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Effects of soaking conditions on total phenolic and antioxidant activity of black tea and black tea combined (50:50) with bush tea

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ABSTRACT

Black tea (*Camellia sinensis*) and black tea combined with bush tea (*Athrixia phylicoides*) were analyzed for their polyphenol content, antioxidant activity and tannin contents. These teas were brewed at several combinations of temperature and soaking duration [30°C for 3 and 10 min., 60°C for 3 and 10 min. and 90°C]. Solvent extraction methods were used for extracting polyphenols, antioxidant activity and tannin contents. A completely randomized design (CRD) with three replications for each treatment was employed. Results showed that total polyphenols, antioxidant activity and tannin content of black tea decreased with decrease in temperature. At 90°C for 3 min., 7.68 mg/100 g of total polyphenols, 3.85 µmol/g of antioxidants and 2.81 mg/ 100 g of tannins were achieved and these amounts decreased to 5.50 mg/100 g for total polyphenols, 1.31 µmol/g of antioxidant activity and 0.72 mg/100 g of tannins at 30°C for 10 min. Combining two teas (50:50) resulted in significant ($p<0.005$) decrease in total polyphenols, antioxidant and tannin content. At high temperature of 90 °C for 3 min, 2.64 mg/100 g of total polyphenol, 2.48 µmol/g of antioxidants and 1.8 mg/ 100 g of tannins were achieved and these amounts decreased to 1.39 mg/100 g for total polyphenols, 0.35 µmol/g of antioxidant activity and 0.64 mg/100 g of tannins at 30 °C for 10 min. Hence, it is recommended to brew tea at 90°C for 3 min. which gives optimum polyphenols and antioxidant activity in the brewed liquor.

Key words: Brewing temperature, bio-chemical analysis, black tea, bush tea.

INTRODUCTION

Potential health benefits of tea, together with its popularity as a beverage, have prompted numerous investigations on the chemical constituents of tea and their biological properties such as antimutagenic (Halder *et al*., 6), anticarcinogenic and antioxidant (Sarkar and Bhaduri, 14) and antiallergic (Maeda-yamamoto *et al.,* 12) properties. Antioxidants have many favourable effects on human health, they may reduce the incidence of cancer and heart diseases by inhibiting oxidation of low density lipoprotein, boost the immune system, detoxify contaminants and pollutants, and reduce inflammation (Bonilla *et al*., 3). The antioxidant action of tea polyphenol compounds depends on their free radical scavenging capacity and iron reducing properties. Depending on manufacturing process, teas can be classified into three major groups: non-fermented green tea, semi-fermented oolong tea and fermented black tea. The major phenolic compounds in tea are catechin (flavonols and flavanol gallates) which can be oxidized to form theaflavins (TF), and thearubigins (TR) (Lakenbrink *et al*., 10).

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The standard processing techniques used in China and South West Asia for oolong tea is to soakt in hot water (>90°C) using a covered ceramic pot (Gong and Gu, 5) carefully followed by stirring and steeping conditions for few minutes in order to avoid extraction of catechin or theaflavins (Su *et al*., 18, 19). Too high water temperature has the undesirable effect of 'overcooking' the tea in confinement, resulting in a yellowish and cloudy infusion which is very much bitter in taste. Its substantial vitamin content could easily be destroyed. Zhu *et al*. (25) reported that 100 g of extract of dry oolong tea using boiling water (1l) had greater antioxidant activity and free radical scavenging capacity than 0.02% butylated hydroxyanisole (BHA). Benzie and Szeto (2) tested 25 different tea extracts including five oolong teas, and found that one cup (about 20 ml) of tea extract (12% w/v tea in water) had the same antioxidant activity potential as 150 mg of ascorbic acid.

Herbal tea quality is one of the critical factors determining the price of tea for export. The sensory quality attributes are astringent taste, bitterness, sweetness and aroma (Hu *et al*., 8). Research indicates that extraction conditions, variety, extracting solvent and processing methods can influence total polyphenol, antioxidant and tannin content of tea. Turkmen *et al*. (2007) reported that aqueous extract of black tea

extracted for a long time using aqueous acetone contained high level of polyphenols and antioxidants activity whereas lower levels of polyphenols and antioxidant activity were obtained when using absolute acetone as an extracting solvent for a short time. The aim of this study was to determine the total polyphenol content and antioxidant activity of black tea and combined black tea and bush tea at various brewing temperatures.

