

## RESEARCH ARTICLE

# Effects of ultrasound-assisted extraction procedure on total phenolics, catechin and caffeine content of green tea extracts

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## ABSTRACT

This study was conducted to investigate the changes encountered in water soluble dry matter (<sup>o</sup>Brix), total phenolics, caffeine and some important catechins of green tea extracts extracted through ultrasound-assisted extraction procedures. Extractions were conducted at 70°C temperature, different tea: water ratios (5:100; 10:100) and brewing times (5, 10 and 20 min) and resultant extracts were also supplemented with tannase enzyme. Total phenolics of green tea extracts varied between 2.68 - 3.87 g GAE/100 g dry green tea. The greatest total phenolics (3.87 g GAE/100 g dry green tea) was obtained from 20 min brewing at 70°C and 10:100 tea: water ratio of tannase enzyme-supplemented extract. As compared to the control samples, decreasing EGCG and ECG contents and increasing EGC and EC contents were observed with tannase enzyme supplementations.

**Keywords:** Catechin; Green tea; Phenolics; Tannase; Ultrasonic extraction

## INTRODUCTION

Production and extraction processes are the first issues to be taken into consideration while assessing bioactive compounds of green tea (Lante and Friso, 2013). In conventional extraction, solubility and mass transfer ratio of tea compounds are tried to be increased through heating or boiling. It was reported in previous studies that increasing temperatures during heating resulted in epimerization of tea catechins and alter chemical structure of catechins. Tea volatile components and majority of polyphenols get into unstable state and are subjected to alteration and reductions, then negative changes are encountered in taste, color and aroma of the tea (Xia et al., 2006; Lante and Friso, 2013). Although cold-extraction may improve chemical and sensory quality of green tea, the process is a long-term and low-impact process, it is not suitable for industrial tea production. The primary objective in tea production is to achieve a high extraction efficiency and a sensory quality. Primary expectations from extraction process include high yields and extraction of compounds at shorter periods and lower temperatures (Xia et al., 2006; Shao et al., 2020).

In ultrasound-assisted extraction, as an alternative of conventional extraction, extraction can be carried out at lower temperatures, thus heat-induced degradations are not encountered and volatile compounds are not removed from the ambient through boiling. It has been suggested that the improvement of solvent extraction from plant material by ultrasound is due mainly to the mechanical effects of acoustic cavitation, which enhances both solvent penetration into the plant material and the intracellular product release by disrupting the cell walls (Pico, 2013). Ultrasound present several advantages in terms of shortening the time of the process, decreasing the volume of solvent and increasing the yield of the extract in comparison with conventional methods (Fan et al., 2022). Both et al., (2014) indicated that the ultrasound-assisted extraction increases the content of polyphenols approximately 15% in comparison with the conventional method in black tea. Mason et al., (1996) indicated that the use of ultrasound improves the extraction of tea solids from leaves at 60°C by nearly 20%.

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Polyphenols have the greatest role in widespread consumption of green tea as a healthy beverage worldwide. Dry matter weight of green tea leaves is composed of 15-30% polyphenols including flavanols, flavonoids and phenolic acids (Balci and Özdemir, 2016). Catechines are the most common flavonoids of green tea and they are gallic acid derivatives (Naidu, 2000; Balci and Özdemir, 2016). Catechins are colorless and water-soluble compounds giving bitter harsh taste to green tea. Chemically, they are flavan-3-ol (Wang et al., 2000). Major tea catechins, constituting basic phenolic building stones of green tea, include (-)-epigallocatechingallate (EGCG), (-)-epicatechingallate (ECG), (-)-epigallocatechin (EGC) and (-)-epicatechin (EC). Minor tea catechins are epimers of major tea catechins including (-)-gallocatechingallate (GCG), (-)-catechingallate (CG), (-)-gallocatechin (GC) and (±)-catechin (C) (Perumalla and Hettiarachchy, 2011; Ghasemzadeh-Mohammadi et al., 2017; Shao et al., 2020).

Green tea catechin quantities vary with the chemical composition of tea leaves, type of tea, geographical origin, climate, environmental conditions, harvest season, processing methods, storage conditions and times (Sağlam and Türkyılmaz, 2007; Türkmen, 2007). Khokhar and Magnusdottir (2002) and Zaveri (2006) indicated that EGCG constituted about 60% of total phenolics of tea and it was respectively followed by EGC, ECG, EC, CG, GC, and C (Perva-Uzunalic et al., 2006; Hong et al., 2020).

Tannin acyl hydrolase (EC3.1.1.20), generally named tannase, tannase (EC 3.1.1.20) is often used to catalyze the hydrolysis of gallated catechins (EGCG, (-) - (GCG) and ECG), resulting in non-gallated catechins ((-)-(EGC), (-)-(GC) and (-)-(EC)) and gallic acid (GA) (Zhang et al., 2016; Govindarajan et al., 2021). Tannase is an industrial enzyme with extensive applications in preparing instant tea, soft drinks, wine to lower the tea cream and to improve color appearance (Lekshmi et al., 2021; Shakir et al., 2022).

In present study, green tea extracts were obtained through ultrasound-assisted extraction at different brewing times and tea: water ratios and changes in total phenolics, catechins and caffeine quantities of resultant extracts were investigated.

## MATERIALS AND METHODS

### Materials

Green tea samples used in present experiments were supplied from Rize Karaali Tea Factory, which is

among the largest tea factories of Turkey. Dry green tea samples were stored at room temperature in dark storages. Distilled water was used as solvent. The tannase enzyme (activity = 500 U/g or greater; optimum pH = 5.0 - 5.5, temperature = 40 °C) used in present experiments was supplied from Kikkoman Co., Japan.

