Change of bioactive content in seed propagated tea plants at different shooting periods

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ABSTRACT

Tea production is vital for east Black Sea region for farmers because it is dominant agricultural product. The majority of tea gardens in the region were multiplicated by farmers using seeds in the last 50 years resulted a great variation for most of the plant characteristics. The present study describes variation in shooting periods on the bioactive content of seed propagated tea plant. Results indicated significant differences among most of the searched parameters among shooting periods. Total phenolic content was the highest at the first shooting period (between 6.45-7.03 g gallic acid equivalent /100 g) dry weight (DW) basis. Among the catechin, Epigallocatechin gallate (EGCG) was found to be dominant in black tea samples (0.756-0.941 g/100 g DW) and followed by epigallocatechin (0.590-0.752 g/100 g DW) and epicatechine gallate (0.510-0.654 g/100 g DW), respectively. In general first shooting period (May) gave the highest catechin content that improve tea quality and with following shooting periods catechin content decreased steadily. Total phenolic content were found between 6.45-7.03 g GAE/100 g DW among shooting periods. The results suggested that first shooting period of black tea samples had higher bioactive content.

Keywords: Tea, diversity, phytochemicals, seed propagation

Introduction

Tea is one of the most important beverages in the world and black tea production in the world increased steadily and reached 4.8 million tons in recent years. On an average, during the last two decades in the production front, China contributed 35% of the world tea production and followed by India (21%), Kenya (8%) and Sri Lanka (7%). Turkey is the 5th

biggest tea producer in the world with 225,000 tons black tea production per year. The country shares 5% world tea production (FAO, 2019).

Tea (*Camellia sinensis*) is the most consumed drink in the world except for water, owing to its pleasant sensory properties, broad health benefits and unique sociocultural characteristics. Usually, tea could be classified into six categories, i.e., green tea (unfermented), yellow tea (slight-fermented), white tea (mild-fermented), oolong tea (semi-fermented), black tea (fermented) and dark tea (post-fermented) according to the varying fermentation extent (Zhao et al., 2019).

At the start of tea cultivation in Turkey, the primary goal was to meet the domestic demand only. Today, Turkey holds a significant place among the world's largest tea producers. But still great percentage of products is marketed in domestic market. All of the tea plantation areas are located in the eastern part of Black Sea Region. Tea cultivation in Turkey contributes significantly to the economic income of local farmers in the east Black Sea region (Cakmakci et al., 2012)

The main tea production area in Turkey is Rize province with 50.583 hectares area and followed by Trabzon (15.328 hectares), Artvin (8430 hectares) and Giresun-Ordu (2299 hectares). Approximately 66% of total tea plantation areas located in Rize province whilst the rest of them are located in Trabzon, Artvin, Giresun and Ordu respectively (Seyis et al., 2018).

Tea growing areas of Turkey is far away from Ecuador line compared the other most important the producers such as China, Kenya, India, Sri Lanka etc. indicating great differences from the rest of the countries. Because temperature does not falls up to minus degree in tea production areas and tea production is covering the whole year in those countries. However in Turkey, tea plantations active 6 months and snowfalls on Turkish tea plantations in winter months bring them an extra important characteristic. Tea plants harvested in in general 3 times in May, July and September, respectively. Shooting (harvesting) period, which is specific to tea cultivation in Turkey, significantly affected almost all of the quality parameters of black tea. Due to winter dormancy and short cultivation period, Turkish tea plantations are pesticide free and Turkish tea called 'natural tea' compared to other tea produced in the world (Kafkas et al., 2009; Yurteri et al., 2019).

Tea was introduced into Turkey with the efforts of Zihni Derin and Ali Rıza Erten at the end of the 1930s. The most of the tea plantations (90-95%) established by seeds and this tradition still have ongoing. Continuous seed propagation in Turkey has produced populations with different yield and quality properties, reflecting wide genetic variation.

Thus clonal selection studies were previously conducted on those germplasms in the Black Sea region and several promising tea clones such as ‘Tuglali-10’, ‘Derepazari-7’, and ‘Pazar-20’ have been released (Oksuz, 1987). Unfortunately these clones have not become widespread so far.

Previous studies clearly revealed that black tea had one of the richest sources of bioactive phytochemicals including theaflavins, thearubigins, catechins, and phenolics (Serpen et al., 2012; Kelebek, 2016; Ozdemir et al., 2018).

The aim of the current study was to evaluate and compare the bioactive content of seed propagated tea populations to reveal if there are any differences among populations.

Material and Methods

The fresh tea leaves (*Camellia sinensis* var. *sinensis*) were harvested as a group of two or three leaves and a bud or mature leaves without a bud during three consecutive shooting periods (May, July, and September) in 2014 from a single seed propagated tea population found in tea garden in Ikizdere town belongs to Rize province located on the Black Sea coast in Turkey. After plucking, the leaves were immediately processed into black tea according to the Turkish Tea Board standards.

Preparation of extract

Phenolic extracts were prepared according to Zuo et al. (2002). First 50 mL of aqueous methanol (80%) containing 0.15% HCl was poured onto 2 g of tea sample, and the phenolic compounds were extracted at 40 °C for 3 h and filtered through coarse filter paper. The filtrate was used for the total phenolic content determination by Folin– Ciocalteu assay and the filtrate was further filtered through 0.45 µm of nylon membrane filter before HPLC analysis (Ozdemir et al., 2018).

