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Total polyphenols, catechin profiles and antioxidant activity of tea products from purple leaf coloured tea cultivars

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ABSTRACT

Black (aerated) and green (unaerated) tea products, processed from 10 green and 18 purple leaf coloured cultivars of Kenyan origin, and two tea products, from the Japanese cultivars, Yabukita and Yutakamidori, were assayed for total polyphenols (TP) content, individual catechin profiles and *in vitro* antioxidant capacity (AA). In addition, the phenolic content of the tea products was determined using the Folin–Ciocalteu phenol reagent. Catechin fractions were identified using reverse phase high performance liquid chromatography (HPLC) with a binary gradient elution system.

The AA% of the tea products was determined using a 2,2′-diphenyl picrylhydrazyl (DPPH) radical assay method. The results showed that TPs, catechin profiles and antioxidant activities were significantly ($p \leqslant 0.05$) higher in unaerated than in aerated teas. Tea products from the purple leaf coloured tea cultivars had levels of TPs, total catechin (TC) and antioxidant activities similar to those from the green leaf coloured cultivars, except for teas from the Japanese cultivars that were very low in the assayed parameters. Caffeine content was significantly ($p \leqslant 0.05$) lower in products from the purple leaf coloured cultivars than in those from the green leaf coloured tea cultivars. Antioxidant activity (%) was higher in tea products from the Kenyan germplasm than in those from the Japanese cultivars. Antioxidant potency of tea products was significantly ($r = 0.789^{**}$, $p \leqslant 0.01$) influenced by the total anthocyanin content of the purple leaf coloured cultivars. Cyanidin-3-*O*-glucoside was the anthocyanin most highly correlated with AA% ($r = 0.843^{**}$, $p \leqslant 0.01$ in unaerated tea). Total catechins in the unaerated products from the green leaf coloured tea cultivars were also significantly correlated with antioxidant capacity ($r = 0.818^{**}$, $p \leqslant 0.01$). Results from this study suggest that the antioxidant potency of teas is dependent on the predominant flavonoid compound, the type of tea cultivar and the processing method.

1. Introduction

Worldwide, aerated (black) and unaerated (green) products are the most widely consumed types of teas, though tea processing has diversified to the production of several specialty types of products (Reeves, Owuor, & Othieno, 1987). Aerated and unaerated teas are both processed from the tender shoots of the tea plant. The quality of the processed product depends on the chemical composition of the tea shoots and the manufacturing technique employed.

Unaerated tea contains significant quantities of the unoxidised catechins: (catechin (+)-C, (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin (GC), (−)-epicatechin gallate (ECG) and (−)-gallocatechin gallate (GCG); the oxidised derivatives of the catechins, theaflavins (TFs) and thearubigins (TRs), are found in fully aerated and semi-aerated (Oolong) teas. In addition, tea also contains amino acids (theanine, gamma amino butyric acid), carbohydrates, proteins, minerals, trace elements, volatile compounds, carotenoids and alkaloids, namely caffeine, theophylline and theobromine.

Initiatives to develop specialty teas have been driven by the desire to provide more healthful tea products. Indeed, some specialty tea products have been demonstrated to be more pharmacologically active owing to their high levels of biologically active molecules. Some of these specialty teas are made even more appealing to the consumer by addition of colour additives and flavours. Examples of such teas include white tea, flavoured teas (ginger, lemongrass, lemon, vanilla, strawberry), scented tea, herbal teas and decaffeinated teas. Other types of tea products are produced by process modification, such are black and green teas enriched with anthocyanins, the amino acid theanine, specific catechins, for example epigallocatechin gallate (EGCG), and gamma

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aminobutyric acid (GABA). Additionally, industrial products have also been commercialised from tea. These include catechin, theaflavin, thearubigin, anthocyanin, theanine, polysaccharide, and saponin extracts and concentrates, as well as tea seed oil, which are used in the food, pharmaceutical and fast moving consumer goods (FMCG) industries.