### Methods

#### Green tea extraction process

Elma Sonic- S100H brand ultrasonic water bath operating at 37 Khz frequency was used for extraction of green tea. During the extraction process at 70°C brewing temperature;

1. Different brewing times (5, 10 and 20 min),
2. Different tea: water ratios (5:100 and 10:100) were used.

Based on specified tea: water ratios (5:100 and 10:100), tea samples were weighed and placed into beakers, then 70 °C distilled brewing water was added and mixed with a glass stirrer for 30 seconds. Mixtures were left for brewing in ultrasonic water bath for different times (5, 10 and 20 minutes). At the end of specified brewing times, samples were filtered through double-layer simple filter papers. Resultant extracts (First Extract (F.E.)) were placed into centrifuge tubes at equal volumes (40 ml) and supplemented then with 1.25 U/g enzyme solution. Enzyme-supplemented samples were instantly kept in water bath at 40°C (optimum operational temperature of the enzyme) for an hour. Following an hour of incubation, samples were initially subjected to pre-cooling process for 5 minutes, then kept in water bath at 2°C for 2 hours for enzyme inactivation. At the end of relevant periods, sample tubes were centrifuged at -2°C and 9000 rpm for 20 minutes. Clear and creamy portions were then separated (Fig. 1). Resultant clear green tea extracts were subjected to following analyses. At the same time, the samples prepared without the supplemented of tannase were named as Control (CNTRL) samples and were subjected to the same analyzes.

#### Applied analyses

Following analyses were applied to green tea samples and/or green tea extracts obtained under different extraction conditions.

#### Water soluble dry matter

Water soluble dry matter content of green tea extracts were determined with the use of Hanna HI 96801 (Romania) brand digital refractometer and results were expressed in °Brix (Cemeroğlu, 2010).

### Total phenolics

Total phenolics of green tea samples and tea extracts were determined in accordance with ISO 14502-1 (Anonymous, 2005). Results were expressed in gallic acid equivalent (g GAE/100 g dry green tea).

### Catechin composition of green tea extracts and determination of caffeine contents with HPLC

HPLC device was used to determine individual catechins (EGC, EC, EGCG, and ECG) and caffeine content of green

tea extracts (Modified from Liang et al., 2002). Green tea extracts filtered through 0.45 µm membrane filters and injected into HPLC device (Perkin Elmer Series-200). Operational conditions were as follows: Two mobile phases were used. Mobile phase A: Trichloroacetic Acid (TCA): acetonitrile: water (0.15 TCA; 5% acetonitrile) and Mobile phase B: acetonitrile: TCA (0.1% TCA), analysis duration was 40 minutes, column (5 µm, C18 (4.6mm x 250mm)) temperature was 25°C, wavelength was 280 nm. Results were calculated from standard graphs generated for each compound and expressed in mg/L.

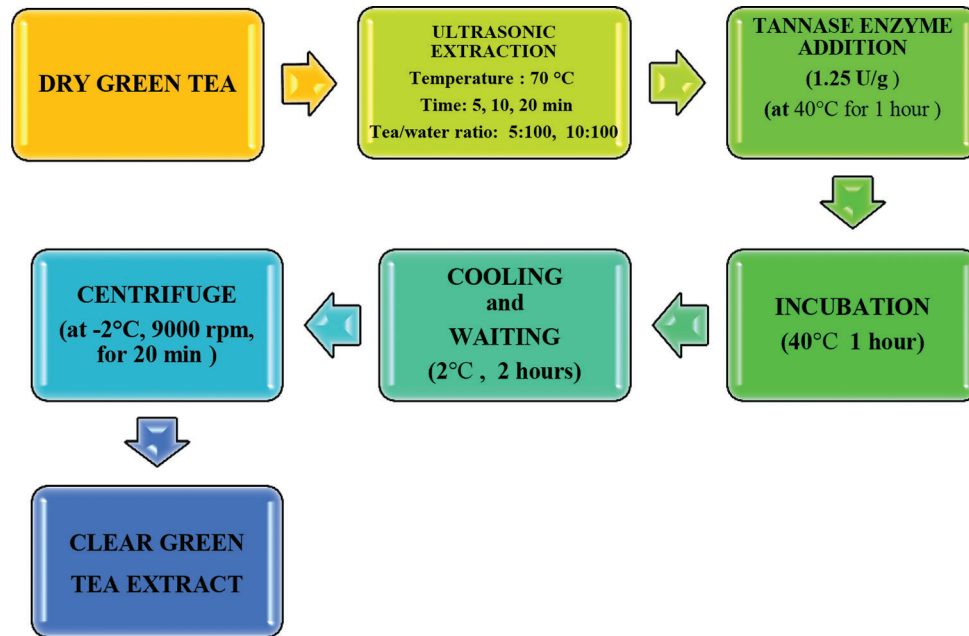


Fig 1. Flow chart for green tea extraction.