MATERIALS AND METHODS

Black tea leaves were collected at Mukumbani tea estates 22°53'60S and 30°25'0E 724 a.m.s.l. (sub-tropical type climate of summer rainfall and cold and dry winter). The tea was processed using standard commercial practice. Wild bush tea leaves were collected from the wild at Hazeyview 25°1'60S and 31°7'0E 524 a.m.s.l., and dried under the shade for three to four weeks (subtropical type climate that is summer and winter rainfall, cold and dry winter). Treatments consisted of brewing temperature (30, 60 and 90°C) and brewing duration (3 and 10 min.). Completely randomized design (CRD) was used for each treatment and experiment was replicated three times.

Black tea and combined black tea and bush tea were brewed using water baths at different temperatures and time, thermometer was used to measure the temperature of water inside the beaker. Distilled water (600 ml) was boiled and poured to a 1000 ml beaker in thermostat water bath, when desired temperature was attained; six g of black tea was measured and added. Tea was then brewed at 30°C for 3 and 10 min., 60°C for 3 and 10 min., 90°C for 3 and 10 min. Magnetic stirrer was used and stop watch was used to record time. Tea solutions were dried below 70°C under reduced pressure on an Eyela CA-1111 Rotavapor to give a crude powder.

Methanol was used as the extraction solvent for the determination of total phenols. Duplicates of 2 g of samples were extracted using 30 ml of the solvent. An amount of 10 ml of methanol was added to 2 g of sample in centrifuge tubes and the samples were vortex mixed every 10 min. for 2 h to improve extraction efficiency. The samples were then centrifuged at 3500 rpm for 10 min. (25°C) using centrifuged tubes. Each sample residue was rinsed three times with 10 ml of solvent. Three supernatants (30 ml) were combined and used for analysis. The Folin-Ciocalteau method (Singleton and Rossi, 15), modified by Waterman and Mole (22), was used to determine total phenols. This method was based on the reducing power of phenolic hydroxyl groups (Hahn *et al*., 7), which react with the phenol reagent to form chromogens that can be detected spectrophotometrically. In brief, methanol extract (0.5 ml) was added to a 50 ml volumetric flask containing distilled water and mixed. Folin-Ciocalteau phenol reagent (2.5 ml) was then added and mixed, followed by 7.5 ml sodium carbonate solution (20 g/100 ml) within one to eight min. after addition of the Folin-Ciocalteau phenol reagent. The contents were mixed and the flask made up to volume with distilled water, stoppered and thoroughly mixed. Absorbance of the reactants was read after 2h at 760 nm using UV-visible spectrophotometer. Catechin was used as standard to prepare a standard curve and results were expressed as mg eqv/100 mg of samples on dry weight basis.

Tannin assay was done following the vanillin-HCl method of Prince *et al*. (13). The extracts and reagents were maintained at 30°C. The methanolic extract (1 ml) was added to 5 ml vanillin reagent (4% HCl in methanol and 0.5 ml vanillin in methanol) and mixed. Sample blanks were done with 4% HCl in methanol replacing the vanillin reagent. The reactants were maintained at 30°C and absorbance read at 500 nm after 20 min. Catechin was used as a standard and results were expressed as mg catechin eqv/100 mg sample on a dry weight basis.

Antioxidant activity of the extracts was determined using Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Awika and Rooney (1). TEAC is a spectrophotometric technique that measures the relative ability of hydrogen-donating antioxidants to scavenge the $ABTS^+$ radical cation chromogen in relation to that of Trolox, the water soluble vitamin E analogue which is used as an antioxidant standard. The $ABTS^+$ was produced by mixing equal volume of 8 mM ABTS with 3 mM potassium persulfate prepared in distilled water and allowed to react in the dark for at least 12 h at room temperature before use. The $ABTS^+$ solution was diluted with a phosphate buffer solution (pH 7.4) prepared by mixing 0.2 M of NaH_2PO_4, 0.2 M $NaHPO_4$ and 150 mM NaCl in 1 litre of distilled water, with pH adjustment using NaOH, where necessary. This solution was made fresh for each analysis. The $ABTS^+$ solution (2900 µl) was added to the methanol extracts of tea (100 µl) of Trolox in a test tube and mixed. Absorbances reading were done at 734 nm and were taken after 30 min. (for the samples) and 15 min. (for the standard) of the initial mixing of the samples and standard respectively. The results were expressed as µM Trolox eqv/g of sample on a dry weight basis.

