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Total Antioxidant Capacity of Selected Grades of Black Tea Grown in Different Geographical Elevations in Sri Lanka

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ABSTRACT. *Antioxidant (AO) potential of tea has been suggested by several researchers and flavonoids in tea are thought to contribute to this AO activity. The aim of the present study was to evaluate the possible effects of infusion time and amount of tea on total antioxidant capacity (TAC) of black tea extracts obtained from tea grades manufactured in different elevations in Sri Lanka. Low-(LG), mid- (MG) and high-grown (HG) black tea grades of Broken Orange Pekoe (BOP), Flowery Broken Orange Pekoe (FBOP), Orange Pekoe (OP), Flowery Fannings (FF) and Dust (D) and HG and LG Broken Orange Pekoe Fannings (BOPF) were evaluated for TAC determined using ferric reducing ability of plasma (FRAP) assay. Results showed that TAC was significantly increasing with increasing amount of tea and infusion time taken to brew the tea ($P<0.05$). Also, different grades manufactured from teas grown in three elevations showed significant differences in TAC ($P<0.05$). Dust of all three elevations gave the highest TAC at 1 min infusion irrespective of the amount. When 5 g of each grade was tested for TAC, BOPF showed maximum TAC in 2 and 4 min infusions among high grown tea grades. Infusions of FF showed the highest TAC among the tea grades of low and mid country at all amounts at 2 and 4 min infusion times. Of all grades, OP showed significantly the lowest TAC of all three elevations among all tea grades during all infusion times irrespective of the amount. Overall, MG FF showed the highest TAC ($108.5 \pm 1.5 \times 10^3$ µM) when 5 g was infused for 4 min. It was concluded that, regardless of the elevation and grades, maximum antioxidant capacity of tea could be obtained from 5 g of tea infused for 4 min. However, there was an interaction between grade and elevation. Among all grades Dust was the quickest in exerting TAC and 5 g, 4 min infusion of FF showed the highest TAC.*

INTRODUCTION

Infusion of traditional tea (*Camellia sinensis*) is characterized by a high content of flavonoids with levels approaching 200 mg/cup for a typical brew of black tea (Lakenbrink *et al.*, 2000). Flavonoids are a large group of plant metabolites with a variety of phenolic structures. They have unique biological properties and may be responsible for many of the health benefits of tea including its strong antioxidant (AO) property. Many in vitro studies showed that the flavonoids present in tea possess strong AO and metal chelating properties and may therefore protect cells and tissues against reactive oxygen species. A large number

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of studies support the hypothesis that oxidative damage to DNA, lipids and proteins may contribute to the development of cardiovascular disease, cancer and neurodegenerative diseases. Reactive oxygen and nitrogen species are formed in the human body and endogenous AO defense mechanism are not always sufficient to counteract them completely. Diet-derived AO may therefore be particularly important in protecting against chronic diseases (Halliwell, 1996; Vendemiale *et al.*, 1999).

The flavonoid concentration of any particular tea beverage depends on the type of tea (*e.g*., blended, decaffeinated, instant etc) and preparation (e.g., amount used, brew time, temperature). Decaffeinating slightly reduces the catechin content of black tea, while herbal infusions (often called "herbal teas") contain neither catechins nor caffeine (Wehrwein, 1999). The highest concentration of flavonoids (541–692 µg/ ml) is found in brewed hot tea (Hakim *et al.*, 2000), less in instant preparations (90–100 µg/ml) and lower amounts in iced and ready-to-drink tea (Arts *et al.*, 2000). Black tea infusate gave maximal antioxidant potential by 5 min and for the green teas, even at 15 min of infusion time, it was impossible to measure maximal antioxidant potential (Langley-Evans, 2000).