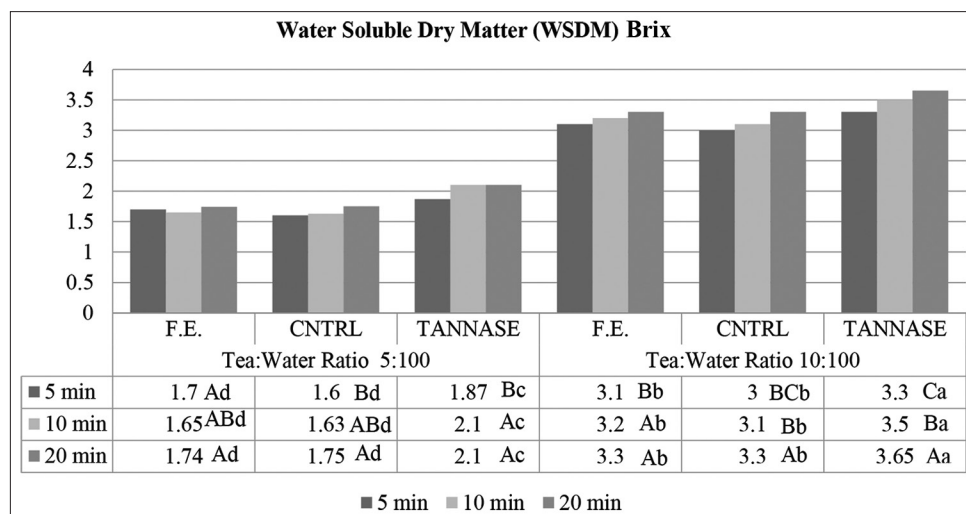


Fig 2. WSDM values of green tea extracts (F.E: First Extract; CTRL: Control Samples; TANNASE: Tannase Supplemented Samples). The difference between the means shown with the same letter in the same row or column is not statistically significant ( $p > 0.05$ ). Different lowercase letters shown in the same line indicate the statistical difference between the WSDM values of the first extract, control, and enzyme-treated samples using different tea: water ratios. Different capital letters shown in the same column show the statistical difference between the WSDM values of green tea extracts obtained with different brewing time applications using the same tea: water ratios.

## Statistical analyses

Experimental data were subjected to analysis of variance in accordance with factorial experimental design with the use of Statistical Package for the Social Sciences (SPSS) statistical software. Significant means were compared with the use of Duncan's multiple range test at 0.05 significance level (Yıldız and Bircan, 1994).

## RESULTS AND DISCUSSION

### Water soluble dry matter (WSDM) content of green tea extracts

WSDM values of green tea extracts obtained through ultrasonic extraction method under different extraction conditions are presented in Fig. 2. WSDM contents of green tea extracts varied between 1.6 - 3.65 °Brix.

WSDM values increased with increasing tea: water ratios and such increases were found to be significant ( $p < 0.05$ ). In all tea: water ratios, increases were observed in WSDM contents of enzyme-supplemented samples, such increases and differences from the control samples were found to be significant ( $p < 0.05$ ).

It is thought that the increase in WSDM may be caused by the hydrolysis of gallated catechins with large molecular structure to catechins with small molecule structure after the use of tannase.

### Total phenolics of green tea extracts

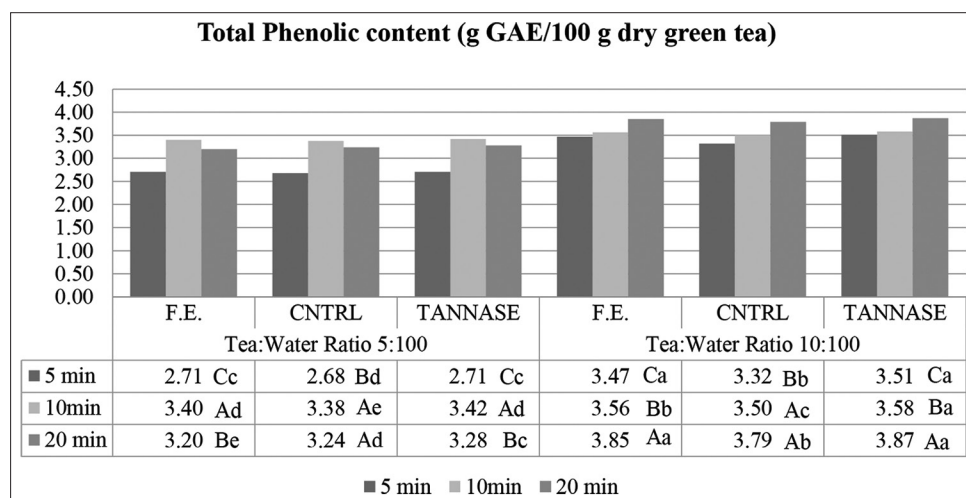
Changes encountered in total phenolics of green tea extracts are presented in Fig. 3. Total phenolics of green tea samples extracted through ultrasonic method under

different extraction conditions varied between 2.68 - 3.87 g GAE/100 g dry green tea. The greatest total phenolics was obtained from 10:100 tea: water ratio of 20 minutes extraction time of tannase enzyme supplemented samples.

The difference between the means shown with the same letter in the same row or column is not statistically significant ( $p > 0.05$ ). Different lowercase letters shown in the same line indicate the statistical difference between the Total Phenolic content values of the first extract, control, and enzyme-treated samples using different tea: water ratios. Different capital letters shown in the same column show the statistical difference between the Total Phenolic content values of green tea extracts obtained with different brewing time applications using the same tea: water ratios.

Total phenolics generally increased in all brewing times with increasing tea ratio of the extraction process and such differences were found to be significant ( $p < 0.05$ ). As compared to the control samples, increases were observed in total phenolics of enzyme-supplemented samples and such differences were also found to be significant ( $p < 0.05$ ).

Shao et al., 2020 reported that while the total phenolics contents by Folin-Ciocalteu Method of green tea extract was  $137 \pm 1$  g/kg, it increased  $291 \pm 5$  g/kg with the addition of tannase enzyme. Balcı and Özdemir (2016) investigated the effects of different extraction temperatures (75, 85 and 95 °C) and different extraction times (3, 5, 10, 15, 20 min.) on bioactive compounds of Turkish green tea and reported total phenolics of green tea as between 6.81 - 13.13 g GAE/100g dw, also reported that total phenolics increased with increasing extraction temperatures



**Fig 3.** Total phenolics of green tea extracts (g GAE/100 g dry green tea) (F.E: First Extract; CTRL: Control Samples; TANNASE: Tannase Supplemented Samples). The difference between the means shown with the same letter in the same row or column is not statistically significant ( $p > 0.05$ ). Different lowercase letters shown in the same line indicate the statistical difference between the WSDM values of the first extract, control, and enzyme-treated samples using different tea: water ratios. Different capital letters shown in the same column show the statistical difference between the WSDM values of green tea extracts obtained with different brewing time applications using the same tea: water ratios.



and times and the greatest total phenolics was obtained from 95°C extraction temperature and 20 min extraction time. It was reported that total flavonoid and phenolics increased with increasing extraction time and temperatures, then antioxidant capacity increased accordingly. It was reported in a previous study that total phenolics of green tea varied between 114.2 - 271.0 mg GAE g<sup>-1</sup> dw (Balci and Özdemir, 2016). Khokhar and Magnusdottir (2002) conducted extraction of different tea types and reported total phenolics of green tea extracts as between 6.58 – 10.62 g GAE/100g dw.