The potential health benefits of tea have been ascribed to the flavonoid component which has potent antioxidant activity. The antioxidant activity of tea flavonoids is indeed thought to account for teas' protective role against such conditions as cardiovascular disease (Cabrera, Artacho, & Gimenez, 2006; Nagao, Hase, & Kokimitsu, 2007), cancer (Cabrera, Gimenez, & Lopez, 2003; Hakim & Chow, 2004), low density lipoprotein oxidation (Hans et al., 2007), inflammation (Karori, Ngure, Wachira, Wanyoko, & Mwangi, 2008), poor oral health (Wu & Wei, 2002), and diabetes (Vinson, Wu, Teufel, & Zhang, 2001). The antioxidant activity of tea has also been shown to exert antimicrobial effects on several disease-causing pathogens (Paola et al., 2005). Besides the above potentially health-enhancing properties of tea, research has shown that co-administration of drugs with catechins (EC and EGCG) inhibits glucuronidation and sulfonation of orally administered drugs, thereby increasing their bioavailability in the body (Hang et al., 2003).

In efforts to enhance the health potency of tea, purple leaf coloured tea cultivars were recently developed in Kenya for the manufacture of a "health tea product" (Kamunya, Wachira, Nyabundi, Kerio & Chalo, 2009). Leaves from these cultivars were recently characterised by their anthocyanin profiles. Results from this study showed that indeed, these cultivars contained anthocyanins and anthocyanidins, with the predominant anthocyanidin being malvidin (Kerio, Wachira, Wanyoko, & Rotich, 2012). Anthocyanins have also been found to have important biological activities, which include; antioxidant (Choi, Chang, Cho, & Hyan, 2007), anti-inflammatory (Dai, Patel, & Mumper, 2007) and anticarcinogenic (Wang & Stoner, 2008) properties. Anthocyanins have also been shown to induce apoptosis in cancerous cells (Lee et al., 2009), besides having the capacity to protect cells against oxidative stress-induced apoptosis (Elisia & Kitts, 2008). However, like catechins, anthocyanins are also products of the phenyl propanoid pathway. It is not clear whether anthocyanin-rich cultivars also have the same profiles of catechins as have the ordinary green leaf coloured tea cultivars.

In the present study, aerated and unaerated tea products, processed from 30 tea cultivars, were assayed for their biochemicals. Ten cultivars were from ordinary green leaf coloured tea cultivars (controls), two from Japanese cultivars and 18 from anthocyanin-rich purple leaf coloured cultivars (test clones) (Kerio et al., 2012). Tea products from the cultivars were analysed for total polyphenols, catechin profiles and *in vitro* antioxidant activities.

2. Materials and methods

2.1. Materials

2.1.1. Tea samples

The plant materials from which the assayed processed tea was made were obtained from the Tea Research Foundation of Kenya (TRFK), Kangaita substation in Kirinyaga District (0°26′S, 37°15′E, 2020 a.m.s.l). The youngest two leaves, plus a terminal bud, were hand-plucked from a total of thirty (30) tea cultivars and processed into both aerated (black) and unaerated (green) tea products in a miniature factory using the standard tea manufacture protocols described below. Of the 30 tea cultivars, 12 were comprised of widely cultivated green leaf coloured tea cultivars, which also included two Japanese tea cultivars, Yabukita and Yutakamidori, and eighteen purple leaf coloured test clones.

2.2. Sample preparation

2.2.1. Processing of tea samples

Tea was manufactured from the harvested leaf in a miniature tea factory at the TRFK, Kericho. Unaerated teas were manufactured using physical wither for 18 h to attain a moisture content of 50–65%. Aeration ("fermentation") was carried out for 1–2 h at 24 °C and the leaf fired in a fluid bed drier at 120 °C for 20–25 min. Unaerated teas were manufactured by steaming the leaf for 1 h; crushing, tearing and curling and finally firing in a fluid bed drier at 120 °C.

2.3. Determination of dry matter content

Five grammes (5 g) each of the aerated and unaerated tea products were weighed to the nearest 0.001 g, placed in pre-weighed aluminium dishes and dried in an oven (Oven Memmert, UND300, Germany) at 103 ± 2°C for 16 h to constant weight. Percentage dry matter (DM) content for each sample was calculated from the weight differences.

