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Seasonal Variation of Total Phenolic, Antioxidant Activity, Plant Nutritional Elements, and Fatty Acids in Tea Leaves (*Camellia sinensis* var. *sinensis* clone Derepazari 7) Grown in Turkey

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Abstract

Seasonal variations of total phenolic, antioxidant activity, PNE (Plant Nutrient Elements), and fatty acids in fresh tea leaves grown in Turkey were studied. Fresh tea leaves sampled from Derepazari 7 clone [*Camellia sinensis* L.var. *sinensis* (Theaceae)] were analyzed and compared during the three commercial harvest seasons (May 15, July 15, and September 15) in both 2005 and 2006. The levels of total phenolics and antioxidant activity was higher at 2nd harvest time (62.88 μg/mg and 89.27%). The seasonal variations of the individual fatty acids were significant ($P < 0.05$) between the three harvest seasons. The amount of N and P in tea leaves was the highest at 1st harvest; however K, Ca, Mg, S, and Mn were highest at 2nd harvest time. This study revealed that total phenolics could be used as quality descriptors for monitoring the seasonal variations in Turkey-grown tea leaves.

Keywords: *Camellia sinensis* var *sinensis,* fatty acids, phenolics, seasonal variation.

Introduction

Tea (*Camellia sinensis* L.) belongs to Theaceae family and is one of the most popular beverages in the world (Fernandez et al., 2002). China and India are major tea producers (941.000 and 831.000 tons) followed by Sri Lanka (308.000 tons), Kenya (295.000 tons) and Turkey (202.000 tons), respectively (Anon., 2005). The production of tea in Turkey began in the early years of the Republic along the Eastern Black Sea Region and most of the tea plantations are centered on the Rize city in the country (Mendilcioglu, 2000).

Tea is drunk in almost every country around the world and has reached a ceremonial status in many places both as a social and medicinal beverage. Since 3000 B.C., traditional Chinese medicine has recommended green tea for headaches, body aches and pains, digestion, enhancement of immune system, detoxification, as an energizer, and to prolong life (Xie et al., 1998). The health benefits of tea are confirmed and the therapeutic value of tea for the prevention and treatment of many diseases has become more and more commonly known (Zaveri et al., 2002).

Total phenolic content present in young tea shoots (also referred to as fresh green leaves, fresh tea shoots, or flushes) are known to be one of the main factors in determining the quality of the resulting tea drink (Hara, 1995). The content of total phenolics in tea shoots grown in Kenya has been found to correlate significantly with Kenyan plain tea quality parameters and clones with low total phenolic content produced low quality black teas (Obanda et al., 1992, 1997). In other words, the quality of black tea is dependent in the first instance on the chemical composition, in particular, the flavanols of the harvested shoots, and in the second instance by the way in which they are handled, processed, and stored (Serafini et al., 1996). Tea leaves also have high antioxidant activity (Hara, 1995). Dietary intake of antioxidant compounds is important for health (Duh et al., 1999). Although there are some synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which is commonly used in processed foods, it has been reported that these compounds have some side effects (Ito et al., 1983). Another healthy function of some plants is their essential fatty acid composition, which humans cannot synthesize, but must obtain through diet. Essential fatty acids regulate numerous body functions including blood

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pressure, blood viscosity, immune and inflammatory responses (Pawlosky et al., 1996; Simopoulos & Salem, 1996). Tea also contains minerals and trace elements such as K, Mn, Cr, Ni, and Zn which are essential to human health. The regular consumption of tea may contribute to the daily dietary requirements of several elements and tea could be an important source of manganese, and the large amount of potassium in comparison with sodium that could be beneficial for hypertensive patients (Xie et al., 1998; Fernandez et al., 2002).

There were studies related to chemical composition of tea leaves (Xie et al., 1998; Ferrara et al., 2001; Fernandez et al., 2002). However, the variation of chemical content present in tea leaves in different harvest time in Turkey has not been studied so far. Moreover, to our knowledge, fatty acid contents of tea leaves have not been reported in literature.

Materials and Methods

Collection and preparation of tea leaf samples

Tea leaves were harvested from dominant clone Derepazari-7 belongs to *Camellia sinensis* var. *sinensis* from Rize city of Turkey at three commercial harvest times (May 15, July 15, and September 15) in both 2005 and 2006 year. Plant materials were further identified by senior taxonomists, Dr. Meryem Sengul, from Department of Botany, Ataturk University, Erzurum, Turkey. There were no statistical differences between the two years when the results were analyzed, therefore the data of the different years on total phenolics, antioxidant activity, fatty acids, and plant nutrient elements were pooled (this could be a consequence of similar growing and climatic conditions in both years where the plants grow). After harvest, the leaves were cleaned and cut into small pieces before being dried in a hot air-blowing oven at 45°C. All samples after drying were ground to a fine powder prior to extraction.