Statistical analysis was done by using Waterman and Mole (224) method. All the analyses were done at Limpopo Agro-food Technology Station (LATS) at the University of Limpopo, South Africa. The collected data were subjected to analysis of variance (ANOVA) and the means were tested by confidence interval of 95% probability. Means were compared by least significant difference (LSD) test following SAS (2003).

RESULTS AND DISCUSSION

Effects of soaking conditions on total phenolic and antioxidant activity of black tea and black tea combined (50:50) with bush tea. Total polyphenols of black tea: total polyphenols decreased with decreasing temperature (Table 1). Brewing black tea at 90 °C for 3 min. achieved 7.68 mg/100 g total polyphenols and they decreased to 5.50 mg/100 g at 30°C for 10 min. These results indicate that at different temperatures there were different amounts of tea polyphenols obtained, however tea boiled at 90°C for 3 min. had significantly ($p<0.05$) higher amount of polyphenols than tea boiled at 30°C for 10 min.

Several studies done indicate that tea polyphenols possess wide range of biological and pharmaceutical benefits, including anticarcinogenic, antioxidative, and hypolipidemic activities (Buschman, 4). This suggests that tea boiled at 90°C for 3 min. will offer more health benefits to the consumer since it contains highest amount of polyphenols. Su *et al.* (18) reported increase of polyphenol with increase in temperature, but at highest temperature (100°C) with longer steeping period there were decrease in polyphenol content of oolong tea. By contrast, soaking temperature of 100°C and longer soaking time was associated with a higher percentage of polyphenols concomitant with a lower anticlastogenic efficacy. Liebert *et al.* (11) reported increase of black tea polyphenol with increased soaking times; polyphenols ranged from 33.8 mg/100 ml after 0.5 min. up to 68.4 mg/100 ml after 10 min. of brewing time.

The amount of polyphenol of black tea obtained in this study (Table 1) are not exactly the same as that reported by Libert *et al.* (11), the difference may be due to variety, growing environment, manufacturing conditions, and grade (particle size) of the tea leaves as each influences the tea leaf and final infusion compositions. The composition of the tea infusion was shown to be influenced by whether the tea was contained in a teabag and, if so, the size and material of construction of the bag. Finally, the preparation method, including the amounts of tea and water used, infusion time, and amount of agitation, was shown to be a major determinant of the component concentrations of tea beverages as consumed.

Results (Table 1) show that antioxidant activity also decreased with decrease in temperature, tea boiled at 90°C for 3 min. had significantly ($P<0.05$) strongest TEAC radical scavenging activity of 3.85 than 3.54 µmol/g obtained from tea boiled for 10 min. at the same temperature. The lowest TEAC antioxidant activity was obtained at tea boiled at 30°C for 3 and 10 min. (1.32 and 1.31 µmol/g respectively), which were not significantly different from each other. These results suggest that brewing tea at 90°C for 3 min. is giving optimum total antioxidant activity. Xie *et al.* (23) reported that water extract of oolong tea had higher antioxidant activity than black tea. Furthermore, Su *et al.* (19) reported that oolong tea exhibit strong radical scavenging activity with increase in soaking temperature and duration. Langley-Evans *et al.* (9) reported that both green and black teas released significant levels of antioxidants into the hot water within 2 min. of infusion and increasing the temperature increased antioxidant potential by 4 to 9.5-fold.

Tannin contents of black tea: tannin contents decreased with decrease in temperature (Table 1), at 90°C for 3 min. highest amount of tannin (2.81 mg/100 g) was obtained, which were not significantly different from 2.79 mg/100g obtained at tea boiled for 10 min. at the same temperature. Tannin decreased to 1.45 mg/ml at 60°C for 3 min. and this amount is significantly different from 1.32 mg/ml obtained for tea boiled for 10 min. at the same temperature. The lowest amounts of tannin obtained was 0.72 mg/100g, which were obtained from tea boiled at 30°C for 10 min. and significantly different from 0.81 mg/ml obtained from tea boiled for 3 min. Tannin contents are the main potential indicators of tea quality. They also influence the astringent taste of tea. Other researchers found that tea leaves have higher tannin content

Table 1. Extraction yields of total polyphenols, antioxidants activity and tannin contents of black tea.