2.4. Preparation of extracts

2.4.1. Total polyphenols and catechins

Coarse granules of processed tea leaves were milled into a fine powder. A sample of 0.2 ± 0.001 g of tea was weighed into graduated extraction tubes (10 ml) and 5 ml of 70% hot water/methanol extraction mixture, at a temperature of 70 °C, dispensed into the extraction tubes using a dispenser (Dispensette Brand Germany) stoppered and mixed on a vortex mixer (Rotamixer, Huck and Tucker, England). The extraction tubes were incubated in the water bath for 10 min and vortexed after 5 min and 10 min, respectively. The tubes were removed from the water-bath, allowed to cool and then centrifuged for 10 min at 3500 rpm, using a centrifuge (Heraeus Sepatech, Germany). A second extraction was done, as above; the extracts were then combined and made up to 10 ml with cold methanol/water extraction mixture and mixed on a vortex mixer.

2.4.2. Anthocyanin extracts

Five grammes (5 g) of ground tea samples were weighed into 250 ml conical flasks, covered with a foil, and mixed with 50 ml of MeOH/HCl (99:1 v/v) and magnetically stirred at 900 rpm for 4 h at room temperature. The resultant solution was filtered and evaporated to dryness using a Rotavapour (Buchi Rotavapour R-300, Switzerland) under reduced pressure at 35 °C. The extract was dissolved in 10 ml of distilled water and passed through a membrane filter 0.45 μM and kept in an ice bath for analysis. The extracts were passed through reverse phase (RP) C18 solid phase extraction (SUPELCO, SPE) (Sigma–Aldrich, USA) cartridges previously activated with 10% MeOH/HCl. Anthocyanins were adsorbed into the column while sugars, acids and other water-soluble compounds were washed out using 0.01% HCl in distilled water. Anthocyanins were then recovered using acidified methanol (10% formic acid v/v). The cartridges were washed with ethyl acetate (Fischer Scientific, UK) to remove phenolic compounds, except anthocyanins. The purified extracts were stored at −10 °C prior to further analysis.

2.5. Analysis of total polyphenols (TPs)

The total phenolic content of the tea samples was determined according to the International Standards Organisation (ISO) ISO 14502-1-2005E procedure for determination of total polyphenols in tea, using Folin–Ciocalteu's reagent. From the sample extract, 1 ml was pipetted into a 100 ml volumetric flask and made up to

the mark with distilled water. One millilitre of the diluted sample was complexed with 5 ml of 10% Folin–Ciocalteu's phenol reagent and 4 ml of 7.5% sodium carbonate solution for 1 h before spectrometric analysis. Gallic acid standards were used for quantification and the results were expressed as percent gallic acid equivalents (GAE).

2.6. Analysis of catechins and caffeine content

Catechin analysis, by high performance liquid chromatography (HPLC), was carried out according to the ISO 14502-2-2005E procedure. One millilitre (1 ml) of the sample extract was made up to 5 ml with stabilizing solution (10% v/v acetonitrile, 500 μg/ml EDTA and ascorbic acid). The solution was filtered through a 0.45 μm nylon membrane filter and put into vials. An aliquot of 20 μl of the solution was injected into the HPLC system by an auto-sampler. Reverse phase HPLC analysis was used. The system, comprised of a Shimadzu LC 20 AT HPLC fitted with a SIL 20A auto-sampler, a SPD-20 UV-Visible detector, with a class LC10 chromatography workstation, with UV detection at 278 nm, using a Gemini C_6 ODS column (250 mm × 4.6 mm i.d.) (Phenomenex Inc. Torrance CA, USA), fitted with a Gemini C_6 ODS (4.0 mm × 3.0 mm i.d.) (Phenomenex Inc. Torrance CA, USA) guard column. Column temperature was set at 35 °C. The flow rate of the mobile phase was 1 ml/min and injection volume was 20 μl. Binary gradient elution conditions were as follows: 100% mobile phase A for 10 min, then (over 15 min) a linear gradient to 68% mobile phase A, 32% mobile phase B and held at this composition for 10 min. It was then reset to 100% mobile phase A and allowed to equilibrate before the next injection. Individual catechins were identified by comparing the retention times of unknown peaks with those identified from peaks obtained from the mixed catechin standards. Catechin and caffeine quantifications were done using a caffeine calibration curve, together with the consensus relative response factors (RRFs) with respect to caffeine, calculated on a dry matter basis. The total catechin content of the teas, as a percentage by mass on a sample dry matter basis, was determined by the summation of individual catechins as;

$$\%\text{Total catechins} = (\%\text{EGC}) + (\% + \text{C}) + (\%\ \text{EC}) + (\%\text{EGCG}) + (\%\text{ECG}) \quad (1)$$

