in the tea: water ratio, infusion temperature and duration, and these increases were significant ($p<0.05$), statistically. With the tannase enzyme application at all tea: water concentrations, infusion temperatures and times, it was discovered that there were drops in the EGCG amounts of the samples, which was significant ($p<0.05$), statistically.

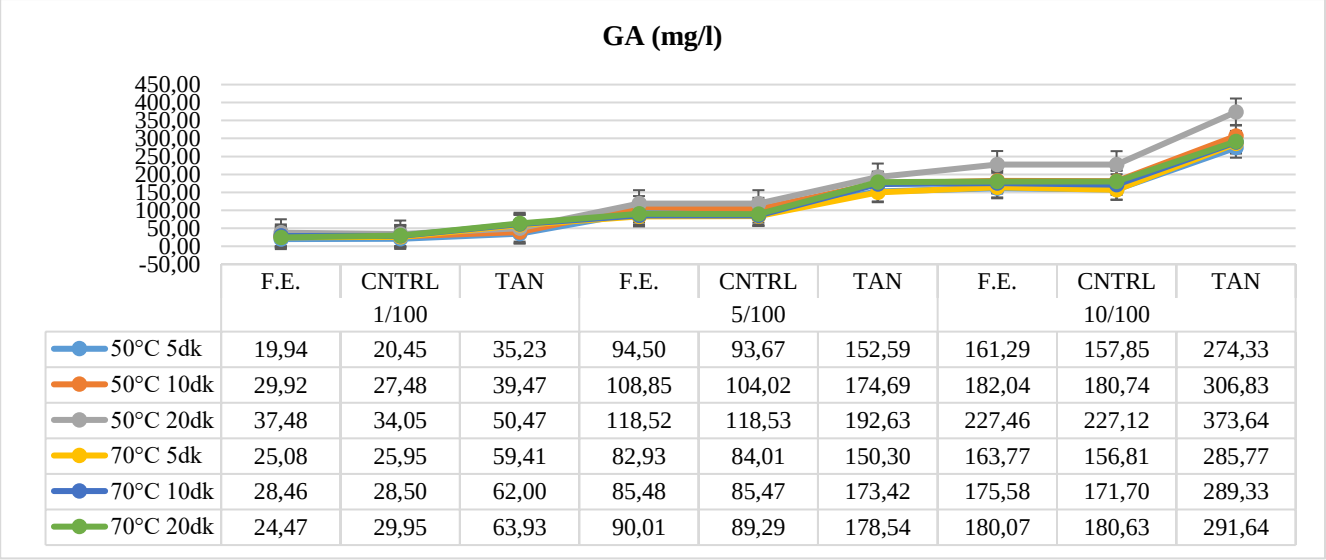
EGC values of black tea extracts obtained by different tea: water ratios, infusion temperatures and times, and tannase enzyme supplementation are presented in Figure 4. The amount of EGC in the samples of the ultrasonic extraction method was between 5.45-81.66 mg/l in the first extracts, 17.69-158.16 mg/l, and 5.07-78.65 mg/l in the samples with and without enzyme treatment, respectively.

The findings also revealed that the tea: water ratio and the infusion temperature had a significant ($p<0.05$) effect on the EGC values of the samples, statistically. An increase in the amount of EGC was detected when the temperature increased. With tannase enzyme application at all different infusion temperatures and times, the EGC values of the samples increased, which was found significant ($p<0.05$).



	F.E.	CNTRL 1/100	TAN	F.E.	CNTRL 5/100	TAN	F.E.	CNTRL 10/100	TAN
50°C 5dk	5,45	5,07	17,69	25,93	21,61	85,17	48,90	41,53	122,10
50°C 10dk	9,38	8,74	25,33	33,89	30,14	105,49	63,31	55,60	142,93
50°C 20dk	13,62	9,61	32,10	41,88	39,00	109,50	65,58	64,41	144,82
70°C 5dk	5,64	5,09	20,72	31,74	27,83	87,49	72,89	72,24	130,94
70°C 10dk	13,48	12,12	26,35	35,19	33,96	111,31	75,20	75,02	153,67
70°C 20dk	13,94	12,28	28,15	36,62	35,47	127,08	81,66	78,65	158,16

Figure 4. EGC amount of black tea extracts (mg/l)
Şekil 4. *Siyah çay ekstraktlarının epigallokateşin (EGC) miktarı (mg/l)*



