

Full Length Research Paper

Antioxidant capacity of different types of tea products

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In the present study, twelve different types of commercial tea samples were assayed to determine their phenolic composition and antioxidant activity. Reverse phase high performance liquid chromatography using a binary gradient system was used for the identification and quantification of individual catechins. Subsequently, total phenolic content was determined spectrophotometrically according to the Folin-ciocalteus method. Total theaflavins and thearubigins were also determined. The radical scavenging behavior of the polyphenols on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was also studied spectrophotometrically. The results showed that total polyphenols, total catechins and antioxidant activity were significantly ($P < 0.05$) different in the commercial tea samples. Green tea had the highest levels of catechins, total polyphenols and total antioxidant activity. White tea (silvery tip) a rare specialty type of tea was not significantly different from green tea. Statistical analysis showed an essential catechin content influence of the tea extracts on antioxidant activity. Epigallocatechin gallate (EGCG) was the most potent catechin and the most potent in antioxidant activity ($r = 0.989^{***}$). Epigallocatechin (EGC) ($r = 0.787$, $P < 0.001$), epicatechin (EC) + catechin (+C) and epicatechigallate (ECG) also showed significant ($P < 0.05$) antioxidant activity. Black tea contained high levels of theaflavins and thearubigins, which accounted for most of the antioxidant potential in this type of tea product ($r = 0.930^{***}$ and $r = 0.930^{***}$ respectively). These results suggest that conversion of catechins during black tea processing did not affect the free-radical potency of black tea. Gallic acid (GA) also showed significant ($r = 0.530^*$) contribution to the antioxidant activity in black tea. Green, black and white tea products processed from Kenyan tea cultivars originally selected for black tea had significantly ($P < 0.05$) higher antioxidant activity than green tea processed from tea cultivars from Japan and China. These results seem to suggest that the cultivar type is critical in determining the antioxidant potency of tea product and that black teas processed from suitable cultivars could be potent in antioxidant activity when compared to green teas.

Key words: Antioxidant capacity, DPPH, catechins, polyphenols, EGCG, theaflavins.

INTRODUCTION

Processed tea, which is one of the most popular beverages, is manufactured from the young tender leaves of the plant *Camellia sinensis* (Cabrera et al., 2003). Two types of tea products are most widely consumed; green and black tea. In both cases, it is the chemical composition of the tea shoots and the reactions that occur during processing that determine the nature of the finished product and its quality. Though most of the tea produced in the

world can be classified as non-fermented/aerated green tea, semi-fermented (oolong) tea and fermented black tea (Reeves et al., 1987), processing has diversified to the production of specialty teas e.g. white tea, flavored teas, organic teas, decaffeinated teas, herbal teas, scented teas and various other blends. The manufacturing techniques of the above types of tea products, which may either be orthodox or non-orthodox, vary considerably and have a pronounced impact on the formative and degradative patterns of various cellular components. The conventional orthodox method which consists of rolling the leaf on a rolling bed, stretching and tearing the leaf has in some cases been replaced with non-orthodox me-

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thods or curl, tear and crush (CTC) which have a quicker and more severe leaf disruption leading to production of smaller fragments and consequently more oxidation (Mahanta and Hemanta, 1992).

In the preparation of green tea, the withered leaves are steamed and then dried relatively rapidly after plucking to minimize chemical and enzymatic reactions. This stops the polyphenol oxidase [PPO] enzyme [EC 1.10.31] catalyzed oxidation of tea leaf catechins (Wilson and Clifford, 1992). In contrast, during black tea processing, tea shoots are macerated to initiate oxidation by PPO before firing. This reaction enables the catechins to condense with the orthoquinones arising from the oxidation of the B ring di- and trihydroxylated catechins to form thea-flavins (TFs). TFs are homogenous substances, which give a yellow red coloration in fermented black tea and contribute to the briskness and brightness of tea liquor. They act as oxidizing agents for substrates like gallic acid to form theaflavic acids. These acids combine with TFs to produce the chemically heterogeneous substances called thearubigins (TRs) that are responsible for the color, body and taste of tea. Molecular oxygen is essential in the formation of these compounds that are characterized by a benzotropolone ring structure (Obanda et al., 2001).

The manufacturing process for semi-fermented oolong tea consists of solar withering, panning, rolling and drying. During this process, the characteristic floral aroma of oolong tea is produced. White tea is a rare specialty tea that gets its name from a specific tea plant variety, as well as a particular post-harvest processing method that raises small silvery hairs on the dried buds. White tea contains a higher proportion of the buds that are covered with fine "silvery" hairs that impart a light white colour to the tea. The brew from white tea is pale yellow in colour with no "grassy" undertones sometimes associated with green tea. True white tea is lightly fermented, rapidly steamed and dried leaving the leaves "fresh". Unlike black, green and oolong teas, white tea is not rolled or crushed but it is steamed rapidly and air-dried to preserve most of the polyphenols. This unique processing produces a rare and expensive but highly refreshing drink. The differences between the various processes of manufacture result in differences in the polyphenol profile between black, green, oolong and white tea. Figure 1 outlines the processing of tea in more details.

