The Bioactive Compounds of Tea and Decaffeinated Tea (*Camellia sinensis)*

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***Abstract*—** In this study, phenolic compounds of tea and decaffeinated green and black tea was determined by HPLC method and the effects of the parameters investigated on the amount and distribution of these compounds was shown. The antioxidant activities of these teas were determined by DPPH method and total phenolic analysis in tea was also performed spectrophotometrically. In the investigated caffeinated green tea GA (0.66-2.657 mg/g); GC (0-0.998 mg/g); EGC (17.99-33.71 mg/g); EC (7.181-13.02 mg/); EGCG (32.14-89.709 mg/g); in the investigated decaffeinated green tea GA (1.152-2.74 mg/g); GC (0-1.335 mg/g); EGC (0.841-21.321 mg/g); EC (0.661-7.884 mg/g); EGCG (3.958-59.526 mg/g) as phenolic compounds were detected. In the investigated caffeinated black tea GA (1.764-3.378 mg/g); EC (0.012-7.101 mg/g); EGCG (0.187-5.599 mg/g); in the investigated decaffeinated black tea GA (1.377-3.954 mg/g); EC (0-5.075 mg/g); EGCG (0-4.38 mg/g) as phenolic compounds were detected.

***Keywords*—**Tea, *Camellia sinensis*, caffeine, decaffeination, HPLC, phenolic compound.

I. Introduction

TODAY, the tea obtained from the leaves of the plant known as camellia sinensis is considered as a healthy beverage due to its high antioxidant capacity and bioactive molecules [1]. Tea is divided into two main types of tea as black and green tea. Both types of tea contain small amount of xanthine alkaloids as well as caffeine from 1% to 5%. Tea also contains quite high rates of phenolic substances or tannins (5% - 27%), which contain catechin (flavonol) and gallic acid. These rates are higher in green teas when compared to black teas [2].

It must not be ignored that consuming excessive amounts of caffeine component contained in tea causes toxic effects. Due to the adverse effects of caffeine, the tendency to consume caffeine-free products [3] increases day by day. Within the scope of the present study, the changes in the bioactive components of tea, the second most consumed beverage in Turkey after water, that take place with the decaffeination process, as well as the antioxidant activities of different brands of teas sold in the domestic and foreign markets and the same of decaffeinated teas were examined.

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II. Material and Method

A. Material

Caffeinated and decaffeinated green and black teas were used as the materials of the study. A total of 8 green tea samples were used as four ordinary green teas (Doga, Clipper, Yama Moto and Yogi) and four decaf green teas (Doga Decaf, Clipper Decaf, Yama Moto Decaf and Yogi Decaf). On the other hand the number of black tea samples used was 6 as three ordinary black teas (Windsor, Tetley, PG) and three decaf black teas (Windsor decaf, Tetley Decaf and PG Decaf).

B. Method

The teas obtained from both the domestic and international market were ground in a laboratory type disk mill and filtered through a sieve with 0.300 and 0.150 µm pore diameter. Tea samples within this range were used in the analyses. Right after grinding, extraction process was carried out and the remaining samples were preserved in a fridge. By using different parameters (solvent, solvent/material rate, etc.) for the extraction of phenolic compounds, extraction conditions were optimized and the most convenient solvent and the rate of solvent/material were selected. According to this, 0.2 g of each of the tea samples preserved in the fridge in ground state were weighted and extracted within 10 ml and 80% (v/v) methyl alcohol solution for 2 hours in a shaker at room temperature. By the end of the duration, the extracts were filtrated through a Whatman no 1 filter. The obtained filtrates were passed through a 0.45 µm microfilter. All extracts were diluted 4 folds with 80% methyl alcohol solution and kept within the fridge and in dark until the time of analysis. For the determination of antioxidant activity the method suggested by Turkmen et al. [4] was employed. For the analyses, DPPH method was implemented with the use of the samples diluted with methyl alcohol with a rate of 1:4 (v/v). After the samples obtained in consequence of phenolic substance extraction were mixed with vortex, they were injected into a HPLC column. All analyses were carried out with 2 repetitions. Phenolic substance analysis was conducted by means of the method suggested by Turkmen and Velioglu [5]. In this method, samples diluted with 1:20 (v/v) methyl alcohol were used. With the modification of the Folin Ciocalteu spectrophotometric method of Obanda and Owuor [6], the mentioned method was followed with 2 repetitions. According to this method 10 ml of Folin Ciocalteu solution is supplemented up to 100 ml with methanol. Sodium carbonate solution, on the other hand, was prepared with 7.5% NaCO3.