Total phenolic content

The total phenolic content was determined by the Folin– Ciocalteu method (Asami et al., 2003). The results were expressed as mg gallic acid equivalent (GAE) per 100 g dry weight (DW) basis.

Catechins content

The catechin group including epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), epicatechin gallate (ECG), catechin gallate (CG), gallocatechin gallate (GCG), catechin (C) and caffeine were determined by HPLC. The chromatographic separation, described by Zuo et al. (2002), was used with some modifications in the mobile phase and UV detection. The absorbance of the catechins EGCG, EGC, EC, ECG, CG, GCG and C) were determined at 280 nm and the results were expressed g/100 g dry weight (DW) basis.

Statistical Analysis

All data were analyzed using SPSS software and procedures. Analysis of variance tables were constructed using the Least Significant Difference (LSD) method at $p<0.05$.

Results and Discussion

Table 1 shows individual catechin content of the black tea samples obtained from 3 shooting periods (May, July and September).

As indicated in Table 1, there were statistically significant differences among shooting periods on individual catechins except CG (Table 1). All of the catechins decreased significantly from the first to the third shooting periods. According to obtained results Epigallocatechin gallate (EGCG) was the predominant catechin in black tea samples and EGCG content was the highest at first shooting period (0.941 g/100 g DW), and followed by second shooting period (0.855 g/100 g DW) and finally it was the lowest at the last shooting period (0.756 g/100 g DW), respectively.

The other individual catechins are also revealed similar patterns (Table 1). For example epigallocatechin (EGC) content was also the highest at first shooting period (0.752 g/100 g DW), and it was decreased with prolonged harvest period (0.640 g/100 g DW at second harvest and 0.590 g/100 g DW at last shooting period), respectively.

EC and ECG content were the highest at first shooting period (0.411 and 0.654 g/100 g DW) and decreased to 0.367 and 0.605 g/100 g DW at second shooting period. At third shooting period, those values decreased again to 0.307 and 0.510 g/100 g DW), respectively (Table 1).

Ozdemir et al. (2018) reported EGCG, EGC, EC and ECG amounts in black tea samples between 0.819-0.976 g /100 g DW, 0.661-0.785 g/100 g DW, 0.274-0.329 g/100 g DW and 0.437-0.523 g/100 g DW, respectively at three shooting periods. Our results are good agreement with above study. The results also in accordance with previously reported studies by Khokhar and Magnusdottir, (2002) and Serpen et al., (2012).

CG, GCG and C content were found to be the highest at first shooting period (0.033, 0.044 and 0.307 g/100 g DW) while it was the lowest at the last shooting period (0.026, 0.032 and 0.222 g/100 g DW) (Table 1).

Catechins and their derivatives are thought to contribute to the beneficial effects ascribed to tea. Tea catechins and polyphenols are effective scavengers of reactive oxygen species in vitro and may also function indirectly as antioxidants through their effects on transcription factors and enzyme activities. The fact that catechins are rapidly and extensively metabolized emphasizes the importance of demonstrating their antioxidant activity in vivo (Higdon and Frei, 2003). EGCG was the richest catechins. It has been reported that EGCG possessing the most number of phenolic hydroxyl groups manifests the strongest antioxidant activity in catechins, which is even stronger than vitamins C and E (Siddiqui et al., 2004). It has been reported that EGCG could exhibit multiple bioactivities, especially anticancer effects, through inhibiting cancer stem cells and modulating molecular events associated with cancer cell proliferation, apoptosis, immunity and so on (Gan et al., 2018; Zhao et al., 2019).

Total phenolic content were found between 6.45-7.03 g GAE/100 g DW among shooting periods. Ozdemir et al. (2018) found that total phenolic contents of different Turkish black tea samples were in the range of 4.75 to 6.97 g GAE per 100 g. Karadeniz and Koca (2009) reported that Turkish black tea samples had total phenolic content between 7.29-8.39 g GAE per 100 g DW. The total phenolic content of this study were found to be similar to Ozdemir et al. (2018) and was lower than those reported previously in the literature.

It is well known that plucking standards and processing conditions could be affect total phenolic content of tea samples. Total phenolic content decreased during the shooting periods, which can be directly related to the total phenolic content of the fresh tea leaves (Zhang et al., 2018).

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Table 1. Individual catechins and total phenolic content in seed propagated black tea samples at different shooting periods (g/100 g DW)

Catechins (g/100 g DW)	Shooting period		
	May	July	September
EGCG	0.941a	0.855b	0.756c
EGC	0.752a	0.640b	0.590c
EC	0.411a	0.367b	0.307b
ECG	0.654a	0.605b	0.510c
CG	0.033[NS]	0.030	0.026
GCG	0.044a	0.042a	0.032b
C	0.307a	0.278b	0.222c
Total phenolics (g GAE/100 g DW)	7.03a	6.89b	6.45c

There were significant ($P < 0.05$) differences among the different letters in the same lines.
NS: Non Significant