The chemical composition of tea is complex and includes polyphenols, alkaloids (caffeine, theophylline and theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, minerals, trace elements and other unidentified compounds. Among these, polyphenols constitutes the most interesting group and are the main bioactive molecules in tea (Cabrera et al., 2003). The major polyphenolic compounds in tea are the flavan-3-ols called catechins which include: (-)-epicatechin (EC), (-)-Epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epigallocatechingallate (EGCG), (-)-Galocatechins

(GC) and (-)-galocatechin gallate (GCG). Catechins are present in large amounts in green tea (Peterson et al., 2005). Based on their chemical structure, catechins that contain three hydroxyl groups in the B ring (positions 3', 4' and 5') are called galocatechins while gallic acid substitution in position 3 of the ring is characteristic of catechin gallate (Pellilo et al., 2002). Catechins account for 6 - 16% of the dry green tea leaves with EGCG constituting 10 - 50% of catechins and being the most potent due to its degree of gallation and hydroxylation (Stewart et al., 2004). TFs and TRs are another group of polyphenolic compounds found in both black and oolong teas (Obanda et al., 2001).

The tea beverage has continued to be considered a medicine since the ancient times because of its polyphenols. Research on the effects of tea on human health has been fuelled by the growing need to provide naturally healthy diets that include plant-derived polyphenols. In line with this, there is need to elucidate how known functional components in foods could expand the role of diet in disease prevention and treatment (Mandel et al., 2006). There is already growing evidence that tea polyphenols reduce the risk of heart diseases and cancer in humans (Vanessa and Williamson, 2004). In some studies, tea has been associated with antiallergic action (Yamamoto et al., 2004) and antimicrobial properties (Paola et al., 2005). Further studies have demonstrated that the co-administration of drugs with catechins (EC and EGCG) inhibits glucoronidation and sulfonation of orally administered drugs thereby increasing the bioavailability of such drugs (Hang et al., 2003). Moreover some epidemiological studies have associated consumption of tea with a lower risk of several types of cancer including those of the stomach, oral cavity, oesophagus and lungs (Cabrera et al., 2003; Hakim and Chow, 2004). Therefore, tea appears to be an effective chemopreventive agent for toxic chemicals and carcinogens.

The ability to scavenge for free radicals by tea polyphenols due to possession of a phenolic hydroxyl group attached to the flavan-3-ol structure has been associated with teas' therapeutic action against free radical mediated diseases thereby attracting tremendous research interest (Amie et al., 2003). Free radicals are known to contribute to numerous disorders in humans including cancer, arterosclerosis, arthritis, ischemia, Central Nervous System (CNS) injury, gastritis, dementia, renal disorders and Acquired Immune Deficiency Syndrome (AIDS) (Pourmorad et al., 2006; Rao et al., 2006). Free radicals are constantly generated due to environmental pollutants, radiation, chemicals, toxins, physical stress and the oxidation process of drugs and food. Many plant phenolics have been reputed to have antioxidant properties that are even much stronger than vitamins E and C. In addition, currently available synthetic antioxidant like butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT) and gallic acid esters have been suspected to cause or prompt negative health effects and hence the

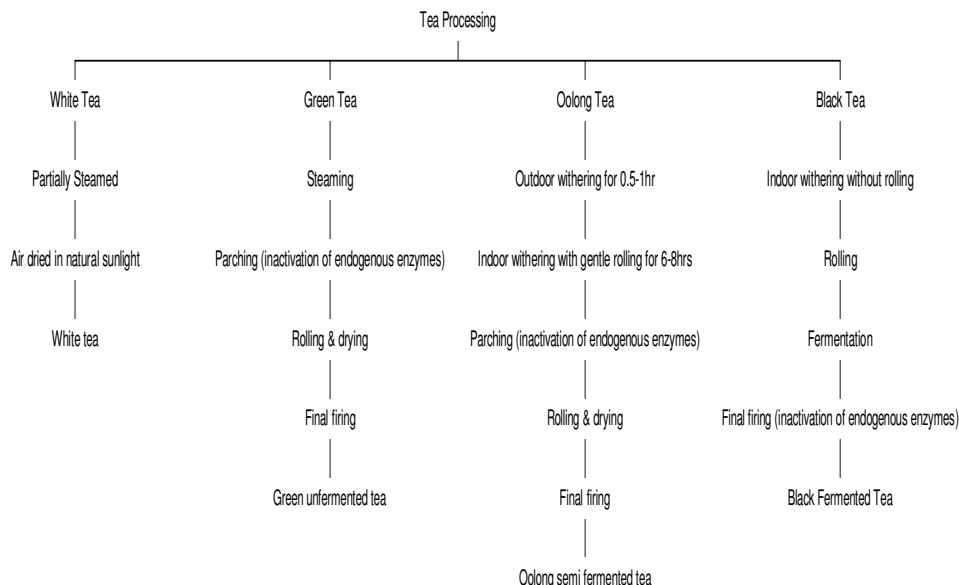


Figure 1. Schematic diagram of the conventional manufacture process of green, black, oolong and white tea.

need to substitute them with naturally occurring antioxidants (Amie et al., 2003; Aqil et al., 2006; Pourmorad et al., 2006).