It is seen that the total phenolic substance amounts determined in our study are lower than the phenolic amounts determined by other researchers. In this difference in the amount of phenolic substances; it is thought that differences in extraction conditions, tea type, geographical origin, process conditions and harvest season may be effective.

### Caffeine and catechin quantities of green tea extracts

Caffeine and catechins (EGCG, ECG, EGC and EC) of green tea extracts obtained through ultrasonic extraction method under different extraction conditions were determined and results are presented below.

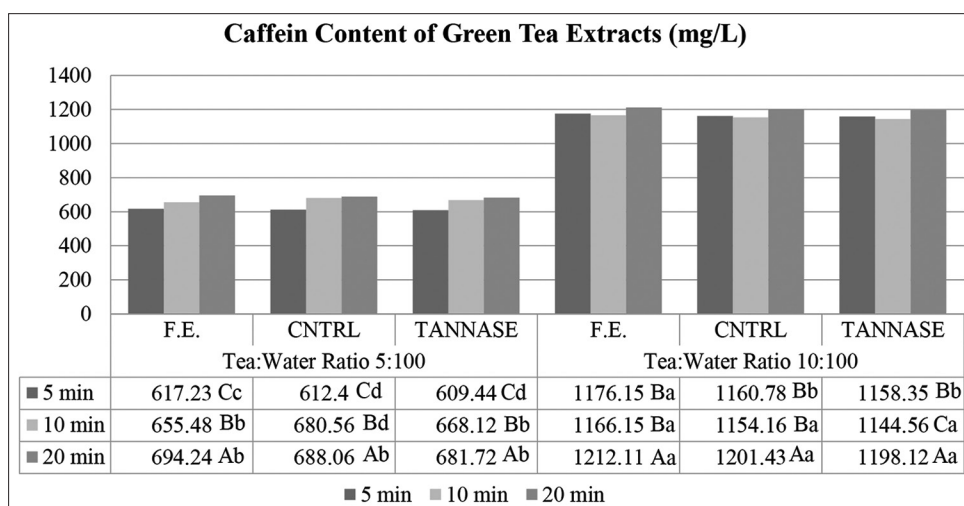
### Caffeine content of green tea extracts

Changes encountered in caffeine contents of green tea extracts under different extraction conditions are presented in Fig. 4. Caffeine contents of the samples obtained through ultrasonic extraction varied between 609.44 - 1212.11 mg/L. Sample caffeine contents increased with increasing tea: water ratios and brewing times and such increases were found to be significant ( $p < 0.05$ ).

The difference between the means shown with the same letter in the same row or column is not statistically significant ( $p > 0.05$ ). Different lowercase letters shown in the same line indicate the statistical difference between the Caffeine Content values of the first extract, control, and enzyme-treated samples using different tea: water ratios. Different capital letters shown in the same column show the statistical difference between the Caffeine Content values of green tea extracts obtained with different brewing time applications using the same tea: water ratios.

The greatest caffeine content (1212.11 mg/L) was obtained from 10:100 tea: water ratio at 70°C for 20 minutes extraction process. Generally decreasing caffeine contents were observed with tannase enzyme additions, but the differences in caffeine contents of enzyme-supplemented and control samples were not found to be significant ( $p > 0.05$ ).

Hong et al. (2020) reported in their study that while the caffeine content in green tea extract was  $18.29 \pm 0.98$  (mg/g), it provided a decrease of  $14.95 \pm 0.85$  mg/g with the addition of tannase enzyme. Shao et al., 2020 reported that while the caffeine content of green tea extract was  $600 \pm 9$  mg/L, it increased  $612 \pm 8$  mg/L with the addition of tannase enzyme. Xu et al. (2019) reported that the green tea extracts (85 °C, 50 g/L tea: water ratio, 30 min) with ultrasonic method and the addition of tannase, the caffeine value was 17.00 mg/g DW, while it was 17.59 mg/g DW with only tannase application. The same value was found 16.60 mg/g DW when only ultrasonic method was used. Researchers indicated that the caffeine were changed slightly with the use of ultrasound or tannase. Hong et al. (2014) investigated physical stability of green tea extracts



**Fig 4.** Caffeine content of green tea extracts (mg/L) (F.E: First Extract; CTRL: Control Samples; TANNASE: Tannase Supplemented Samples). The difference between the means shown with the same letter in the same row or column is not statistically significant ( $p > 0.05$ ). Different lowercase letters shown in the same line indicate the statistical difference between the WSDM values of the first extract, control, and enzyme-treated samples using different tea: water ratios. Different capital letters shown in the same column show the statistical difference between the WSDM values of green tea extracts obtained with different brewing time applications using the same tea: water ratios.

supplemented with 5% tannase enzyme and reported that 55.6 mg/g caffeine content decreased to 51.5 mg/g with enzyme supplementation. Authors also indicated that the relative amounts of caffeine were not changed after tannase treatment. Labbe et al. (2006) investigated the effects of brewing temperature and time on solubility of green tea catechins and reported the best extraction conditions for green tea extracted at 1:50 tea: water ratio as 70-80 °C and 20-40 minutes. It was also reported that caffeine solubility slightly increased with increasing brewing temperature and caffeine concentration merely influenced by brewing temperature. Perva-Uzunalic et al. (2006) conducted a study on extraction efficiency for caffeine and catechins of green tea and reported caffeine content as 36 g/kg DM and caffeine extraction efficiency as between 61-64% when 85°C water was used in extractions. Khokhar and Magnusdottir (2002) reported caffeine contents of green tea extracts as between 11-20 mg/g dry matter.