Caffeine content was quantified as follows:

$$\%\text{Caffeine} = \frac{(A_{\text{sample}} - A_{\text{intercept}}) \times RRF_{std} \times V \times d \times 100}{\text{Slope}_{\text{caffeine}} \times m \times 1000 \times DM} \quad (2)$$

where

A_{sample} – Peak area of the individual component in the test sample.
$A_{\text{intercept}}$ – Peak area at the point of interception on y-axis.
$\text{Slope}_{\text{caffeine}}$ – Caffeine calibration line slope.
V – Sample extraction volume
d – Dilution factor.
m – Mass in grams of test sample.
DM – Dry matter content of test sample.

2.7. Analysis of anthocyanins

Anthocyanin analysis by the spectrophotometric and HPLC methods was done as described by Kerio et al. (2012). For HPLC analysis, the column used was Gemini C_{18} ODS (250 mm × 4.6 mm i.d.) (Phenomenex Inc. Torrance CA, USA), fitted with a Gemini C_{18} ODS column (4.0 mm × 3.0 mm i.d.) and guard column (Phenomenex Inc. Torrance CA, USA).

2.8. Determination of antioxidant capacity

The stable 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH•) (Sigma–Aldrich, UK) was used for the determination of free radical-scavenging activity of the tea extracts, using a modified method of Brand-Williams, Cuvelier, and Berset (1995).

2.9. Extractability of tea anthocyanins in water and methanol

A standard tea infusion was prepared in boiling distilled water for 2 min, 5 min, 7 min and 10 min. Monomeric anthocyanin content was determined using the pH differential method of Giusti and Wrolstad (2001) and the results compared with the methanol extract method described by Kerio et al. (2012).

2.10. Data analyses

All the determinations were carried out in triplicate and the data were subjected to analysis of variance, and the means separated by the least significant difference (LSD) test, using MSTAT Version 2.10.

3. Results

3.1. Total polyphenols (TP)

Data on TP content of the assayed teas are presented in Table 1. Unaerated tea products from the different cultivars gave levels of TP significantly ($p \leqslant 0.05$) higher than those of aerated teas, with the exception of TRFK 6/8 (a high black tea quality cultivar) and TRFK KS3 whose TP contents in the unaerated tea were not significantly different from those in the aerated tea cultivars. Aerated and unaerated tea products from cultivar GW Ejulu-L, another high black tea quality cultivar, exhibited the highest contents of polyphenols. The two Japanese cultivars, Yabukita and Yutakamidori, produced teas with the lowest total polyphenol contents, 16.8% and 19.1% for unaerated tea and 12.9% and 14.8% for aerated tea, respectively (Table 1). The level of total polyphenols in processed teas from the purple leaf coloured cultivars ranged from 17.1% to 21.1% for aerated and from 18.8% to 24.6% for the unaerated teas. These levels of TP were comparable to those of processed teas from the widely grown green leaf coloured cultivars, e.g. TRFK 6/8, TRFK 31/8, TRFK 303/577 and AHP S15/10. These results also showed that the purple leaf coloured cultivars produced aerated teas whose levels of total polyphenols were not significantly different from those of the ordinary green leafed cultivars.

3.2. Individual catechins

3.3.1. General

The following catechins were identified in the processed teas from the 30 test cultivars: epigallocatechin (EGC), catechin (+C), epicatechin (EC), epigallocatechin gallate (EGCG) and epicatechin gallate (ECG). The order of elution of the catechins was as follows: gallic acid, 4.72 min; epigallocatechin, 8.40 min; (+)-catechin, 10.42 min; caffeine, 12.30 min; epicatechin, 17.32 min; epigallocatechin gallate, 18.20 min; epicatechin gallate, 22.37 min. A representative chromatogram derived from the assay of unaerated tea from cultivar TRFK 6/8 presented in Fig. 1. The non-gallated catechins, epigallocatechin (EGC) and catechin (+C) eluted before the gallated catechins, epicatechin gallate (ECG) and epigallocatechin gallate (EGCG).