There is therefore an increased quest to obtain natural antioxidants with broad-spectrum action. Despite the upsurge of interest in the therapeutic potential of plants as sources of natural antioxidants few studies have been carried-out using black tea. In addition information on the antioxidant properties of white tea, which is a rare specialty tea is grossly lacking.

In the present study, a set of twelve tea samples; eight commercial Kenyan tea samples that included black, green, oolong and white tea and two of each Japanese and Chinese green teas were analyzed for total polyphenols, total catechins, total TFs, total TRs and their antioxidant activity. Additionally, two popularly consumed fresh unprocessed vegetables namely onion and spinach were analyzed for antioxidant activity and compared to the tea samples. The objective of the study was to investigate the relationship between the phenolic content with the antioxidant activity of the tea samples.

MATERIALS AND METHODS

Samples

A set of twelve tea samples; eight commercial Kenyan teas that included fermented (black), semi fermented (oolong), non-fermented (green) and white tea from different tea factories in Kenya and two of each Japanese and Chinese green teas were analyzed. The samples had been manufactured in commercial factories using standard manufacturing conditions. Black teas had been manufactured using physical withering up to 50 - 65% moisture content for 18 hours; fermentation at 24°C for 1 - 2 h and a final firing in a fluid bed drier at 120°C for 20 - 25 min. The oolong teas had been processed using outdoor withering under sunlight for 30 - 60 min;

indoor withering at room temperature with turned over treatment for 6 - 8 h and then rolling and final firing at 100°C for about 30 min. The green teas had been manufactured using standard green tea manufacturing procedures of steaming for 1 h and then final firing in a fluid bed drier at 120°C for 20 - 25 min. White tea had been processed from the hairy tip buds only by partial steaming and air-drying in natural sunlight. Preliminary assay were carried out to establish the appropriate amount of sample for analysis and to ensure that the samples had not been damaged or destroyed during transportation. All biochemical analysis was carried out in duplicate.

Sample treatment for polyphenol and catechin analysis

Tea samples of a coarse granular structure were minced and ground to a fine powder. 2 g of the sample was placed on a pre-weighed moisture dish and left for 16 h at 103°C in the oven to dry for the determination of dry matter. Of these, 0.2 ± 0.001 g was weighed into an extraction tube. Five milliliter of hot 70% v/v methanol/water was dispensed into the sample as an extraction mixture and vortexed. Heating of the extraction tube continued in the water bath for 10 min with mixing in the vortex mixer after every 5 min. The extraction tubes were then removed from the water bath and allowed to cool. The tubes were then placed in a centrifuge at 3500 rpm for 10 min. The supernatant was decanted into a graduated tube and the extraction procedure repeated. The extracts were combined and made up to 10 ml with cold methanol/water mixture. One milliliter of the sample extract was transferred into a graduated tube and diluted to 5 ml with a stabilizing solution (10% v/v acetonitrile with 500 µg/ml EDTA and ascorbic acid). The solution was further filtered through a 0.45 µm nylon membrane filter. A 20 µl aliquot of this solution was injected into HPLC for analysis.

Catechin analysis using HPLC

A modified method of Zuo et al. (2002) was used. A Shimadzu LC 20 AT HPLC fitted with a SIL 20A auto sampler and a SPD-20 UV-Visible detector with a class LC 10 chromatography workstation was used for the analysis of the prepared samples. A Luna TM 5

μM C18, 25 cm x 4.6 i.d (Phenomenex, Torrance, CA, USA) column with a Reodyne precolumn filter 7335 model was used. All solvents were filtered through a 0.45 μm millipore membrane filter disk and degassed before injection into a HPLC system. A gradient elution was carried out using the following solvent systems: Mobile phase A (acetonitrile/acetic acid/double distilled water- 9/2/89 v/v/v), Mobile phase B (acetonitrile/acetic acid/double distilled water - 80/2/18 v/v/v). The mobile phase composition for a binary gradient condition started at 100% solvent A for 10 min then over 15 minutes a linear gradient to 60% mobile phase A, 32% mobile phase B and held at this composition for 10 min. The condition was reset to 100 % mobile phase A and allowed to equilibrate for 10 min before the next injection. The flow rate of the mobile phase was 1 ml/ min and the temperature at the column was performed at $35 \pm 0.5^\circ\text{C}$. The identification of individual catechins was carried-out by comparing the retention times and UV- absorbance of unknown peaks with peaks obtained from the mixed known standards under the same conditions. The quantification of catechins was performed at 278 nm and was achieved using a caffeine external standard with a calibration curve $R^2 = 0.9984$ in conjunction with the consensus individual catechin relative response (RRF) values with respect to caffeine calculated on dry matter basis. Total catechin as percentage by mass on a sample dry matter basis was given on the summation of individual catechins.