### EGC content of green tea extracts

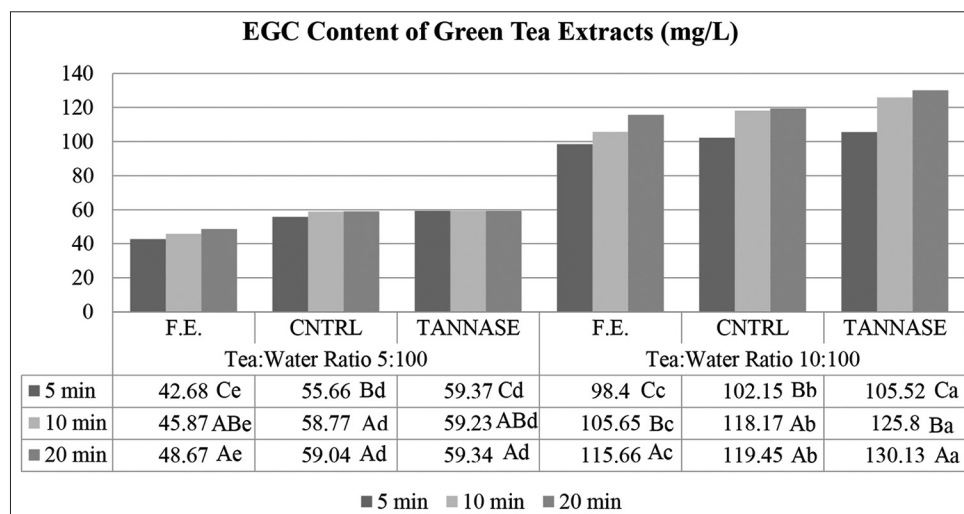
Changes encountered in EGC contents of green tea extracts are presented in Fig. 5. EGC contents of the samples obtained through ultrasonic extraction varied between 42.68-130.13 mg/L.

The difference between the means shown with the same letter in the same row or column is not statistically significant ( $p>0.05$ ). Different lowercase letters shown in the same line indicate the statistical difference between the EGC values of the first extract, control, and enzyme-treated samples using different tea: water ratios. Different capital letters shown in the same column show the statistical difference between the EGC values of green tea extracts

obtained with different brewing time applications using the same tea: water ratios.

Increasing EGC contents were observed with increasing tea: water ratios and brewing times the differences in EGC contents of the samples were found to be significant ( $p<0.05$ ). Increasing EGC contents were observed with tannase enzyme supplementation to green tea extracts obtained at different tea: water ratios and brewing times and such increases were found to be significant ( $p<0.05$ ).

Shao et al. (2020) reported that while the EGC content of green tea extract was  $361 \pm 5$  mg/L, it increased  $1951 \pm 5$  mg/L with the addition of tannase enzyme. Cao et al. (2019) stated that the EGC concentration increased from 0.50 mg/ml to 1.29 mg/ml with the addition of 0.5% tannase in autumn green tea extracts. Xu et al. (2019) reported that the green tea extracts (85 °C, 50 g/L tea: water ratio, 30 min) with ultrasonic method and the addition of tannase, the EGC value was 2.02mg/g DW, while it was 2.07mg/g DW with only tannase application. The same value was found 0.29mg/g DW when only ultrasonic method was used. Ong and Annuar (2017) reported that while the EGC content of green tea extract (0.25 g/100 ml, 80 °C, 5 min) was  $136.6 \pm 3.1$  (mg/g), it increased  $397.7 \pm 1.1$  mg/g with the addition of tannase enzyme. Balcı and Özdemir (2016) investigated the effects of different extraction temperatures (75, 85 and 95 °C) and different extraction times (3, 5, 10, 15, 20 min.) on bioactive compounds of Turkish green tea and reported EGC contents as between 28.03 - 59.42 mg/g dw. Zhang et al., (2016) indicated that EGC concentrations increased 2.12 mmol/L to 5.29 mmol/L in green tea extracts (1:30 tea:



**Fig 5.** EGC contents on green tea extracts (mg/L) (F.E: First Extract; CNTRL: Control Samples; TANNASE: Tannase Supplemented Samples). The difference between the means shown with the same letter in the same row or column is not statistically significant ( $p>0.05$ ). Different lowercase letters shown in the same line indicate the statistical difference between the WSDM values of the first extract, control, and enzyme-treated samples using different tea: water ratios. Different capital letters shown in the same column show the statistical difference between the WSDM values of green tea extracts obtained with different brewing time applications using the same tea: water ratios.

water ratio, 80 °C, and 15 min) supplemented with tannase enzyme (1:5000 w/v). Hong et al. (2014) investigated physical stability of green tea extracts supplemented with 5% tannase enzyme and reported that 1557.9 mg/g EGC content increased to 5284.4 mg/g with enzyme supplementation. Lu et al. (2009) reported increasing EGC concentrations of green tea supplemented with tannase enzyme at different concentrations due to enzymatic hydrolysis. Labbe et al. (2006) reported that solubility kinetics of catechins varied based on brewing time and temperature and achieved the best EGC yield at 50°C and 20- 40 minutes brewing times.

It is seen that the increases in EGC amounts determined by the use of tannase enzyme in the study are similar to the increases in EGC amounts determined by other researchers. In this increase in EGC amounts; it is thought that tannase enzyme hydrolyzes EGCG, which has a large molecular structure, to EGC and GA, which has a smaller molecule.