Tea produced in Sri Lanka is sorted into ‘grades’ according to size and/or appearance of teas. Sri Lankan teas are divided into two groups: (1) larger ‘Leaf’ grades and (2) smaller ‘Broken’ grades. There are two types of ‘Leaf’ grades: Orange Pekoe (O.P), Pekoe (Pek.), and Souchong (Sou.). Broken grades are divided mainly into five categories: Broken Orange Pekoe (B.O.P.), Broken Pekoe (B.P.), Broken Pekoe Souchong (B.P.S.), Broken Orange Pekoe Fannings (B.O.P.F.), and Dust (D) (Sivapalan *et al.*, 1986)

The AO potential of tea probably depends on environmental factors such as sunlight, variety, geographical location, processing methods etc. It is generally believed that among several grades of Sri Lankan black tea, dust which has the smallest particles may have the highest AO capacity. Dust is the cheapest tea grade which is commonly consumed by local consumers, whereas ‘leafy’ and other ‘broken’ grades have high demand in the export market and among affluent consumers locally. However, the beneficial effects of tea in terms of AO are not known by the consumers as well as the producers. The objective of the study was to evaluate the effect of different tea grades, infusion time and amount of tea used on total antioxidant capacity (TAC) of black tea grown in different geographical elevations in Sri Lanka.

MATERIALS AND METHODS

Selection of tea grades

The following black tea grades were selected from high (>1200 m above mean sea level), mid (600-1200 m above mean sea level) and low (<600 m above mean sea level) grown teas in Sri Lanka: Broken Orange Pekoe (BOP; particle size between 1.4-0.85 mm), Flowery Broken Orange Pekoe (FBOP; particle size between 2.0-1.70 mm), Orange Pekoe (OP; particle size between 2.0-1.70 mm), Flowery Fannings (FF; particle size between 1.0-0.5 mm) and Dust (D; particle size between 0.5-0.42 mm) of all three elevations and Broken Orange Pekoe Fannings (BOPF; particle size between 0.85-0.5 mm) of tea grown in high and low elevations.

Dried black tea samples of different grades were obtained from St. Coombs Estate of the Tea Research Institute (TRI) of Sri Lanka, Thalawakele and Kelani Valley Plantations Limited from their factories located at different tea growing areas. All the tea samples were received at the laboratory less than 2 weeks after processing. All samples were kept in amber colour, air-tight plastic bottles until analysis.

Sample preparation

One, three and five grams of tea from each tea grade from different locations were weighed and 150 ml of deionized boiling water was added to each sample. The temperature of the sample was maintained at 90 ºC keeping in a water bath with constant stirring. One milliliter samples of the tea were drawn at one, two and four minutes. One milliliter of deionized water was added to the drawn 1 ml infusion to dilute. Samples were centrifuged at 4000 rpm immediately for 10 min after withdrawal to remove tea flakes and sediments. Supernatant fractions were immediately frozen and stored at -70ºC. All tea infusions were further diluted 40:1 using deionised water prior to assay.

Ferric reducing ability of plasma (FRAP) assay

The antioxidant potential of the tea samples was determined using a modified ferric reducing ability of plasma (FRAP) assay (Benzie and Strain, 1996). Briefly, freshly prepared 3 ml of FRAP reagent (10 mM TPTZ in 40 mM HCl solution plus 20 mM $FeCl_3.6H_2O$ and 300 mM acetate buffer) was mixed with 100 µl of test sample, deionized water (blank), or standard as appropriate. Standard solution of $FeSO_4.7H_2O$ of known concentrations in the range of 100-2000 µM was used for the standard curve. Absorbance readings were taken within 6 min after mixing using the spectrophotometer (Jenway model 6305, Jenway Ltd, England) and the values were determined against the standard curve.

Statistical analysis

Four-factor factorial ANOVA was performed to identify the main effects of different tea grades, elevations, amounts used and infusion times on TAC and the interactions among tea grades, amount of tea and infusion time. Duncan's New Multiple Range Test (DNMRT) was carried out to identify the statistically significant differences in the mean total antioxidant capacity values. Statistical analysis was performed using the Minitab version 14.0 and SPSS version 10.0 software packages.