Determination of fatty acid content

Fatty acid composition was analyzed according to a previous method (Anon., 2000). Fatty acids were designated (e.g., 18:1 ω9c) so that the figures represent, from left to right, the total number of carbon atoms (i.e., 18), the number of double bonds (i.e., 1), the position of the double bond from the ω end of the fatty acyl chain (i.e., ω9) and the configuration of the double bond (i.e., c for cis).

Determination of antioxidant activity

The antioxidant activity of methanol extract of tea leaves was determined according to the β-carotene bleaching method described by Kaur and Kapoor (2002). Grounded sample (10 mg) was mixed with 10 ml methanol and stirred for 30 min on a magnetic stirrer. The suspension was filtered through Whatman No. 1 filter paper. Final solutions were used as stock solution for the antioxidant activity and phenolic analysis. Briefly, 4 ml of β-carotene solution (0.1 mg in 1 ml chloroform), 40 mg of linoleic acid and 400 mg of Tween 40 were transferred to a round-bottom flask. The mixture was then evaporated at 50°C by means of a rotary evaporator to remove chloroform. Then, 100 ml of oxygenated distilled water were added slowly to the residue and vigorously agitated to give a stable emulsion. Then, 800 μl of extracts were added to 3 ml aliquots of β-carotene/linoleic acid emulsion. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. The mixtures were incubated at 50°C for 90 min. The measurement was carried out at 15 min intervals for 90 min. Methanol were used as control. A blank, devoid of β-carotene, was prepared for background subtraction. BHA and BHT were used as a standard. All samples were assayed in duplicate. Degradation rate (DR) was calculated according to first order kinetics, using the following equation based on:

$$\ln(a/b) \times 1/t = \mathrm{DR}_{\mathrm{sample}} \text{ or } \mathrm{Dr}_{\mathrm{standart}}$$

Where ln is natural log, a is the initial absorbance (470 nm) at time 0, b is the absorbance (470 nm) at 100 min and t is time. Antioxidant activity (AA) was expressed as percent of inhibition relative to the control, using the following formula:

$$\mathrm{AA} = (\mathrm{DR}_{\mathrm{control}} - \mathrm{DR}_{\mathrm{sample}} \text{ or } \mathrm{DR}_{\mathrm{standart}}/\mathrm{DR}_{\mathrm{control}}) \times 100$$

Determination of total phenolics in tea leaves

The concentration of total phenolics in the methanol extract of tea leaves was determined by the Folin–Ciocalteau colorimetric method (Naczk & Shahidi 1989). Briefly, 1 ml of the solution (contains 1 mg) extract in water was pipetted into a flask. Then 46 ml of distilled water and 1 ml of Folin-Ciocalteu's reagent was added and mixed thoroughly. The mixture was left to stand for 3 min and 3.0 ml of 2% sodium carbonate were added. After 120 min incubation at ambient temperature with shaking, the resulting absorbance was measured at 760 nm. Measurements were carried out in duplicate and the calibration curve was performed with gallic acid, and the results were expressed as μg of gallic acid equivalents per miligram (μg GAE/mg).

Determination of mineral elements

Total N was determined by the micro Kjeldahl method (James, 1995). In order to determine the mineral composition, samples were burned with a nitric acid and perchloric acid solution, on the hot plate, at 200°C. Then, the absorbance of the extract was measured by the Atomic Absorbance Spectrophotometer. The amounts of minerals were

Table 1. Fatty acid content (%) of tea leaves.

Fatty acid		Fatty acid content (%)			
Molecular Name	Common Name	1st Harvest	2nd Harvest	3rd Harvest	Average
16:0	(Palmitic acid)	25.26a	17.06b	16.51b	19.61
18:3 ω6c	(Linoleic acid)	13.20a	9.16b	10.88ab	11.08
18:3 ω3c	(α-Linoleic acid)	29.02a	19.82b	29.05a	25.96
24:1 ω9c	(Nervonic acid)	16.62b	24.75a	23.28a	21.55
23:0 3OH	(Tricosanoic acid)	15.90c	24.38a	20.28b	20.19
Total peak areas (%)		100.00	95.17	100.00	98.39

*The a, b, c or ab in same lines the are result of statistical analysis and show that there are significant differences among harvest dates on fatty acids at $P < 0.05$ statistical level.

calculated with a standard curve of each element. Phosphorus content of the extract, however, was analyzed by determining the absorbance of the color yellow, obtained from the Barton reaction, using a spectrophotometer (Thermo, Nicolet 100, UV) at 680 nm wavelengths, and comparing the results to the Standard curve.