#### EGCG contents of green tea extracts

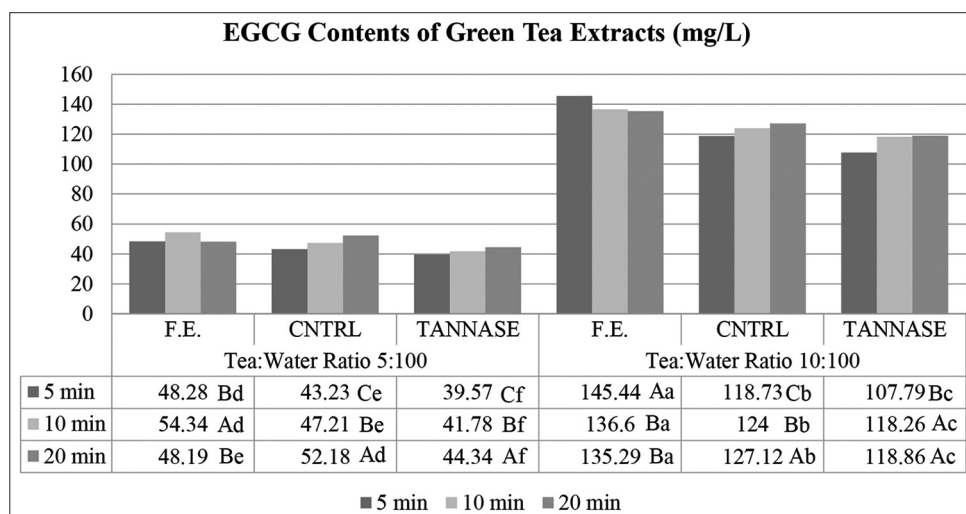
Changes encountered in EGCG contents of green tea extracts are presented in Fig. 6. EGCG contents of samples extracted through ultrasonic extraction varied between 39.57-145.44 mg/L.

The difference between the means shown with the same letter in the same row or column is not statistically significant ( $p>0.05$ ). Different lowercase letters shown in the same line indicate the statistical difference between the EGCG values of the first extract, control, and enzyme-treated samples using different tea: water ratios. Different

capital letters shown in the same column show the statistical difference between the EGCG values of green tea extracts obtained with different brewing time applications using the same tea: water ratios.

Decreasing EGCG contents were observed in tannase enzyme-supplemented green tea extracts. The differences in EGCG contents of tannase supplemented samples and the samples obtained at 5:100 and 10:100 tea: water ratios were found to be significant ( $p<0.05$ ).

Cao et al. (2019) reported that the EGCG concentration decreased from 1.47 mg/ml to 0.25 mg/ml with the addition of 0.5% tannase in autumn green tea extracts. Xu et al. (2019) reported that the green tea extracts (85 °C, 50 g/L tea: water ratio, 30 min) with ultrasonic method and the addition of tannase, the EGCG value was 0.16 mg/g DW, while it was 0.16 mg/g DW with only tannase application. The same value was found 11.55 mg/g DW when only ultrasonic method was used. Ong and Annuar (2017) reported that while the EGCG content of green tea extract (0.25 g/100 ml, 80 °C, 5 min) was  $67.3 \pm 0.7$  (mg/g), it decreased  $0.7 \pm 0.1$  mg/g with the addition of tannase enzyme. Balci and Özdemir (2016) investigated the effects of different extraction temperatures (75, 85 and 95 °C) and different extraction times (3, 5, 10, 15, 20 min.) on bioactive compounds of Turkish green tea and reported EGCG contents as between 38.05-69.66 mg/g dw. Wang and Lee (2016) indicated optimum extraction conditions of green tea for EGCG content as 40 °C and 10 minutes and reported that EGCG content primarily changed with the temperature. Zhang et al. (2016) indicated that



**Fig 6.** EGCG contents of green tea extracts (mg/L) (F.E: First Extract; CTRL: Control Samples; TANNASE: Tannase Supplemented Samples). The difference between the means shown with the same letter in the same row or column is not statistically significant ( $p>0.05$ ). Different lowercase letters shown in the same line indicate the statistical difference between the WSDM values of the first extract, control, and enzyme-treated samples using different tea: water ratios. Different capital letters shown in the same column show the statistical difference between the WSDM values of green tea extracts obtained with different brewing time applications using the same tea: water ratios.

EGCG concentrations decreased 3.21 mmol/L to 0.04 mmol/L in green tea extracts (1:30 tea: water ratio, 80 °C, and 15 min) supplemented with tannase enzyme (1:5000 w/v). Baik et al. (2015) indicated that degallated catechins of tea extracts significantly increased, but EGCG content decreased with tannase and pectinase enzyme supplementations. Hong et al. (2014) investigated physical stability of green tea extracts supplemented with 5% tannase enzyme and reported that 3990.9 mg/g EGCG content decreased to 3.3 mg/g with enzyme supplementation. Lu et al. (2009) reported decreasing EGCG concentrations of green tea supplemented with tannase enzyme at different concentrations due to enzymatic hydrolysis. Labbe et al. (2006) reported EGCG content at 50 °C brewing temperature and 5, 20 and 40 min brewing times respectively as 15.5, 245.3 and 489.3 µg/ml, the values at 90 °C brewing temperature and 5, 10 and 40 minutes brewing times were respectively reported as 489.6, 763.8 and 1071.4 µg/ml.

It is seen that the decreases in EGCG amounts determined by the use of tannase enzyme in the study are similar to the decreases in EGCG amounts determined by other researchers. This decrease in EGCG amounts; it is thought that tannase enzyme converts EGCG, which has a large molecular structure, to non-hydrolysed catechins with smaller molecules.