%Total catechin = [%ECG + (%+C) + (%EC) + %EGCG + %ECG] content.

Total polyphenols determination

The Folin-ciocalteu reagent method was used to determine total polyphenols as described by Pourmorad et al. (2006).

Total theaflavins (TF) content analysis/flavognost

Total TF were determined by the flavognost method of Hilton (1973).

Spectrophotometric measurement of total Thearubigins (TR)

Total thearubigins were determined using the method of Roberts and Smith (1961).

Free radical scavenging activity determination

The stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of free radical scavenging of the tea extracts using a modified method of Brand-Williams et al. (1995). 5 g of tea was infused in 100 ml of boiling double-distilled water followed by stirring with a magnetic stirrer and additional steeping for 30 min at room temperature. The extracts were strained through a nylon mesh (120 μm) followed by a filter paper (Whatman No. 54). Aliquots of the extracts were kept frozen (-1°C) until further use. The soluble solid extract was standardized to give stock solutions of 50 mg soluble solids per 100 ml of 50% methanol. A methanolic solution (50 μl) of the antioxidant was placed in a cuvette and 6.0 x 10⁻⁵ M methanolic solution of DPPH (2 ml) was added (DPPH solution was made using 80 % methanol). The decrease in absorbance at 517 nm was determined using a CE 393 digital grating spectrophotometer until the absorbance stabilized. Reading was done at 15 or 30 min interval. The DPPH solution was prepared afresh and kept in the dark to minimize the loss of free radical stock solution. All determinations were performed in duplicate. The (%) inhibition of DPPH radical was calculated from the absorbance data according to (Yen and Duh, 1994).

% Inhibition against DPPH = $[(AB - AA)/AB] \times 100$

Where AB is the absorbance of the blank sample (50 μl double distilled water and 2 ml DPPH) and AA is the absorbance of the tested sample after 15 min.

Statistical analysis

All determinations were carried out in duplicates and data were subjected to analysis of variance using SPSS version 11.5 software. The Duncan's Multiple Range Test (DMRT) was used to separate the means.

RESULTS AND DISCUSSION

Separation chromatograms of the major catechins by reverse phase (RP) HPLC is as shown in Figure 2. To obtain an adequate resolution of the peaks within a reasonable time of analysis, a gradient elution program was developed. The best results were obtained with double distilled water-acetonitrile-acetic acid at a flow rate of 1 ml /min which allowed the separation of catechins within 18 min. To avoid interaction of the free hydroxyl groups of catechins and the stationary phase, all solutions were prepared in an acidic media because catechins are stable in acidic media (Pellilo et al., 2002). The Luna TM Phenomenex column was chosen because of its high stationary phase surface and a constant support dimension that permitted a complete separation of catechins within a short time. The column technique employed was exclusively RP because of its high resolution for separation and quantification of phenolic substances. In addition, 70% aqueous methanol solution was used for extraction because of its protective role on phenolic substances from being oxidized (Proestos et al., 2006). The major catechins were identified by a comparison of their retention times with those of authentic standards at UV absorption spectra of 278 nm. Under these operating conditions, the retention times in minutes for the studied compounds were as follows: EGC (8.1), +C (10.2), caffeine (13.5), EC (14.8), EGCG (16.4), ECG (21.7), GC (6.1) and GA (4.3) (Figure 2). Previously, results for green tea (Ferruzzi and Green, 2005) and for clonal fresh leaves (Ender et al., 2004) were comparable to the observed results.

The total catechin content was significantly higher ($P < 0.05$) in green tea than oolong and black tea as indicated in Table 1. This demonstrates clearly that the degree of fermentation during the manufacturing process had an influence on the Catechin content of the final product. Black tea is obtained by a post-harvest fermentation which is an auto-oxidation reaction catalyzed by the enzyme polyphenol oxidase, whereas green teas are steamed to inactivate oxidation. Oolong tea is obtained by a partial oxidation of the leaf, an intermediate process between green and black tea manufacture (Peterson et al., 2005). White tea is a product of partial steaming and air-drying of the hairy tips. This unique processing pre-