Statistical analysis

The experiment was a completely randomized design with four replications. Data were subjected to analysis of variance (ANOVA) and means were separated by Duncan multiple range test at $P < 0.05$ significant level.

Results and Discussion

Fatty acid composition of tea leaves

Fatty acid analysis has shown that tea leaves studied contained five major compounds and a much greater variation of fatty acids was found among different harvest times (Table 1). α-Linoleic acid was the dominant fatty acid (29.02%) in tea leaves at 1st harvest time followed by palmitic acid (25.26%) and nervonic acid (16.62%), respectively. In 2nd harvest time this figure was changed and nervonic acid determined as major fatty acid in tea leaves (24.75%) followed by tricosanoic acid (24.38%) and α-linoleic acid (19.82%), respectively. At 3rd harvest time α-linoleic acid again was major fatty acid in tea leaves (29.05%) followed by nervonic (23.28%) and tricosanoic (20.28) acid, respectively (Table 1). Total peak areas of the mentioned fatty acids in tea leaves were 100.00% at 1st harvest, 95.17% at 2nd harvest, and 100.00% at 3rd harvest time (Table 1). The fatty acid compounds contribute to the flavor of tea leaves, which has distinct taste rather than aroma. According to literature searched, there is no study on fatty acid content of tea leaves. Thus, our fatty acid results may be the first study to provide data that the tea leaves possess fatty acids.

Antioxidant activity of tea leaves

Antioxidant activity of tea leaves are given in Figure 1. The differences among harvest times, BHA and BHT were found statistically important. Antioxidant activity of tea leaves were high and found to be 84.43% at 1st harvest and increased to 89.27% at 2nd harvest and decreased to 85.17% at 3rd harvest time. The antioxidant activity of BHA (200 mg/l) and BHT (200 mg/l) were 88.24 and 91.18%, respectively (Figure 1). In previous studies conducted on different tea samples using different solvents, the antioxidant activity was found between 2–83% (Turkmen et al., 2006). Juliani and Simon (2002) reported that antioxidant activity of green tea 4–5 times higher than cinnamon (*Ocimum basilicum* L., Lamiaceae) and oregano (*Origanum vulgare* L., Labiatae). Tavazzi & Offord (2001) revealed that

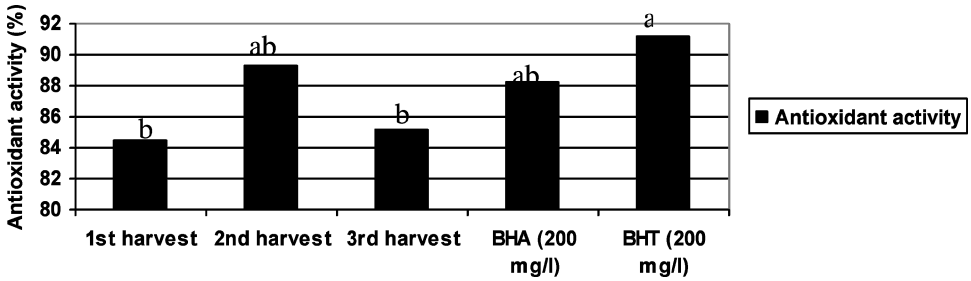

Figure 1. Seasonal variation of antioxidant activity in tea leaves.
**The a, b, c or ab are the result of statistical analysis and show that there are significant differences among harvest dates, BHA and BHT on antioxidant activity at $P < 0.05$ statistical level.

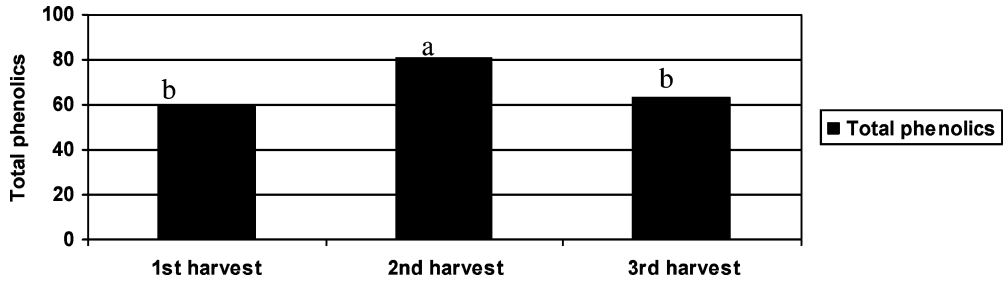

Figure 2. Seasonal variation of total phenolics (μg/mg) in tea leaves.
**The a, b, c or ab are the result of statistical analysis and show that there are significant differences among harvest dates on total phenolics at $P < 0.05$ statistical level