Total phenolic substance content was expressed in terms of mg/g as the mg gallic acid equivalent (GAE)/g (r^2=0.9987).

III. Results and Discussion

A. Determination of Bioactive Components in Caffeinated and Decaffeinated Green Teas

HPLC chromatograms of caffeinated and decaffeinated teas are presented in Figure 1 and Figure 2. As it is seen in Figure 1, the components detected in green tea are gallic acid (GA), gallocatechin (GC), epigallo catechin (EGC), epicatechin (EC), epigallo catechin gallat (EGCG) and caffeine.

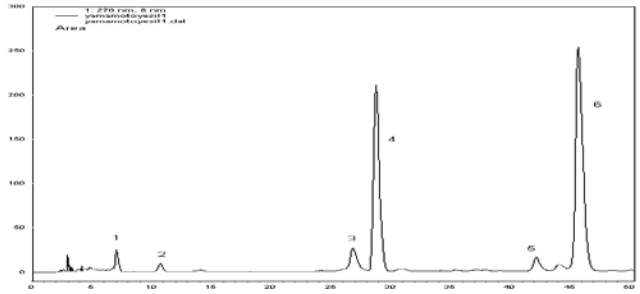

Fig. 1. Chromatogram of Yama Moto brand green tea. Peaks: 1, GA; 2, GC; 3, EGC; 4, Caffeine; 5, EC; 6, EGCG

Examining the chromatograms of caffeinated and decaffeinated green teas (Figure 1 and Figure 2) shows that both caffeine and phenolic substances decrease prominently. As it is seen in Table 1, in caffeinated green teas the amount of gallic acid varies between 0.66 and 2.657 mg/g, while gallocatechin amounts are between 0 and 1.335 mg/g, epigallo catechin amounts are between 17.99 and 33.71 mg/g, epicatechin amounts are between 7.181 and 13.02 mg/g and finally epigallo catechin gallat amounts are between the values of 32.14 and 89.709 mg/g. As for the decaffeinated green teas gallic acid amounts are between 1.152 and 2.74 mg/g, gallocatechin amounts are between 0 and 1.335 mg/g, epigallo catechin amounts are between 0.841 and 21.321 mg/g, epicatechin amounts are between 0.661 and 7.884 mg/g and epigallo catechin gallat amounts are between 3.958 and 48.526 mg/g. As it is also understood from the values in Table 4.1, with the decaffeination process, the phenolic substance concentrations in teas decrease by some amount.

As for the caffeine rates in the normal green tea samples we have examined, they were determined to vary within the range of 15.438 and 35.38 mg/g. The same rate in decaffeinated green teas varied between the values of 1.217 and 3.409 mg/g. Examining the caffeine rates in the study conducted by Henning et al. [7] shows that the caffeine rate in caffeinated green teas varied within the range of 21.8 and 33.6 mg/100 ml. The same were found out to be within the range of 0.7 and 5.8 mg/100 ml in decaffeinated green teas.

TABLE 1. PHENOLIC SUBSTANCE AND CAFFEINE CONCENTRATIONS DETERMINED THROUGH HPLC IN CAFFEINATED AND DECAFFEINATED GREEN TEAS (MG/G)

Name	CA	GA	GC	EGC	EC	EGCG
Doga Green 1	15,438	0,660	0,277	17,990	7,181	32,140
Doga Green Decaf 1	1,823	2,740	-	0,841	0,661	3,958
Yogi Green 1	29,523	0,928	-	24,276	8,367	55,520
Yogi Green Decaf 1	1,323	1,274	-	21,321	7,884	59,526
Clipper Green 1	35,380	2,657	0,998	33,710	13,020	89,709
Clipper Green Decaf 1	3,207	1,376	0,512	10,885	4,665	33,698
Yama Moto Green 1	29,863	1,543	0,569	32,169	9,650	80,419
Yama Moto Green Decaf 1	2,987	1,806	1,335	16,564	5,600	47,608

CA: Caffeine, GA: Gallic acid, GC: Gallocatechin, EGC: Epigallo catechin, EC: Epicatechin, EGCG: Epigallo catechin gallat

