# SEASONAL VARIATION OF TOTAL PHENOLIC, ANTIOXIDANT ACTIVITY AND MINERALS IN FRESH TEA SHOOTS (*CAMELLIA SINENSIS* VAR. *SINENSIS*)

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**ABSTRACT**
Seasonal variation of total phenolics, antioxidant activity and minerals in fresh tea shoots, consisting of one apical bud and two adjoining leaves sampled from Muradiye, Tuglali, Gundogdu, Enstitu 9, Hamzabey, Hayrat and Pazar 20 clones grown in Eastern Black Sea region in Turkey was investigated during three harvest season (May, July and September) in both 2006 and 2007 years. The total phenolics of all clones were lower in cool months of May in both years (average 33.00-90.27 mg GAE/g dry weight basis). Thereafter, the levels of total phenolics increased throughout the warmer months from July to September. Antioxidant activity determined by β-carotene bleaching assay showed similar trends which increased from 1$^{st}$ harvest (May) to 3$^{rd}$ harvest (September). All clones showed nearly 100% antioxidant activity at 2$^{nd}$ and 3$^{rd}$ harvest season which higher than standard synthetic antioxidant BHA (butylated hydroxyanisole). However, seasonal variation of minerals (N, P, K, Ca, Mg, Na, Fe, Cu, Mn, Zn) showed different results according to clones used. These results seem to suggest that the harvest time is crucial to determining the antioxidant potential of fresh tea shoots.

**Keywords**: Fresh tea, *Camellia sinensis* var. *sinensis,* minerals, phenolics, seasonal variation.

## INTRODUCTION

Tea (*Camellia sinensis* L.) is one of the world's oldest beverages. The tea plant was first discovered by the ancient Chinese, who then spent many centuries perfecting the art of tea production, which resulted in a variety of types available today (Fernandez *et al*., 2002).

The tea plant is an evergreen of the *Camellia* family that is native to China and Northern India. There are two main varieties of the tea plant. The small leaf variety, known as *Camellia sinensis var sinensis*, thrives in the cool, high mountain regions of central China and Japan. The broad leaf variety, known as *Camellia sinensis var assamica*, grows best in the moist, tropical climates found Northeast India and the Szechuan and Yunnan provinces of China (Mendilcioglu, 2000).

China is one of the major tea producers (941.000 tons) and exporters and followed by India (831.000 tons), Sri Lanka (308.000 tons), Kenya (295.000 tons) and Turkey (202.000 tons), respectively (Anon., 2005).

The production of tea in Turkey mainly started in the early years of the Republic along the Eastern Black Sea Region. Many of the tea plantations are centered around the Rize city, and from the Georgian border to Trabzon, Arakli, Rize, Karadere and Fatsa (near Ordu), reaching in some places 30 kilometers inland and reaching the altitude of around 1000 m (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 therapeutic value of tea for the prevention and treatment of many diseases has become more and more commonly known (Staag and Millin, 1975; Zaveri *et al*., 2002).

Tea is 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).

Recently, there has been increasing interest in finding plants with high antioxidant capacities since they can

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protect the human body from free radicals and retard the progression of many chronic diseases (Moeller *et al*., 2000; Miliauskas *et al*., 2004). Young tea shoots contain more than 35% of their dry weight in polyphenols. Non-fermented green tea contains predominantly flavanols, flavandiols and phenolic acids like gallic acid, coumaric acid or caffeic acid, with those in green tea being higher than those in black tea (Serafini *et al*., 1996). The phenolic compounds that are 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 *et al*., 1995).

There were studies related to chemical composition of tea shoots (Xie *et al*., 1998; Ferrara *et al*., 2001; Fernandez *et al*., 2002). However the variation of chemical content present in tea shoots in different harvest time in particular in Turkey has not been studied so far. Therefore the aim of the study to investigate the seasonal variation of total phenolics, antioxidant activity and minerals in young tea shoots of seven tea clones in Turkey.

## MATERIALS AND METHODS

### *Chemicals*

All chemicals used were analytical grade and were bought from Sigma (USA).

### *Collection and preparation of tea samples*

Fresh tea shoots (*C. sinensis*), consisting of one apical bud and two adjoining leaves, around 500 g, were hand-plucked from commercial clones, namely Muradiye, Tuglali, Gundogdu, Enstitu 9, Hamzabey, Hayrat and Pazar 20. The clones are found together in the same plantation in Ataturk Tea and Horticulture Research Institute located at Rize city in Eastern Black Sea region of Turkey. The clones were same age. The samples were collected from these commercially produced clones at three harvest season (15 May, 15 July and 15 September) in both 2006 and 2007 year. There were no statistically differences between years in terms of chemical profiles among shoots therefore the results of two years were pooled. The collected fresh shoots from 10 plants per clones were cleaned and cut into small pieces before being dried in a hot air-blowing oven at 45 °C for 3 days. Dried samples were ground to a fine powder with a mortar and pestle and kept at room temperature prior to extraction. The dried shoots were used for the analysis of antioxidant activity, total phenolics and minerals.