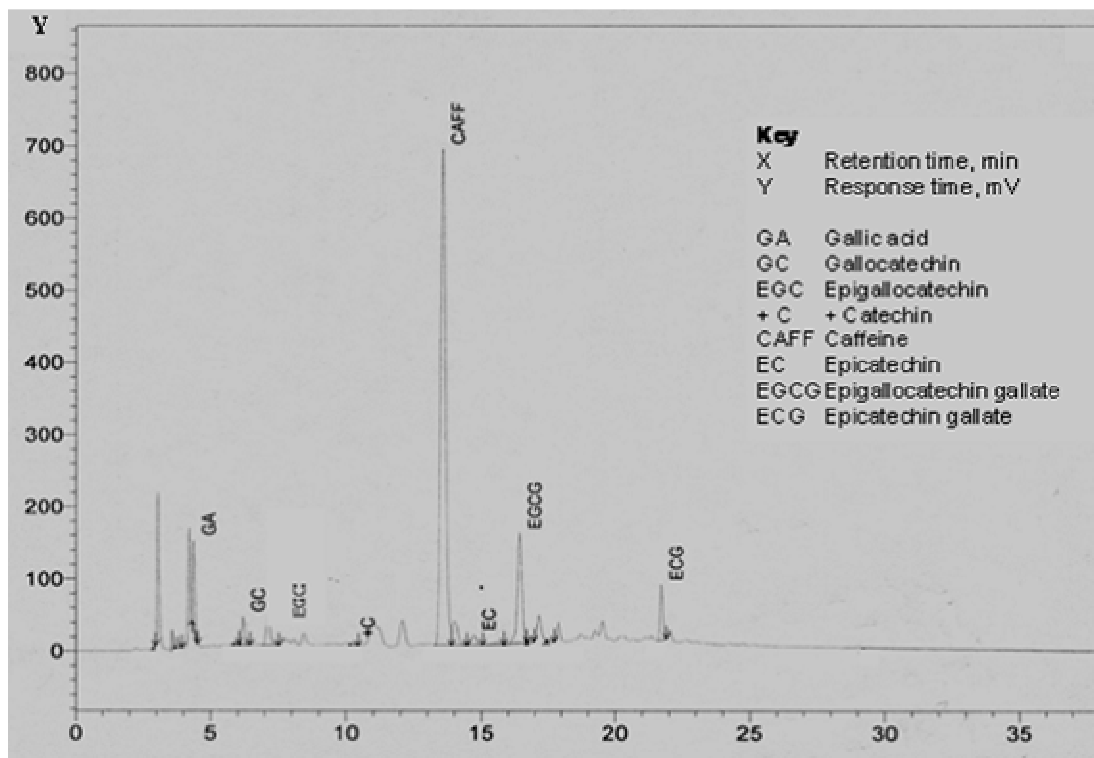


Figure 2. A representative high performance liquid chromatogram of green BP CTC tea.

serves most of the catechins in white tea (Table 1).

Individual catechins varied significantly ($P < 0.05$) among the teas with EGCG, GC and EGC levels being the highest and +C, ECG and EC being less abundant. These results are similar to those of (Ender et al., 2004). White tea which is predominantly manufactured from the young apical hairy bud only showed high levels of EGCG and ECG that are present in higher amounts in fresh young leaves. This latter result corroborates the result by Saijo et al. (2004) who determined the chemical constituents of young tea leaves and the change occurring during leaf development. The decrease in the gallic acid esters of catechin such as EGCG and ECG during leaf development means that there is a slow biosynthesis of gallic acid moiety in each catechin gallate compared with dry matter production. Since catechin biosynthesis is slower than dry matter production from young leaves to the less young leaves, it is apparent that there is no weight increase in the less young and mature leaves and as a result catechin moves to other young leaves or are metabolized to other products. This accounts for the change in catechin levels in various leaf developmental stages and hence the levels of residual catechins in tea manufactured from different ages as exemplified in the differences in catechin levels between white tea and other types of teas in our study (Table 1).

The variation in the polyphenolic composition of the different tea products resulted from the leaf maceration

during manufacturing. The rolling and cutting of the tea shoots in non-orthodox manufacture causes a release of polyphenol oxidase which interacts with phenolic compounds, one simple catechin and one galocatechin, to produce theaflavins and thearubigins that possess a benzotropolone skeleton (Reeves et al., 1987; Mahanta and Hemanta, 1992). Owuor and Obanda (2006) investigated the use of green tea flavan-3-ols in predicting black tea quality potential and revealed that a correct balance of the trihydroxylated flavan-3-ols and dihydroxylated flavan-3-ols was necessary to ensure maximum formation of the theaflavins. The trihydroxylflavan-3-ols are oxidized faster during the fermentation phase of black tea processing explaining the high levels of EGCG and ECG in green tea and the subsequent reduction in black tea. Theaflavins are further oxidized to form thearubigins that are heterogeneous in nature and contribute significantly towards taste, color and body of tea (Obanda et al., 2004; Li et al., 2005). Black tea therefore has high levels of TFs and TRs that are the main fermentation products as evident in Table 1.

Results from the present study however clearly showed that TRs were present in green tea (Table 1). Further observation revealed that in green tea, TRs were formed in the presence of low levels of TFs unlike in black tea where the levels were almost similar (Table 1). This may suggest that theaflavins are not the only source of thearubigins. Wilson and Clifford (1992) explained the factors

Table 1. Phenolic composition and antioxidant activity (%) of various teas.