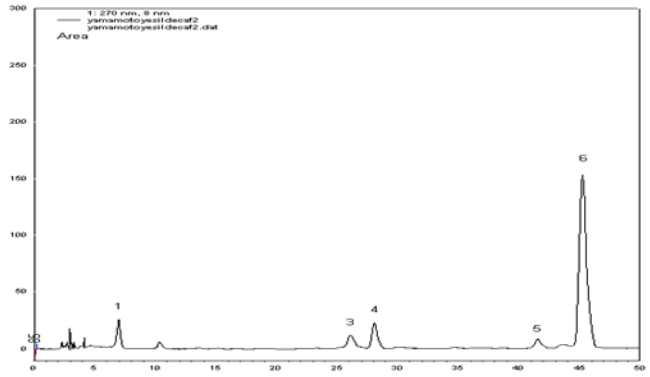

Fig. 2. Chromatogram of Yama Moto brand decaffeinated green tea. Peaks: 1, GA; 2, GC; 3, EGC; 4, Caffeine; 5, EC; 6, EGCG

B. Determination of Bioactive Components in Caffeinated and Decaffeinated Black Teas

HPLC chromatograms of caffeinated and decaffeinated black teas are presented in Figure 3.

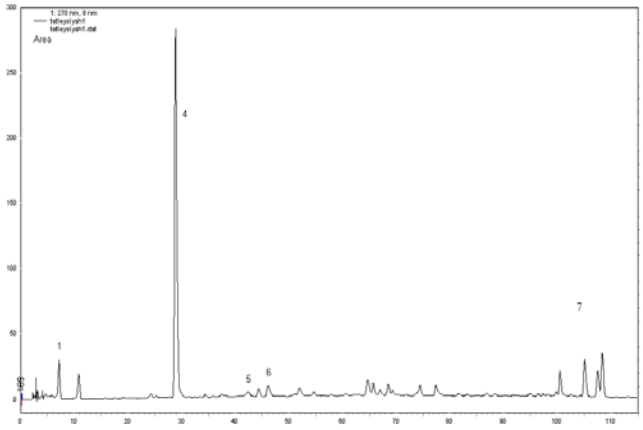

Fig. 3. Chromatogram of Tetley brand black tea. Peaks: 1, GA; 4, Caffeine; 5, EC; 6, EGCG; 7, Theaflavin mixture

In caffeinated black teas GA was found out to be between 1.764 and 3.378 mg/g, while EC was found between 0.012 and 7.101 mg/g and EGCG between 0.187 and 5.599 mg/g. As for decaffeinated black teas while GA was determined to

be between 1.377 and 3.954 mg/g, EC was found between 0 and 5.075 mg/g and EGCG between 0 and 4.38 mg/g. GC and EGC could not be found in black tea samples. It is reported in the literature that EGCG is the highest catechin found in black teas extracted with different solvents (such as 40% ethanol, 80% methanol, water) [8]. In the study conducted by Henning et al. [7], while the flavonol amount in black teas was found out to vary within the range of 21.2 and 68.3 mg/g, the same was found out to be between 4.66 and 5.4 mg/g in decaffeinated black teas. These values are in consistency with the values we have obtained in our study. As for the caffeine rates in the normal black tea samples we have examined, they were determined to vary within the range of 14.137 and 41.696 mg/g. The same rate in decaffeinated black teas varied between the values of 0.055 and 0.644 mg/g.

C. Antioxidant Activities of Caffeinated and Decaffeinated Green Teas

Values belonging to caffeinated and decaffeinated green teas are presented in Table 2. As it is seen from Table 2, except for the Doga brand of tea, the antioxidant activity values of caffeinated green teas and that of decaffeinated green teas are similar. Antioxidant activities of Doga brand of caffeinated green teas were found out to be 95,68% and 95,84%. These values are in consistency with the percentile antioxidant activities of caffeinated and decaffeinated green teas. However, inhibition percentage of Doga brand green tea was found out to be at a lower level (83.36% and 87.2%).

TABLE 2. ANTIOXIDANT ACTIVITY VALUES OF GREEN TEAS

Sample Name	inhibition %
Doga Green 1	95.68
Doga Green Decaf 1	83.36
Yogi Green 1	95.52
Yogi Green Decaf 1	95.36
Clipper Green 1	95.36
Clipper Green Decaf 1	95.2
Yama Moto Green 1	96.00
Yama Moto Green Decaf 1	95.52

D. Antioxidant Activities of Caffeinated and Decaffeinated Black Teas

Values belonging to caffeinated and decaffeinated black teas are presented in Table 3. As it is seen from Table 3, except for the Windsor brand of tea, the antioxidant activity values of caffeinated black teas and that of decaffeinated black teas are similar. Antioxidant activity values of Windsor brand caffeinated tea were found out to be 94.72%. These values are in consistency with the inhibition percentage values of other caffeinated and decaffeinated black teas. However, inhibition % value of Windsor brand decaffeinated tea was found out to be at a quite low level (33.44% and 39.52%).