### *Determination of total phenolics in tea shoots*

Total phenolics in the methanol extracts were determined colorimetrically using Folin-Ciocalteu reagent as described by Slinkard and Singleton (1997). Gallic acid was used as a Standard and results were expressed as mg gallic acid equivalents per g dry weight basis.

### *Determination of antioxidant activity*

Antioxidant activity of samples was determined by using β-Carotene–linoleic acid assay. In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Kaur and Kapoor, 2002). Antioxidant capacities of the samples were compared with those of BHA and the blank.

### *Determination of mineral elements*

The tissues sampled at heading were oven-dried at 68$^{o}$C for 48 h and ground. The Kjeldahl method (Bremner, 1996) and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N. Macro (P, K, Ca and Mg), and micro elements (Na, Fe, Mn, Zn and Cu) were determined after wet digestion of dried and ground sub-samples in a $H_2SO_4$-Se-salisilic acid mixture. In the diluted digests, P was measured spectrophotometrically by the indophenol-blue method after its reaction with ascorbic acid by an Aqumat UV/VIS spectrophotometer (Thermo Electron Spectroscopy LTD, Cambridge, UK). Potassium, Ca, Mg, Na, Fe, Mn, and Zn, analysis were determined by atomic absorption spectrometry (Perkin Elmer 3690) (AOAC, 2005).

## 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

The total phenolic content of young fresh tea shoots belongs to seven clones is given in table 1. The statistically important differences on total phenolics were obtained in different harvest times in tea clones ($p<0.05$).

Total phenolic content were continuously increased from 1$^{st}$ harvest to 3$^{rd}$ harvest in all clones, which found to be 87.55-252.09 mgGAE/g DW in Muradiye clone, 45.73-125.73 mgGAE/g DW in Tuglali clone, 78.46-222.09 mgGAE/g DW in Gundogdu clone, 33.00-111.18 mgGAE/g DW in Enstitu 9 clone, 83.00-194.82 mgGAE/g DW in Hamzabey clone, 62.09-246.64 mgGAE/g DW in Hayrat clone and 90.27-291.18 mgGAE/g DW in Pazar 20 clone, respectively (table 1). Robertson (1991) previously reported that fresh tea shoots are extremely rich in phenolic compounds which can constitute up to 300 mg/g of dry material. Juliani and Simon (2002) are also reported that green tea shoots is very rich for total phenolics and total phenolic content of green tea shoots 4-5 times higher than phenolic rich cinnamon (*Ocimum basilicum*) and oregano (*Origanum vulgare*) plants.

The great difference of tea shoots in terms of total phenolics at different harvest time is supposed to the effect of change of ecological parameters. Rize city takes more sunlight in September month (3rd harvest) than the other harvest times. This may affect the biosynthesis of total phenolics. On the other hand, in this month in Rize region, the differences between day and night temperatures are also higher and the rainfall was irregular. In this stress conditions, tea clones may produce more phenolics.

It has been shown that the biosynthesis of phenolic compounds in tea shoots can be effectively induced by stronger sunlight and length of daytime (Harbowy and Balentine, 1997). That is why in shaded tea flushes the concentrations of polyphenols are much lower (Mahanta and Baruah, 1992). On the basis of this information, the differences in total phenolic levels between fresh tea shoots harvested in different months in Turkey may not be just a temperature effect but also a day length and sunlight effect. However, further studies are required to elucidate the induction of the biosynthesis of total phenolics by day length and sunlight exposure correlating to the UV index. Previously, under field conditions, the phenolic composition of tea shoots varies considerably with seasonal, genetic, and agronomic factors and mechanisms that induce seasonal variations on total phenolic content in tea shoots may include one or all three of the following environmental conditions: day length, sunlight, and/or temperature, which vary markedly across seasons (Hilton and Palmer-Jones, 1973). The highest total phenolic levels in tea shoots are important for public by reducing the risk of atherosclerosis and coronary heart disease, which can be caused by oxidation of low-density lipo proteins (Shahidi and Wanasundara, 1992).

The present total phenolic content results would indicate that there is potential to produce higher quality black tea during the September months in Turkey. These results are in agreement with the findings of Yao *et al*. (2005) which found more phenolics occurred in relatively warmer months in the shoots. On the other hand, the differences were observed on total phenolic content in shoots among tea clones (table 1). Obanda *et al*. (1997) showed the level of phenolics in green tea shoots varied among clones.