RESULTS

There was no significant main effect of elevations and grade on TAC. However the amount of tea and infusion time had a significant effect on TAC. Except for elevation x amount x time interaction, all the other interactions (elevation x grade, elevation x amount, grade x amount, grade x time, amount x time, elevation x grade x amount, elevation x grade x time, grade x amount x time) were statistically significant ($P<0.05$).

Effect of infusion time on TAC

Table 1 shows the changes in TAC of 5 g of teas with different infusion times (1 min, 2 min and 4 min). TAC of tea significantly increased with infusion time. The highest TAC was observed when tea was infused for 4 min in all grades of tea from the three elevations. Dust of all elevations gave the highest TAC at 1 min infusion. Among tea grades of both low- and mid- grown, FF showed the highest TAC at 2 min and 4 min infusion times. Broken Orange Pekoe Fannings showed the highest TAC among high-grown tea grades ($P<0.05$) at 2 min and 4 min infusion times.

Table 1. TAC of 5 g of high, mid, and low grown tea grades with infusion time 1 min, 2 min and 4 min (TAC x 10^3 μM)

Infusion time	BOP	FBOP	FF	OP	BOPF	Dust
High grown tea						
1 min	75.29	33.58	37.42	27.57	68.61	79.65
2 min	86.38	54.66	56.10	43.71	89.92	89.53
4 min	93.23	62.15	72.33	55.11	97.41	93.30
Mid grown tea						
1 min	42.31	31.53	58.49	21.10	Not analysed	82.34
2 min	66.88	47.82	94.03	33.67	Not analysed	91.54
4 min	80.59	59.76	108.49	43.50	Not analysed	96.27
Low grown tea						
1 min	28.64	31.49	39.66	20.59	40.19	58.90
2 min	45.78	48.51	61.26	29.65	56.14	60.69
4 min	60.58	56.17	79.54	38.79	67.85	65.78

Effect of amount of tea on TAC

Table 2 shows the changes in TAC of different amounts of tea infused at 4 min. TAC of tea significantly increased with increasing amount of tea in all grades. The highest TAC was observed when 5 g of tea was infused in all grades of tea from the three elevations.

Elevation x Grade interaction

Overall, the highest TAC values were obtained for different tea grades when 5 g of tea was infused for 4 min. Table 3 shows TAC of different grades of tea grown in different elevations, measured at 4 min of infusion of 5 g of tea.

BOPF had significantly higher TAC than all other high-grown tea grades. FF had the highest TAC among mid-grown and low-grown tea grades. Dust had the second highest TAC in high- (along with BOP), mid- and low- (along with BOPF) grown tea grades. When TAC of tea grades from the three elevations was compared, high-grown BOP, FBOP, OP, BOPF were significantly higher than similar grades from other elevations. Mid-grown FF had significantly higher TAC than FF of other two elevations. There was no significant difference in TAC in high- and mid-grown Dust. Overall, the highest TAC was found in mid-grown FF.

Table 2. TAC of 4 min of high, mid, and low grown tea grades with amounts of 1 g, 3 g and 5 g (TAC x 10^3 µM)

Infusion time	BOP	FBOP	FF	OP	BOPF	Dust
High grown tea						
1 g	31.06	14.26	18.50	12.44	19.98	13.87
3 g	63.41	40.66	44.96	37.44	57.06	52.16
5 g	93.23	62.15	72.33	55.11	97.41	93.30
Mid grown tea						
1 g	20.14	13.19	24.89	9.90	Not analysed	14.52
3 g	52.71	43.87	61.76	25.50	Not analysed	56.60
5 g	80.59	59.76	108.49	43.50	Not analysed	96.27
Low grown tea						
1 g	13.10	12.29	20.51	9.12	16.82	12.59
3 g	34.58	35.77	51.14	24.12	42.37	48.23
5 g	60.58	56.17	79.54	38.79	67.85	65.78

Table 3. Comparison of TAC (x10^3 µM) of tea grades (5g; 4 min) between and within elevation