TABLE 3. ANTIOXIDANT ACTIVITY VALUES OF BLACK TEAS

Sample Name	Absorbance	inhibition %
Windsor Black 1	0.033	94.72
Windsor Black Decaf 1	0.416	33.44
Tetley Black 1	0.030	95.2
Tetley Black Decaf 1	0.032	94.88
Pg Black 1	0.030	95.2
Pg Black Decaf 1	0.034	94.56

E. Phenolic Substance Concentrations of Caffeinated and Decaffeinated Green Teas

Phenolic substance concentrations of caffeinated and decaffeinated green teas are presented in Table 4. As it is seen from Table 4, a significant decrease in the phenolic substance concentration of Doga brand of decaffeinated teas (43.16 - 55.16 mg/kg) was observed in comparison of the caffeinated teas of the same brand (128.22 - 144.22 mg/kg). This result indicates that during the decaffeination process of Doga brand green teas, also phenolic substances are removed at a significant rate. As for Yogi, Clipper and Yama Moto brands of green teas, it is observed that the phenolic substance concentrations of decaffeinated teas decrease proportionally. These results suggest that decaffeinated green teas are obtained by putting the same caffeinated teas through the decaffeination process.

TABLE 4. PHENOLIC SUBSTANCE AMOUNTS OF GREEN TEAS

Sample Name	Absorbance	Concentration (mg/kg)
Doga Green 1	1.175	128.22
Doga Green Decaf 1	0.452	43.16
Yogi Green 1	1.351	148.93
Yogi Green Decaf 1	1.249	139.52
Clipper Green 1	1.892	212.58
Clipper Green Decaf 1	1.106	120.11
Yama Moto Green 1	1.668	186.22
Yama Moto Green Decaf 1	1.168	127.4

F. Phenolic Substance Concentrations of Caffeinated and Decaffeinated Black Teas

Phenolic substance concentrations of caffeinated and decaffeinated black teas are presented in Table 5. According to Table 5, in the phenolic substance concentrations of Windsor brand of decaffeinated black teas (32.81 and 41.87 mg/kg) there is a significant decrease in comparison with the caffeinated teas of the same brand (137.4 - 159.4 mg/kg). On the other hand, no significant difference in the phenolic substance concentrations of the decaffeinated and caffeinated black teas of Tetley and PG brands could be found.

TABLE 5. PHENOLIC SUBSTANCE AMOUNTS OF BLACK TEAS

Sample Name	Absorbance	Concentration (mg/kg)
Windsor Black 1	1.440	159.4
Windsor Black Decaf 1	0.364	32.81
Tetley Black 1	1.177	128.46
Tetley Black Decaf 1	1.155	125.87
Pg Black 1	1.116	121.28
Pg Black Decaf 1	0.958	102.69

When black and green teas are examined in general it is observed that black teas contain lower flavonol than green teas due to the fact that fermentation process generates theaflavins, thearubigins and their gallat derivatives epicatechin polymers [7]. Also, as it is understood from the values in Figure 5, with the decaffeination process phenolic substance concentrations of respective branch decrease.

G. Evaluation of HPLC Chromatograms, Phenolic Substance Concentrations and Antioxidant Activities of Caffeinated and Decaffeinated Green Teas

Antioxidant activities of decaffeinated green teas exhibit approximately the same values in caffeinated green teas of the same brands. Examining Table 4 and Table 5 in general shows that, despite the decrease of 67% and 71% in the phenolic substance amount of Doga Decaffeinated Green Tea, its inhibition % decreases at the rates of 8.48% and 12.68%. This may be an indication that although the phenolic substance presence decreases, the remaining phenolics still have high inhibition. However, examining the chromatograms clearly shows that the chromatograms in green teas are reduced. Total amount of phenolic substance and the chromatograms support each other, however examining the inhibition % values shows that although the phenolic substances are reduced with the decaffeination process there is no decrease in antioxidant activity. It is believed that the total phenolic matter and antioxidant activity need to be supported with chromatograms.