Antioxidant activity of fresh tea shoots in seven clones is given in table 1. There were statistically differences among harvest times in all tea clones except Muradiye ($p<0.05$). Antioxidant activity was increased from 1st harvest to 3rd harvest times in all tea clones (table 1).

Antioxidant activity were found to be between 95.71-98.47% in Muradiye clone, 76.45-97.20% in Tuglali clone, 88.23-97.92% in Gundogdu clone, 85.85-97.98% in Enstitu 9 clone, 85.45-96.53% in Hamzabey clone, 88.70-98.26% in Hayrat clone and 89.53-98.95% in Pazar 20 clone, respectively (table 1). Antioxidant activity of

**Table 1**: Seasonal variation of antioxidant activity and total phenolic content of tea shoots

| Clones | Harvest date | Antioxidant activity (%) | Total phenolic content mgGAE/g DW |
|---|---|---|---|
| Muradiye | May | 95.71ns | 87.55c |
| | July | 95.84 | 113.91b |
| | September | 98.47 | 252.09a |
| Tuglali | May | 76.45c | 45.73c |
| | July | 90.58b | 108.46b |
| | September | 97.20a | 125.73a |
| Gundogdu | May | 88.23b | 78.46b |
| | July | 94.64ab | 83.00b |
| | September | 97.92a | 222.09a |
| Enstitu 9 | May | 85.85b | 33.00c |
| | July | 94.75ab | 83.00b |
| | September | 97.98a | 111.18a |
| Hamzabey | May | 85.45b | 83.00b |
| | July | 95.44a | 91.18b |
| | September | 96.53a | 194.82a |
| Hayrat | May | 88.70b | 62.09c |
| | July | 92.17ab | 122.09b |
| | September | 98.26a | 246.69c |
| Pazar 20 | May | 89.53b | 90.27b |
| | July | 95.33ab | 95.73b |
| | September | 98.95a | 291.18a |

*Values in the same column with different lower-case letters in same clone are significantly different at $P<0.05$. ns:non significant

**Table 2**: Seasonal variation of mineral content of tea shoots

| Clones | Harvest date | N (%) | P (%) | K ppm | Ca ppm | Mg ppm | Na ppm | Fe ppm | Cu ppm | Mn ppm | Zn ppm |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Muradiye | May | 2.13b | 0.199b | 13600b | 1200c | 504ab | 180b | 639a | 298a | 355a | 440a |
| | July | 2.23a | 0.298a | 15600a | 1600b | 450b | 206ab | 609b | 268ab | 345ab | 405b |
| | September | 1.80c | 0.168c | 12000c | 2200a | 630a | 216a | 540b | 252b | 300b | 372c |
| Tuglali | May | 2.94a | 0.274a | 14400b | 1600b | 630a | 270a | 882a | 412a | 490a | 608a |
| | July | 2.82a | 0.263a | 13600c | 1000c | 630a | 180b | 846b | 395ab | 470a | 583a |
| | September | 1.62b | 0.151b | 18800c | 2400a | 576b | 252ab | 486c | 227b | 270b | 335b |
| Gundogdu | May | 1.86b | 0.174b | 16000a | 1000b | 630a | 144b | 588b | 260b | 310b | 384b |
| | July | 1.92b | 0.179b | 13000b | 800c | 594b | 252a | 576b | 269b | 320b | 397b |
| | September | 2.85a | 0.266a | 15800a | 2400a | 630a | 252a | 855a | 399a | 475a | 589a |
| Enstitu 9 | May | 2.64b | 0.246b | 14000b | 1400b | 540b | 144b | 675b | 315b | 375b | 465b |
| | July | 2.76ab | 0.280a | 14600ab | 2000a | 558b | 216a | 900a | 420a | 500a | 620a |
| | September | 2.85a | 0.266ab | 15000a | 2200a | 648a | 180ab | 805ab | 379ab | 470ab | 569a |
| Hamzabey | May | 2.22a | 0.207a | 12000b | 1400b | 612ns | 216b | 666a | 311ns | 370a | 459a |
| | July | 1.65b | 0.154b | 12200b | 1200c | 540 | 288a | 495b | 231 | 275b | 341b |
| | September | 2.25a | 0.210a | 19600a | 2000a | 576 | 180c | 675a | 315 | 375a | 465a |
| Hayrat | May | 2.25ns | 0.210b | 14400ns | 1600c | 540ns | 126c | 792ns | 370ns | 440ns | 546ns |
| | July | 2.43 | 0.260ab | 14000 | 1900b | 570 | 288b | 808 | 400 | 470 | 580 |
| | September | 2.64 | 0.280a | 14200 | 2400a | 612 | 324a | 900 | 420 | 500 | 620 |
| Pazar 20 | May | 1.80b | 0.168b | 15800b | 2000c | 594ab | 180c | 540c | 252c | 300c | 372c |
| | July | 2.61a | 0.244ab | 18000a | 2200b | 486b | 270a | 783b | 365b | 435b | 539b |
| | September | 2.58a | 0.280a | 13800c | 2600a | 648a | 216b | 900a | 420a | 500a | 620a |