Sample	Total polyphenols	Total catechins	Individual catechins						TFs	TRs	AA+
			EGC	EGCG	GC	ECG	+C	EC			
Kenyan teas											
Black PD (CTC)	20.65 ^{cd, 7}	5.91 ^{e, 9}	1.12 ^{de, 9}	2.82 ^{de, 9}	4.88 ^{a, 1}	0.99 ^{b, 3}	0.37 ^{b, 6}	0.62 ^{g, 9}	11.61 ^{c, 3}	10.30 ^{d, 4}	73.31 ^{d, 6}
Black BP (CTC)	17.45 ^{f, 11}	3.07 ^{f, 14}	0.63 ^{e, 11}	1.43 ^{ef, 11}	3.60 ^{cd, 4}	0.57 ^{cde, 9}	0.20 ^{de, 11}	0.25 ^{h, 11}	18.75 ^{a, 1}	15.65 ^{a, 1}	71.19 ^{fg, 10}
Green PF (CTC)	26.85 ^{a, 2}	14.93 ^{a, 1}	5.47 ^{a, 1}	6.78 ^{a, 1}	3.26 ^{cd, 5}	0.75 ^{bc, 4}	0.43 ^{b, 2}	1.56 ^{ab, 2}	0.46 ^{fg, 8}	5.59 ^{h, 11}	77.22 ^{a, 1}
Green BP (CTC)	25.70 ^{a, 4}	10.04 ^{d, 7}	5.17 ^{ab, 2}	3.11 ^{cd, 8}	2.45 ^{ef, 9}	0.28 ^{e, 12}	0.30 ^{b, 4}	1.21 ^{cde, 5}	1.63 ^{e, 6}	8.38 ^{f, 8}	73.31 ^{d, 5}
Black orthodox	22.25 ^{b, 5}	4.62 ^{ef, 10}	1.20 ^{de, 10}	1.87 ^{def, 10}	3.00 ^{de, 7}	0.62 ^{cd, 8}	0.33 ^{bc, 8}	0.79 ^{fg, 9}	13.80 ^{b, 2}	11.51 ^{c, 3}	71.52 ^{ef, 9}
Green orthodox	27.10 ^{a, 1}	11.06 ^{cd, 4}	4.91 ^{ab, 4}	4.72 ^{b, 5}	3.84 ^{bc, 3}	0.35 ^{de, 11}	0.38 ^{b, 5}	0.98 ^{ef, 7}	0.41 ^{fg, 10}	5.77 ^{h, 10}	75.64 ^{b, 3}
Oolong orthodox	26.15 ^{a, 3}	9.49 ^{d, 8}	2.31 ^{c, 7}	4.37 ^{bc, 7}	3.01 ^{de, 6}	1.03 ^{b, 2}	0.57 ^{a, 1}	1.30 ^{bcd, 4}	6.81 ^{d, 5}	8.75 ^{ef, 7}	74.78 ^{c, 4}
White tea/ Silvery tip	21.30 ^{c, 6}	10.20 ^{cd, 6}	1.73 ^{cd, 8}	5.53 ^{ab, 2}	4.46 ^{ab, 2}	1.505 ^{a, 1}	0.41 ^{b, 3}	1.00 ^{def, 6}	0.85 ^{f, 7}	1.75 ^{i, 12}	76.01 ^{b, 2}
Other ungraded teas											
Japanese teas											
Green CTC cultivar Yabukita	19.36 ^{de, 9}	12.69 ^{ab, 2}	4.75 ^{b, 5}	5.05 ^{b, 3}	1.83 ^{fg, 10}	0.70 ^{bc, 7}	0.35 ^{bc, 7}	1.85 ^{a, 1}	0.25 ^{g, 12}	9.06 ^{e, 6}	71.76 ^{ef, 8}
Green CTC cultivar Yutakamidori	19.78 ^{de, 8}	12.24 ^{bc, 3}	4.97 ^{ab, 3}	4.18 ^{b, 4}	1.79 ^{fg, 11}	0.74 ^{bc, 5}	0.23 ^{cde, 10}	1.50 ^{bc, 3}	0.45 ^{fg, 9}	7.69 ^{g, 9}	70.54 ^{g, 11}
Chinese teas											
Green CTC cultivar Hanlu	18.82 ^{ef, 10}	11.19 ^{cd, 5}	4.65 ^{b, 6}	4.64 ^{b, 6}	2.48 ^{ef, 8}	0.71 ^{bc, 6}	0.31 ^{bcd, 9}	0.90 ^{fg, 8}	0.31 ^{fg, 11}	9.30 ^{e, 5}	69.21 ^{h, 13}
Green CTC cultivar Yinghong	11.42 ^{g, 12}	3.31 ^{f, 12}	0.60 ^{e, 12}	0.78 ^{f, 12}	1.20 ^{g, 12}	0.36 ^{de, 10}	0.14 ^{e, 12}	0.22 ^{h, 12}	11.57 ^{c, 4}	13.55 ^{b, 2}	67.62 ^{i, 14}
Fresh unprocessed vegetables											
Spinach	-	-	-	-	-	-	-	-	-	-	69.34 ^{h, 12}
Onion	-	-	-	-	-	-	-	-	-	-	72.35 ^{e, 7}
Grand mean	21.48	9.95	3.12	3.82	2.98	0.72	0.34	1.01	5.62	8.88	72.41
LSD (P _≤ 0.05)	1.423	1.856	0.6995	1.423	0.7431	0.3265	0.1206	0.2953	0.5697	0.5947	0.9021
C.V (%)	3.01	8.30	10.20	16.92	11.34	20.58	4.59	13.26	16.93	3.04	0.55