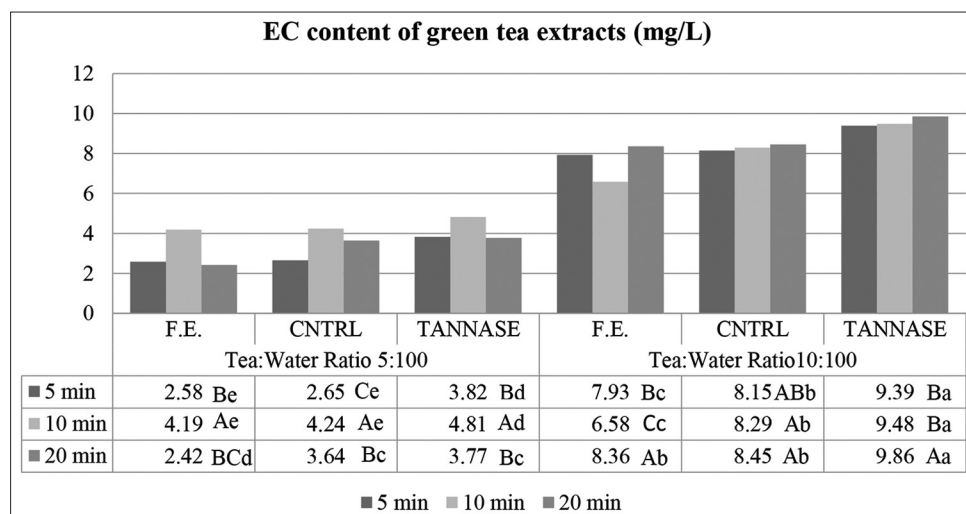
### EC content of green tea extracts

Changes encountered in EC contents of green tea extracts are presented in Fig. 7. EC contents of samples obtained through ultrasonic extraction under different extractions conditions varied between 2.42 - 9.86 mg/L.

The difference between the means shown with the same letter in the same row or column is not statistically significant ( $p>0.05$ ). Different lowercase letters shown in the same line indicate the statistical difference between the EC values of the first extract, control, and enzyme-treated samples using different tea: water ratios. Different capital letters shown in the same column show the statistical difference between the EC values of green tea extracts obtained with different brewing time applications using the same tea: water ratios.

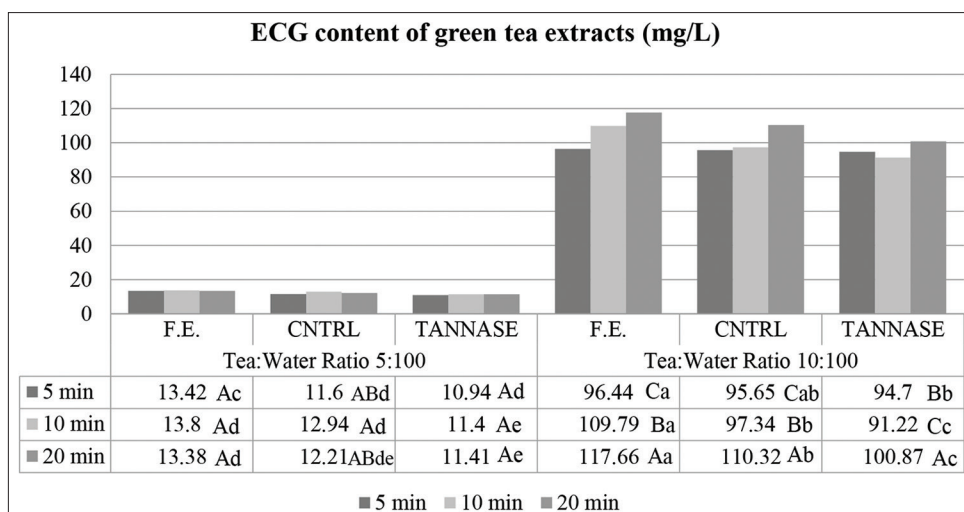
EC contents of the green tea extracts generally increased with changing tea: water ratios and increasing brewing times and the differences in EC contents of the samples were found to be significant ( $p<0.05$ ). Increasing EC contents were observed with tannase enzyme supplementations and such increases were also found to be significant ( $p<0.05$ ).

Hong et al. (2020) reported that while the EC content of green tea extract was  $61.34 \pm 0.82$  (mg/g), it increased  $106.20 \pm 1.43$  mg/g with the addition of tannase enzyme. Shao et al. (2020) reported that while the EC content of green tea extract was  $130 \pm 3$  mg/L, it increased  $878 \pm 14$  mg/L with the addition of tannase enzyme. Cao et al. (2019) reported that the EC concentration increased from 0.20 mg/ml to 0.34 mg/ml with the addition of 0.5% tannase in autumn green tea extracts. Xu et al. (2019) reported that the green tea extracts (85 °C, 50 g/L tea: water ratio, 30 min) with ultrasonic method and the addition of tannase, the EC value was 1.96 mg/g DW, while it was 2.12 mg/g DW with only tannase application. The same value was found 0.70mg/g DW when only ultrasonic method was used. Ong and Annur (2017) reported that while the EC content of



**Fig 7.** EC content of green tea extracts (mg/L) (F.E: First Extract; CNTRL: Control Samples; TANNASE: Tannase Supplemented Samples). The difference between the means shown with the same letter in the same row or column is not statistically significant ( $p>0.05$ ). Different lowercase letters shown in the same line indicate the statistical difference between the WSDM values of the first extract, control, and enzyme-treated samples using different tea: water ratios. Different capital letters shown in the same column show the statistical difference between the WSDM values of green tea extracts obtained with different brewing time applications using the same tea: water ratios.