*Values in the same column with different lower-case letters in same clone are significantly different at $P<0.05$. ns: non significant

BHA (200 mg/l) was 91.18%. All extracts sampled from 3th harvest in all clones had higher antioxidant activity than BHA (table 1). Similar to 3rd harvest, all clones also showed higher antioxidant activity at 2nd harvest time than BHA, except Tuglali clone. However, in 1st harvest time, only Muradiye clone had higher antioxidant activity than BHA (table 1). The other clones had lower antioxidant activity than BHA at 1st harvest time.

In previous studies conducted on tea, the antioxidant activity of different tea products in different solvent was found between 56-83% (Turkmen *et al*., 2006; Karori *et al*., 2007). Tavazzi and Offord (2001) revealed that commonly consumed products such as tea, coffee and cocoa have possessing significant amount antioxidant activity.

The results for antioxidant activity clearly outline that tea shoots could be one of the richest sources among plants in terms of antioxidant activity. The great difference of tea shoots for antioxidant activity at different harvest time is supposed to the effect of change of ecological parameters. The composition of tea shoots varies with climate, season, variety, and age of the shoot (Leung and Foster, 1996). Tea and its constituent catechins are best known for their antioxidant properities, 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 and Ahmad, 2000; Yang *et al*., 2002).

There were strong relationships between antioxidant activity and total phenolics in all harvest dates in all tea clones ($R$=0.998, $p<0.05$). Juliani and Simon (2002) are also reported strong relationships between antioxidant activity and total phenolics in tea shoots which support our findings.

The mineral contents of tea shoots at different harvest times in seven tea clones are shown in table 2. Differences among the different harvest times were observed based on the mineral compositions in all tea clones (table 2).

The amount of N in tea leaves was the highest at 3rd harvest time in Gundogdu, Enstitu 9, Hamzabey and Hayrat clones between 2.25-2.85%, respectively. However, in Muradiye and Pazar 20 clones the amount of N was the highest in 2nd harvest (2.23-2.61%). In Tuglali clone the amount of N was the highest at 1st harvest time (2.94%), respectively (table 2).

The amount of P in tea leaves were between 0.151-0.280% and it was the highest at 3rd harvest time in Gundogdu, Hamzabey, Hayrat and Pazar 20 clones (table

2). Similar to N, the Tuglali clone had highest P content at 1st harvest time as 0.274%.

The K content of fresh tea shoots were between 12000-19600 ppm among clones at different harvest times. Three out of seven clones had the highest K content at 3rd harvest time (Tuglali, Enstitu 9 and Hamzabey). The other clones had higher K content at 2nd (Muradiye and Pazar 20 clones) and 1st harvest (Gundogdu and Hayrat clones) (Table 2).

All tea clones had the highest Ca content at 3th harvest time between 800-2600 ppm (table 2). Mg content was also highest at the 3rd harvest time in most of the clones, except Tuglali and Hamzabey (table 2).

Most of the tea clones had the highest Fe, Cu, Mn and Zn content at 3th harvest time (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. In this study, while the existence of ten minerals was determined in all harvest times, K was predominant, followed by N, Ca, Mg and P, respectively (Table 2). It is previously reported that N content of tea leaves were between 3.0-4.0% (Owuor and Wanyoka, 1983) which is higher than our results. Ozgumus *et al*. (1982) found that P content of tea leaves were between 0.31- 0.40% which close to our P results. In addition, our K results are in accordance with Kacar *et al.* (1979).

It is noteworthy that minerals are important not only for human nutrition, but for plant nutrition as well. Potassium, a mineral essential for controlling the salt balance in human tissue, can be detected. Zinc, a trace mineral that is especially important for normal functioning of the immune system, is present in good levels in tea shoots in seven tea clones. Calcium, a mineral which is essential to bone structure and function, is relatively high in seven tea clones.

## CONCLUSION

it can be concluded that tea shoots are a valuable product, based on its rich and beneficial nutrient composition. It seems that the most important chemical components such as phenolics were highest at the last harvest time in Turkey. However, certain growing conditions and cultural management techniques affecting the chemical components of tea shoots will be the subject of further research projects.

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