DMRT ranking - Means within a column followed by the same letter are not significantly different at P=0.05 according to Duncan's Multiple Range Test –DMRT. Numerical ranking is from the highest value of the parameter to the lowest.

*Data has been arcsine transformed.

Tea grades: PD, pekoe dust; BP, broken pekoe; PF, pekoe fanning's; CTC, curl tear crush.

EGC, Epigallocatechin; EGCG, Epigallocatechingallate; GC, Galocatechin; ECG, Epicatechin gallate; +C, Catechin; EC, Epicatechin; TFs, Theaflavins; TRs, Thearubigins; AA Antioxidant Activity.

affecting the formation and degradation of theaflavins and thearubigins in black tea and observed that maximum synthesis of theaflavins occurs when oxygen is in excess to support benzotropolone ring formation. However, under a limiting oxygen concentration, polyphenol oxidase, which has a high affinity for the substrate, has a preferential demand for oxygen and theaflavins formation is suppressed at the expense of catechin quinone formation. This competition for oxygen is particularly noticeable during the early stages of fermentation when the concentration of the catechins is at its highest and enzyme turnover is unimpeded by substrate availability. This occurs during green tea manufacture since the enzyme is active before deactivation through steaming. For this reason, high enzyme activity in an already low oxygen concentration creates almost total anaerobiosis, which suppresses benzotropolone ring formation. Consequently as a result of this, thearubigins are formed, mainly from gallocatechins since the simple catechins are unable to react in benzotropolone ring formation. Moreover, it might be possible to minimize thearubigins formation by deactivating the enzyme immediately after plucking through a steaming procedure although this is hardly achievable during commercial tea processing. Further research is desirable to explain in details the existence of this thearubigins in green tea and the importance of steaming during tea processing.

The polyphenolic composition of tea and especially its catechins has aroused interest in their potential as radical scavenging compounds. Data on antioxidant capacity is presented in Table 1. Overall, green and white teas' had significantly ($p < 0.05$) higher antioxidant activity compared to black tea. There was no significant difference in the antioxidant capacity of black tea manufactured using orthodox and non-orthodox methods. Table 2 presents data on the correlation between tea polyphenols contents and the antioxidant activity of different types of tea products. Total catechins significantly ($p < 0.001$) correlated with antioxidant activity ($r = 0.959$). EGCG was identified as the most potent antioxidant ($r = 0.989$, $P < 0.001$). EC, EGC, +C and ECG contents also showed significant influence on the antioxidant activity. Therefore, the antioxidant activity was higher in tea extracts containing high levels of EGCG, EC, EGC, +C and ECG. These results are similar to those of Gramza et al. (2006). This antioxidative effect of polyphenols has been attributed to the phenolic hydroxyl groups in their structures that make them potent free radical scavengers (Amie et al., 2003). On the basis of these results, it appears that the most effective radical scavengers are catechins with a 3', 4' and 5'-trihydroxylated substitution pattern on the B ring and/or hydroxyl group at C-3 position of the catechin structure. This hydroxylation confers a higher degree of stability on the catechin phenoxyl radical by participating in electron delocalisation that is an important feature of the antiradical potential. This explains why radical scavenging is high in the gallocatechins i.e. EGCG and EGC

that are potent antioxidants (Zhu et al., 2001; Amie, et al., 2003, and Rao, et al., 2006).