Treatment	Total polyphenols (mg/ 100 g)	Antioxidant activity (µmol/ g)	Tannin (mg/100 mg)
90°C 3 min.	7.68[a]	3.85[a]	2.81[a]
10 min.	7.45[b]	3.54[b]	2.79[a]
60°C 3 min.	6.81[c]	2.48[c]	1.45[b]
10 min.	6.60[d]	2.42[c]	1.32[c]
30°C 3 min.	5.60[e]	1.32[d]	0.81[d]
10 min.	5.50[f]	1.31[d]	0.72[e]
SE ±	0.014	0.022	0.015

Means within each column followed by different letters are significantly different ($P<0.005$)

Table 2. Extraction yields of total polyphenols, antioxidants activity and tannin content of 50:50 combinations of black and bush teas.

Treatment	Total polyphenols (mg/100g)	Antioxidant activity (µmol/g)	Tannin (mg/100 mg)
90°C 3 min.	2.64[a]	2.48[a]	1.80[a]
10 min.	2.11[b]	2.45[a]	1.50[a]
60°C 3 min.	1.85[c]	1.44[b]	1.03[b]
10 min.	1.72[d]	1.43[b]	0.87[c]
30°C 3 min.	1.42[e]	0.40[c]	0.86[c]
10 min.	1.39[e]	0.35[c]	0.63[d]
SE±	0.011	0.019	0.012

Means within each column followed by different letters are significantly different (P<0.005).

as compared to processed tea brands. This maybe influenced by difference in process of manufacture or the aging of the tea leaves.

Effects of soaking conditions on total phenolic, antioxidant activity and tannin contents of black tea and black tea combined with bush tea (50:50). Total polyphenols of combined bush tea and black tea: total phenols are shown in (Table 2), tea boiled at 90°C for 3 min. had significantly (p<0.05) higher total phenols than tea boiled at 30°C for 10 min. Total polyphenols decreased with decreased in temperature. Lowest amount of polyphenols were obtained at tea boiled at 30°C for 10 min. However there was decrease in total polyphenols after combining the two teas, for example black tea yielded 7.68 mg/100 g total polyphenol at 90°C for 3 minutes (Table 2) but black tea and bush tea yielded only 2.64 mg/100 g total polyphenols at the same temperature and time. In a study conducted by (Shanmuga and Sivasamy, 16), to improve the quality of made tea, blending studies were carried out, where different proportions of seedling leaves were blended with known quantity of clonal leaves. When the seedling teas proportion increased (3:2) or equal (1:1) to that of clonal leaves, the values of polyphenols content of made tea was significantly lower. On the other hand, when the clonal leaves proportion enhanced, the polyphenols content enhanced dramatically. According to Subbarao *et al*. (17) polyphenol content of the different plants parts may differ significantly as influenced by growth conditions.

The antioxidant activity decreased with decreases in temperature, higher antioxidant activity were achieved at 90°C for 3 and 10 min. (2.48 and 2.45 µmol/g, respectively), which were not significantly different from each other. Antioxidant activity continued to decrease and at 60°C for 3 and 10 min. (1.44 µmol/g) were achieved which were not significantly different 10 min. Lowest antioxidant activity were achieved at 30°C for 3 and 10 min. (0.40 and 0.35 µmol/g), respectively, which were not significantly different from each other. Liebert *et al.* (11) reported increase of polyphenols of black and green tea with increase in time and brewing temperature. At 90°C, brewing for 3 min. achieved tannins content of 1.80 mg/100 g tannin which was not significantly different from 1.50 mg/100 g achieved at the same temperature. Lowest tannins were obtained at 30°C for 10 min. 0.63 mg/100 g significantly different from 0.86 mg/100g obtained at tea boiled for 3 min. Tabasum *et al*. (20) found that tea leaves and choora tea (unprocessed) had higher tannin contents than processed tea brands. In conclusion it is recommended to brew tea at 90°C for 3 min. since it gives optimum polyphenols and antioxidant activity. Further study is required to determine sensory attributes and blending tea at different ratios.

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