**Fig 8.** ECG content of green tea extracts (mg/L) (F.E: First Extract; CNTRL: Control Samples; TANNASE: Tannase Supplemented Samples). The difference between the means shown with the same letter in the same row or column is not statistically significant ( $p>0.05$ ). Different lowercase letters shown in the same line indicate the statistical difference between the WSDM values of the first extract, control, and enzyme-treated samples using different tea: water ratios. Different capital letters shown in the same column show the statistical difference between the WSDM values of green tea extracts obtained with different brewing time applications using the same tea: water ratios.

green tea extract (0.25 g/100 ml, 80 °C, 5 min) was  $27.1 \pm 0.6$  (mg/g), it increased  $61.5 \pm 0.2$  mg/g with the addition of tannase enzyme. Zhang et al. (2016) indicated that EC concentrations increased 1.71 mmol/L to 3.49 mmol/L in green tea extracts (1:30 tea: water ratio, 80 °C, and 15 min) supplemented with tannase enzyme (1:5000 w/v). Wang and Lee (2016) tried to determine optimum extraction parameters and extracted five components of green tea. Optimum extraction conditions were reported as; 5 min at 20°C for caffeine, 15 min at 30°C for catechin, 10 min at 40°C for EGCG, 15 min at 40°C for EC. EGCG concentrations were primarily influenced by temperature and EC concentrations were influenced both by extraction time and temperatures. Ziadini (2010) indicated decreasing EGC and EC concentrations of tea extracts when the extraction temperature was increased from 80°C to 90 °C. Decreasing EGC and EC concentrations with increasing temperatures were attributed to degradation, oxidation or epimerization of catechins (Balci and Özdemir, 2016).

It is seen that the increases in EC amounts determined by the use of tannase enzyme in the study are similar to the increases in EC amounts determined by other researchers. In this increase in EC amounts; it is thought that tannase enzyme hydrolyzes ECG, which has a large molecular structure, to EC and GA, which has a smaller molecule.

### ECG content of green tea extracts

Changes encountered in ECG contents of green tea extracts are presented in Fig. 8. ECG contents of samples extracted through ultrasonic extraction method varied between 10.94 - 117.66 mg/L.

The difference between the means shown with the same letter in the same row or column is not statistically significant ( $p>0.05$ ). Different lowercase letters shown in the same line indicate the statistical difference between the ECG values of the first extract, control, and enzyme-treated samples using different tea: water ratios. Different capital letters shown in the same column show the statistical difference between the ECG values of green tea extracts obtained with different brewing time applications using the same tea: water ratios.

Increasing ECG contents were observed with increasing tea: water ratios of the extracts and differences in ECG contents of the samples were found to be significant ( $p<0.05$ ). Tannase enzyme supplementations to green tea extracts under different tea: water ratios and brewing times reduced ECG contents and such decreases were also found to be significant ( $p<0.05$ ).

Hong et al. (2020) reported that while the ECG content of green tea extract was  $185.00 \pm 1.2$  (mg/g), it increased  $511.73 \pm 1.8$  mg/g with the addition of tannase enzyme. Xu et al. (2019) reported that the green tea extracts (85 °C, 50 g/L tea: water ratio, 30 min) with ultrasonic method and the addition of tannase, the ECG value was 0.16 mg/g DW, while it was 0.19 mg/g DW with only tannase application. The same value was found 6.33 mg/g DW when only ultrasonic method was used. Balci and Özdemir (2016) extracted 2.83 g tea with 250 mL water and investigated the effects of different extraction temperatures (75, 85 and 95 °C) and different extraction times (3, 5, 10, 15 and 20 min) on bioactive compounds of Turkish green tea and reported ECG contents of green tea as between 8.02 - 14.61 mg/g

dw. Zhang et al., (2016) indicated that ECG concentrations decreased 1.81 mmol/L to 0.04 mmol/L in green tea extracts (1:30 tea: water ratio, 80 °C, and 15 min) supplemented with tannase enzyme (1:5000 w/v). Baik et al. (2015) reported decreasing ECG content of green tea extracts with tannase enzyme supplementations. Hong et al. (2014) investigated physical stability of green tea extracts supplemented with 5% tannase enzyme and reported that 1473.7 mg/g ECG content decreased to 240.2 mg/g with enzyme supplementation. Lu et al. (2009) reported decreasing ECG concentrations of green tea supplemented with tannase enzyme at different concentrations due to enzymatic hydrolysis. Labbe et al. (2006) indicated that ECG contents changed with brewing times and temperatures and reported ECG content at 50 °C brewing temperature and 20 and 80 min brewing times respectively as 7.6 and 33.9 µg/ml, the values at 90 °C brewing temperature and 5, 20 and 80 min brewing times were respectively reported as 48.1, 97.9 and 133.9 µg/ml.

It is seen that the decreases in ECG amounts determined by the use of tannase enzyme in the study are similar to the decreases in ECG amounts determined by other researchers. This decrease in ECG amounts; it is thought that tannase enzyme converts ECG, which has a large molecular structure, to non-hydrolysed catechins with smaller molecules.

## CONCLUSION

In ultrasound-assisted extraction processes, as compared to control samples, EGCG and ECG contents decrease, EGC and EC contents increased in all tea-water ratios and brewing temperatures with tannase enzyme supplementations. The increase in EGC and EC contents were attributed to hydrolysis of EGCG and ECG into EGC and EC through the tannase enzyme activity.

In green tea extracts extracted under different extraction conditions, caffeine contents generally decreased with tannase enzyme supplementation, but the differences in caffeine contents of enzyme-supplemented and control samples without enzyme supplementation were not found to be significant ( $p>0.05$ ).

It was concluded based on present findings that a more functional product richer in functional compounds (phenolics) could be possible with tannase enzyme supplementations. In terms of optimum brewing dynamics for industrial green tea extract production, 20 minutes extraction could be sufficient at high brewing temperature (70 °C) and tea: water ratio for higher total phenolics. Such a case may constitute a significant factor for time and productivity of instant tea sector.

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## Author contributions

Cemal Kaya and Esra Esin Yücel designed the study. Emine Ateş and Esra Esin Yücel performed the research including data analysis and literature search. Esra Esin Yücel, Cemal Kaya and Mustafa Bayram wrote and revised the manuscript. The final version was approved by all authors.

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