Black teas analyzed in this study exhibited some antioxidant activity with a high DPPH radical scavenging activity though less than that of green, white and oolong tea. During black tea manufacture, the gallocatechins are first to be oxidized and dimerised to TFs and TRs because of their high oxidation potential and high concentration in the leaves. These major phenolic compounds in black tea also contributed significantly to the radical scavenging activity that is, TFs ($r = 0.920$, $P < 0.001$) and TRs (0.807, $P < 0.001$) and GA ($r = 0.530$, $P < 0.05$). Interestingly, TFs, which are major phenolic product in black tea, had a higher radical scavenging activity compared to some of its precursors ECG, EGC and EC (Table 2). This confirms that conversion of catechins to TFs during black tea processing did not affect the radical scavenging potency. These observations are consistent with those of Leung et al. (2001) who showed that black tea possesses more or less the same antioxidant potency as catechins present in green tea. EGCG and EGC contribute significantly to the formation of TFs. These are B ring trihydroxylated catechins, which are oxidized at a much faster rate than the B ring dihydroxylated catechins (EC, EGC and +C) due to their lower oxidation potential (Owuor and Obanda, 2006). TFs formed from this reaction have hydroxyl groups (OH) considered necessary for free radical scavenging activity. These additional groups increase the total number of phenyl hydroxyl groups and make the gallate containing catechins and TFs more able to donate protons due to resonance delocalization thereby expressing the observed antioxidant activity of black tea. Similarly, gallic acid contributed significantly to the radical scavenging activity in black tea because it is a potent hydrogen donor to DPPH. Additionally, our study provided evidence of the contribution of TRs towards the antioxidant activity of black tea. A significant correlation ($r = 0.807$, $P < 0.001$) was observed (Table 2). The antioxidant activity of TRs can be explained by the presence of 3-OH groups, which are more or less esterified by gallic acid in the TRs structure. However, this is a highly speculative hypothesis since to date there is no definite data on TRs structures (Li et al., 2005). Oolong (semi-fermented) tea which was intermediate between green and black tea did not contain high levels of the major antioxidative gallocatechins and also did not yet contain a great amount of theaflavins and thearubigins which are found in fully fermented black tea. Consequently, this type of tea had an antioxidant activity that was intermediate of that of green and black tea (Table 1).

A comparison of the antioxidant activity of the Kenyan commercial teas with those of Japan and China was carried out to determine the effect of the variety from which the tea products were processed on antioxidant activity. This study revealed that Kenyan tea products both green and black were rich in total polyphenols as shown in Table 1. The high polyphenol content in Kenyan

Table 2. Correlation coefficient matrix analyses between various tea chemical parameters.

TP	TFs	TRs	EGC	EGCG	ECG	+C	EC	GC	GA	AA	TC	
1.00	0.881***	0.734***	0.919***	0.791***	0.530*	0.832***	0.920***	0.675**	0.637**	0.807***	0.899***	TP
	1.00	0.952***	0.894***	0.908***	0.262	0.766***	0.885***	0.308	0.584*	0.920***	0.955***	TFs
		1.00	0.706**	0.922***	0.658**	0.738***	0.803***	0.450	0.378	0.930***	0.892***	TRs
			1.00	0.782***	0.674**	0.624**	0.860***	0.644**	0.815**	0.787***	0.919***	EGC
				1.00	0.597*	0.769***	0.873***	0.333	0.505*	0.989***	0.965***	EGCG
					1.00	0.578*	0.261	0.757***	0.771***	0.579*	0.255	ECG
						1.00	0.887***	0.401	0.442	0.766***	0.794***	+C
							1.00	0.627**	0.449	0.860***	0.937***	EC
								1.00	0.184	0.400	0.369	GC
									1.00	0.530*	0.645**	GA
										1.00	0.959***	AA
											1.00	TC

*Correlation significant at the $P \leq 0.05$ level.**Correlation significant at the $P \leq 0.01$ level.***Correlation significant at the $P \leq 0.001$ level.

tea products is not unexpected since the tea breeding programme in Kenya has indirectly and consistently selected germplasm for high total phenol content to produce black teas with high levels of TFs and TRs. A previous study had confirmed the superiority of Kenyan tea germplasm in its total polyphenol content (Wachira and Kamunya, 2005). Tea germplasm from Japan and China that is traditionally used for green tea manufacture is selected to be low in astringency and bitterness and consequently low in total polyphenols. A comparison of the antioxidant activity of tea and popularly consumed vegetable such as spinach and onion showed that the antioxidant activity of tea was significantly ($P < 0.05$) higher than that of the fresh unprocessed vegetable (Table 1) which demonstrated the potency of tea as a potentially health enhancing food.

Despite the increasing studies on the antioxidant property of black, green and oolong tea, limited information is available on white tea. In the present study, the antioxidant capacity of white tea was shown to be similar to that of green tea. This can be attributed to the high levels of EGCG, which is the most potent antioxidant, and ECG that is present in large amounts in the young fresh leaves or the hairy bud traditionally used in the manufacture of this rare specialty type of tea (Takeda, 2004; Stewart et al., 2004).

Conclusion

Many phenolics in foods and beverages have been reported to have antioxidant capacities that are much higher than those of vitamin C and E and even those of currently available synthetic antioxidants such as butylated hydroxyl toluene-BHT. In addition, phenolic foods have added advantage over the other antioxidants since they are water-soluble and are therefore excreted by the body unlike fat-based vitamin E that is absorbed and retained even at potentially harmful levels (Aqil et al., 2006). Tea, a widely consumed polyphenolic beverage may play a significant role as a naturally occurring antioxidant substitute and hence contribute to human health.

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