Grade	HG	MG	LG	HG *vs* MG	HG *vs* LG	MG *vs* LG
BOP	93.23^b	80.59^c	60.58^c	*	*	*
FBOP	62.14^d	59.76^d	56.17^d	*ns*	*ns*	*ns*
FF	72.33^c	108.49^a	79.54^a	*	*ns*	*
OP	55.10^e	43.49^e	38.78^e	*	*	*ns*
BOPF	97.40^a	Not analyzed	67.85^b	-	*	-
Dust	93.30^b	96.27^b	65.77^b	*ns*	*	*

Means with similar superscripts within a column are not significantly different (DNMRT)
*$P<0.05$; ns, not significant (Bonferroni comparison of grades)

DISCUSSION

In the present study, black tea grades grown in three elevations of Sri Lanka, namely high-grown, mid-grown and low-grown, were analyzed to evaluate TAC. Land elevation is one of the determining factors of polyphenols in tea which is responsible for TAC. Tea is graded according to the size of the particle and it can also affect the TAC due to differences in surface area responsible to effective extraction. Present study also considered the effects of commonly used infusion time and the amount of tea combination for TAC analysis.

In the present study, increasing both infusion time and amount resulted in an increase in TAC in all tea grades. This indicates substances with AO capacity could have been released from tea with increasing time. As black tea contains 15-30% flavonoids (Hara, 1997) they might contribute to increase in TAC in tea infusion.

Dust released AO into the infusion most rapidly, hence dust showed the highest TAC after 1 min infusions in all grades of tea grown in three elevations. Among the tea grades, Dust has the smallest particle size. Small particles have larger surface area, which allows phenolic substances in tea to be released into the infusion. Release of AO from other grades with larger particle size happens slowly. Among the high-grown tea grades, BOPF showed the highest TAC at 2 and 4 min infusion times. Though BOPF showed lower TAC in 1 min, it started showing higher TAC with increasing infusion time. This suggests that BOPF needs longer infusion times than that of Dust grade to release compounds responsible for TAC within a short period of time. FF of both mid- and low- grown also needs longer infusion time compared to Dust of their elevations. OP is a leafy grade with the largest particle size of all tea grades analysed, whereas the others were broken grades with smaller particles. Smallest surface area of OP among all grades might be the reason for the lowest TAC value. In another study antioxidant potential of the high grown BOPF grade of Sri Lankan black tea (a high demand tea among Sri Lankan tea drinkers) was examined, both *in vitro* and *in vivo*, using rat blood serum (Abeywickrama *et al*, 2005). They found that BOPF possessed mild but amount-dependent antioxidant activity *in vitro* and the *in vivo* antioxidant activity was both amount- and time- dependent.

A large variability of TAC among the black teas grown in different elevations observed in our study can be explained by the influence of different processing, maturation, harvest season, sunlight etc., that would affect the level of AO present in the teas. Astill *et al.,* (2001) found that variety, growing environment, manufacturing conditions, and grade (particle size) of the tea leaves each influence the tea leaf and final infusion compositions

Since 5g of mid-grown FF infused for 4 min showed the highest TAC compared with all other tea samples ($108.5 \pm 1.5 \times 10^3$ µM). It can be suggested that to get similar AO amount as FF, a higher amount of tea from other grades should be infused for a long period of time. When three elevations were compared high-grown BOP, FBOP, OP, BOPF showed the highest TAC compared with mid- and low-grown teas of the same grade. Higher geographical elevation provides lower environmental temperature to tea plants than lower elevations. Lower temperature slows down the growth of the tea plant causing the leaves to develop slowly, and, thus, to be more flavorful. As polyphenols are responsible for astringency and flavour of tea infusion (Lesschaeve & Noble, 2005) it can be expected that high grown tea grades must be having more polyphenols than mid- and low- grown grades.

CONCLUSIONS

According to the findings of this study it can be concluded th at, *in vitro* TAC in black tea mainly depends on the amount and infusion time. Also, tea grades manufactured in different elevations can also affect the TAC. In vivo human studies are suggested to evaluate further effect on TAC of different grades of black tea grown in different elevations in Sri Lanka.

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