	F.E.	CNTRL 1/100	TAN	F.E.	CNTRL 5/100	TAN	F.E.	CNTRL 10/100	TAN
50°C 5dk	19,94	20,45	35,23	94,50	93,67	152,59	161,29	157,85	274,33
50°C 10dk	29,92	27,48	39,47	108,85	104,02	174,69	182,04	180,74	306,83
50°C 20dk	37,48	34,05	50,47	118,52	118,53	192,63	227,46	227,12	373,64
70°C 5dk	25,08	25,95	59,41	82,93	84,01	150,30	163,77	156,81	285,77
70°C 10dk	28,46	28,50	62,00	85,48	85,47	173,42	175,58	171,70	289,33
70°C 20dk	24,47	29,95	63,93	90,01	89,29	178,54	180,07	180,63	291,64

Figure 5. GA amount of black tea extracts (mg/l)
Şekil 5. *Siyah çay ekstraktlarının Gallik Asit (GA) miktarı (mg/l)*

Figure 5 illustrates GA values of black tea extracts discovered by different tea: water ratios, infusion temperatures and times, and tannase enzyme supplementation. The amount of GA in the samples obtained by ultrasonic extraction ranged between 9.94-227.46 mg/l in the first extracts, 35.23-373.64 mg/l and 20.45-227.12 mg/l in the tannase-supplemented and control samples, respectively.

Findings revealed that the tea: water ratio, infusion temperature and time had a significant ($p<0.05$) effect on the GA values of the samples, statistically. The GA amounts of the extracts generally increased depending on the change in the tea: water ratios and the increase in the infusion temperature and time, which was significant ($p<0.05$). Moreover, GA amounts of the samples increased with applying tannase enzyme at all infusion temperatures and times, which was significant ($p<0.05$), statistically.

Previous studies revealed that black tea contains less catechins than green tea. In contrast, black tea possesses higher amounts of gallic acid. This situation is likely due to GA conversion from catechin gallates during fermentation (oxidation) of green tea to black tea. During biochemical oxidation, the amount of catechin (EGCG, ECG, EC, C, EGC, GCG) decreased while the amount of gallic acid increased (Gramza et al., 2005).

Türkmen (2007) declared the amount of EGCG in Turkish black teas by ultrasonic extraction (40 min.) as 0.70±0.01 mg/g dry weight. Further, Raghuwanshi et al. (2013) examined the effects of the tannase enzyme (*Penicillium Charlesii*) on the Kangra black tea extract (CTC method), infusion at 85°C for 20 minutes at a ratio of 5:100 tea: water. They found the amount of EGCG in the control and tannase-supplemented samples as 26.54 mg/g tea and 2.83 mg/g tea, respectively. The amount of EGC in control and tannase-supplemented samples was 18.96 mg/g tea and 42.12 mg/g tea, respectively. They also discovered that the amount of GA in control and tannase-supplemented samples was 10.10 mg/g tea and 113.2 mg/g tea.

What is more, Chandini et al. (2011) stated that the amount of EGC in the extract (at a ratio of 2:100 tea: water at 90°C for 40 minutes) using CTC-type black tea supplied from India was 3.69±0.16% in the control sample, 3.82±0.13% and 3.90±0.00%, respectively with 10 and 20 U/g dosage tannase in black tea.

Furthermore, Li et al. (2017) reported that the ratio of tannase to tea was 1:1 (v/w), and there was a decline in EGCG, from 246.5 µg/ml to 153.9 µg/ml. Yet, an increase was observed in gallic acid, from 21.9 µg/ml to 83.9 µg/ml. After treating inferior Tieguany in oolong tea leaves with tannase, EGCG was minimized by 37.6% .

As a result of the enzymatic treatment, a decrease in the gallated catechins (EGCG), and an increase in ungallated catechins (GA, and EGC) were detected. According to the previous findings of Chandini et al. (2011), after tannase split the ester bonds in ECG and EGCG, the transformation of EGCG to EGC and GA, hydrolyzation of GCG to GC and GA, and degradation of

ECG to EC and GA occurred. The increases in EGC amounts with the use of tannase enzyme in the present study are quite similar to the increases in EGC amounts and decreases in EGCG found by other researchers.

It was also reported by Saklar et al. (2015) that various factors affect the catechin contents of different tea cultivars such as type of tea, age of tea leaves, harvesting seasons and conditions, climate, cultivation practices, drying, and technological processes during tea production.

4. Results

In conclusion, the most significant finding to emerge from this study is that applying the tannase enzyme contributed to the reduction of cream formation, which is one of the problems in RTD tea production. The results indicated that the amount of cream in black tea extracts obtained by ultrasonic extraction decreased by 38.89-59.11% with the tannase treatment. At the same time, thanks to the reduction in cream formation with the application of tannase enzyme, it is seen that products with higher functional properties can be produced thanks to the fact that phenolic substances, especially catechins, which are the functional components of tea extracts, remain in the tea extract. It is hoped that the findings in the present study will make noteworthy contributions to the literature and serve as a base for future and more comprehensive studies.

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