H. Evaluation of HPLC Chromatograms, Phenolic Substance Concentrations and Antioxidant Activities of Caffeinated and Decaffeinated Black Teas

Antioxidant activities of decaffeinated black teas show approximately the same values in the same brand of caffeinated black teas. However, the phenolic substance concentration of decaffeinated black teas decreases in comparison to caffeinated black teas. Examining the chromatograms of black teas shows that, as it is the case in green tea samples, also in decaffeinated black teas chromatograms decrease. The fact that antioxidant activity does not decrease despite this can be explained in two ways. The first explanation is the possible presence, in both green and black teas, of compounds other than phenolic substances that provide antioxidant activity. Another possibility is that despite the reduction in the amount of phenolics with the decaffeination process, only a small amount of reduction takes place in the inhibition % due to the strong antioxidant effects of these phenolics. However, in order to obtain healthy findings total phenolic substances and antioxidant activity need to be supported with chromatograms.

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Alteration of Attitude toward GM-Foods of Urban Consumer Depending Geographical Regions in Turkey

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***Abstract*—**In the present study, descriptive data concerning the attitudes of urban consumers living in different geographical regions of Turkey (Marmara, Aegean, Mediterranean, Central Anatolia, Black Sea and Eastern Anatolia Regions) towards GM and GM foods were obtained and the region-based variances of these data were examined with a sampling error margin of 2.83% in consequence of the research carried out with the participation of the consumers (n = 1222) determined by means of implementing stratified random sampling method to houses and offices. Although different concerns and risks are in question, in all geographical regions there is a negative attitude against GM organism, technology and foods. However, these negative attitudes and viewpoints are based on different sources on the basis of geographical regions. The differences in the regions' socio-economic structures were determined as significant factors in this differentiation.

Keywords— Genetically modification, biotechnology, food, consumer, attitude

I. Introduction

NEW biotechnological methods to improve the quality and quantity of foods have been recently used to meet the demands of an increasing world population [1, 2, 3]. Since the mid-1990's, genetic modification (GM) is a rapidly growing and controversial method that can boost agricultural productivity, but the technology is not fully understood by the consumers [4]. There are also reports about "uncertainties" and "risks" of consuming GM foods and there has been a "doubt" that whether GM food causes allergies on human beings or damages the immune system [5]. On the other hand, when we consider the population growth in the world and its changing climate, it is expected that the production of GM foods will be increased [6], so that consumers' attitude on GM foods will be more important.

Consumers in different regions show different attitude toward adaption of GM food based on ethical ground, American consumers have neutral position toward GM food as they benefits of GM food for both producers and consumers [7]. However, most of the consumers living in developing countries have a negative position toward them [8, 9,10, 11, 12, 13, 14, 15].

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As for the studies carried out in Turkey, while there is a small number of studies intended to reflect the country-wide situation, mostly the attitudes and tendencies of people in different locations or position/duties (such as students, officials of the related ministry) were tried to be determined [6, 16, 17, 18, 19, 20].

In this study, it was aimed to produce descriptive information on consumers' awareness and attitudes concerning GM and GM foods by using the data obtained from face to face questionnaires directed to urban consumers living in several geographical regions of Turkey (Marmara, Aegean, Mediterranean, Central Anatolia, Black Sea and Eastern Anatolia Regions).

II. Material and Method

A. Sampling Method and Selection of Sample

The target population of the research was formed by consumers over the age of 18 from all socio-economic groups (AB, C1, C2, DE), living in the city centers of Adana, Ankara, Antalya, Aydin, Bursa, Erzurum, Gaziantep, Istanbul, Izmir, Kayseri, Kocaeli, Samsun, Tekirdag or Trabzon cities of Turkey and dealing with the shopping of food needs of the house hold they live in. By considering the district and neighborhood distributions of the cities within the sample size distribution determined for the consumers residing in the centers of these cities a total of 1222 questionnaires were implemented.

The following formula was utilized for determining sample number:

$$n = \left(\frac{Z_{\alpha/2}}{d}\right)^2 p.q \quad (1)$$

$$n = \left(\frac{1.96}{0.0283}\right)^2 0.5 \,.\, 0.5 \cong 1200$$

n = Sample size,

$Z_{\alpha/2}$= Confidence coefficient, (for a confidence of 95%, this coefficient was accepted as 1.96)

p = Rate of food consumers residing in the urban centers of 14 cities representing Turkey (Center; central locations with a population bigger than 50000). Taken as 0.5, since there was no preliminary information on the possible availability of the feature intended to be measured within the population.

q = 1 - p = Rate of urban consumers