commonly consumed products such as tea, coffee, and cocoa have possessing significant antioxidant activity. The results for antioxidant activity clearly outline that tea leaves could be one of the richest sources of plants in terms of antioxidant activity. The great difference of tea leaves for antioxidant activity at different harvest time is supposed to the effect of change of ecological parameters. It was previously reported that, the composition of tea leaves varies with climate, variety, and age of the leaf (Leung & Foster, 1996). Tea and its constituent catechins are best known for their antioxidant properties, which has led to their evaluation in a number of diseases associated with reactive oxygen species (ROS), such as cancer, cardiovascular, and neurodegenerative diseases. Several epidemiological studies as well as studies in animal models have shown that green tea can afford protection against various cancers such as those of the skin, breast, prostate and lung (Mukhdar & Ahmad, 2000; Yang et al., 2002).

Total phenolics in tea leaves

The total phenolic content of tea leaves are given in Figure 2. Total phenolic content were 59.44 μg/mg at 1st harvest and increased to 80.69 μg/mg at 2nd harvest and decreased to 62.88 μg/mg at 3rd harvest time (Figure 2). The level of total phenolics has been found to positively correlate with the antioxidant activity in the tea leaves ($R = 0.891$). Robertson (1992) previously reported that tea leaves are extremely rich in phenolic compounds which can constitute up to 300 mg/g of material. Juliani and Simon (2002) are also reported that green tea leaves is very rich for total phenolics and total phenolic content of green tea leaves 4-5 times higher than cinnamon (*Ocimum basilicum*) and oregano (*Origanum vulgare*). They found strong relationships between antioxidant activity and total phenolics in tea leaves which support our findings. It has been shown that the biosynthesis of phenolic compounds can be effectively induced by sunlight (Harbowy & Balentine 1997). That is why in shaded tea flushes the concentrations of total phenolics are much lower. On the basis of this information, the differences in total phenolic levels between fresh leaves harvested in cooler (May and September) and warmer months (July) in Turkey may not be just a temperature effect but also a day length and sunlight effect. The highest total phenolic levels in tea are important for public by reducing the risk of atherosclerosis and coronary heart disease, which can be caused by oxidation of low-density lipoproteins (Shahidi & Wanasundara, 1992).

Mineral elements in gren tea leaves

The mineral contents of tea leaves at different harvest times are shown in Table 2. Differences among the different harvest times were observed based on the mineral compositions (Table 2). The amount of N and P in tea leaves was the highest at 1st harvest; however K, Ca, Mg, S and Mn were highest at 2nd harvest time (Table 2). The N, P, K, Ca values of tea leaves varied from 4.61 to 3.73%; 0.21 to 0.35%; 1.43 to 1.97%; and 0.26 to 0.31%, respectively (Table 2). Mg, S and Mn values of tea leaves at different harvest times are determined as 0.23 to 0.28%; 0.20 to 0.25% and 0.04 to 0.12%, respectively (Table 2).

The mineral composition of plants depended, not only on the species or varieties, but also on the growing conditions such as soil and geographical condition. We used only one cultivar in this study. The research area was same as well. Therefore main effect on mineral content should be harvest times as mentioned above. In this study, while the existence of seven elements was determined in all harvest times, N was predominant, followed by K, Ca, and P, respectively (Table 2). It is previously reported that N content of tea leaves were between 3.0-4.0% (Owuor & Wanyoka, 1983) which in agreement with our results. Kacar et al. (1979) reported that N content of tea leaves was highest at 1st

Table 2. Mineral content of tea leaves.

Harvest time	Mineral elements (%)						
	N	P	K	Ca	Mg	S	Mn
1st	4.61a	0.35a	1.64b	0.26b	0.24ab	0.22ab	0.08b
2nd	4.36b	0.28b	1.97a	0.31a	0.28a	0.25a	0.12a
3rd	3.73c	0.21c	1.43c	0.26b	0.23b	0.20b	0.04c

**The a, b, c or ab in same lines are the result of statistical analysis and show that there are significant differences among harvest dates on mineral elements at $P < 0.05$ statistical level.

harvest and decreased to last harvest which supports our findings. Ozgumus et al. (1982) found that P content of tea leaves were between 0.31-0.40%. Our P results are lower than this literature. This could be explained of low P content of soils. It is previously reported that approximately 30% of tea soils in Black Sea Region in Turkey had P deficiency (Sarimehmet, 1989). In addition, our K results are in accordance with Kacar et al.(1979).

As a conclusion of this study, it can be said that tea leaves are a valuable product, based on its rich and beneficial nutrient composition.

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