Table 1
Content of total polyphenols (%) in aerated and unaerated teas processed from 30 green leaf and purple leaf coloured tea cultivars.

Cultivars	Unaerated (Green) tea	Aerated (Black) tea
Green leaf coloured		
GW Ejulu-L	32.0	26.3
AHP S15/10	21.4	17.9
TRFK 31/8	25.0	21.0
TRFK 31/11	22.7	20.1
TRFK 6/8	24.0	23.9
TRFK 301/2	17.5	15.2
EPK 14–3	22.0	18.7
TRFK 303/577	22.9	18.7
TRFK 303/216	19.7	16.8
Yutakamidori[a]	16.8	12.9
Yabukita[a]	19.1	14.8
TRFK 301/1	20.9	16.6
Mean	22.0	18.6
Purple leaf coloured		
TRFK 306/1	22.8	20.0
TRFK 306/2	22.2	19.6
TRFK 306/3	23.2	19.6
TRFK 306/4	24.2	20.0
TRFK 73/1	21.7	17.6
TRFK 73/2	21.3	17.5
TRFK 73/3	21.3	18.5
TRFK 73/4	22.2	18.8
TRFK 73/5	23.4	19.1
TRFK 73/7	20.5	18.9
TRFK K-purple	24.6	19.2
TRFK KS1	20.8	18.9
TRFK KS2	24.2	19.7
TRFK KS3	20.4	20.5
TRFK 91/1	24.0	21.1
TRFK 91/2	18.8	17.0
TRFK 83/1	20.8	17.4
TRFK 14/1	20.9	17.1
Mean	22.1	18.9
Pooled Mean	22.0	18.8
CV%	5.03	3.18
LSD ($p \leqslant 0.05$)	1.81	0.98

[a] Japanese tea cultivars mainly used for manufacture of green tea.

3.3.2. EGCG

EGCG, the most potent antioxidant catechin was significantly higher in unaerated teas from the green leaf coloured cultivars TRFK 31/8 (7.02%), TRFK 6/8 (6.45%) and AHP S15/10 (5.61%), though the levels were much lower in the aerated teas from the same cultivars (Table 2). Among the purple leaf coloured cultivars, the TRFK 306 series produced teas that had lower EGCG content than those of the TRFK 73 series. For example, unaerated teas of cultivars TRFK 306/1, TRFK 306/2, TRFK 306/3 and TRFK 306/4 had EGCG levels of 2.15%, 1.58%, 2.23% and 2.34%, respectively, while the aerated teas from the same cultivars recorded levels of 1.26%, 0.66%, 0.50%, and 0.59%, respectively. This latter observation may indicate that EGCG was consumed rapidly to form *O*-quinones during the aeration stage of black tea processing, hence giving the large differences in EGCG content of the aerated and unaerated teas.

3.3.3. ECG

Unlike for EGCG, the contents of ECG in the processed teas showed a different trend with the levels not significantly ($p > 0.05$) differing between the aerated and unaerated forms of tea. This was particularly apparent for the cultivars TRFK 31/8, TRFK 6/8, GW Ejulu-L and AHP S15/10 (Table 3).

3.3. Gallic acid

There were significant ($p \leqslant 0.05$) differences in the gallic acid contents between teas from the different cultivars (Tables 2 and 3). The trend was not consistent, since some unaerated teas had lower contents of the gallic acid than had their aerated counterparts, for example clone TRFK 301/1 and vice versa for clone TRFK 306/3. Some purple leaf coloured tea cultivars such as TRFK 91/1 produced aerated and unaerated teas that were high in gallic acid.

3.4. Caffeine content

Data on the caffeine content of the tea products from the 30 tea cultivars are presented in Tables 2 and 3. There were significant

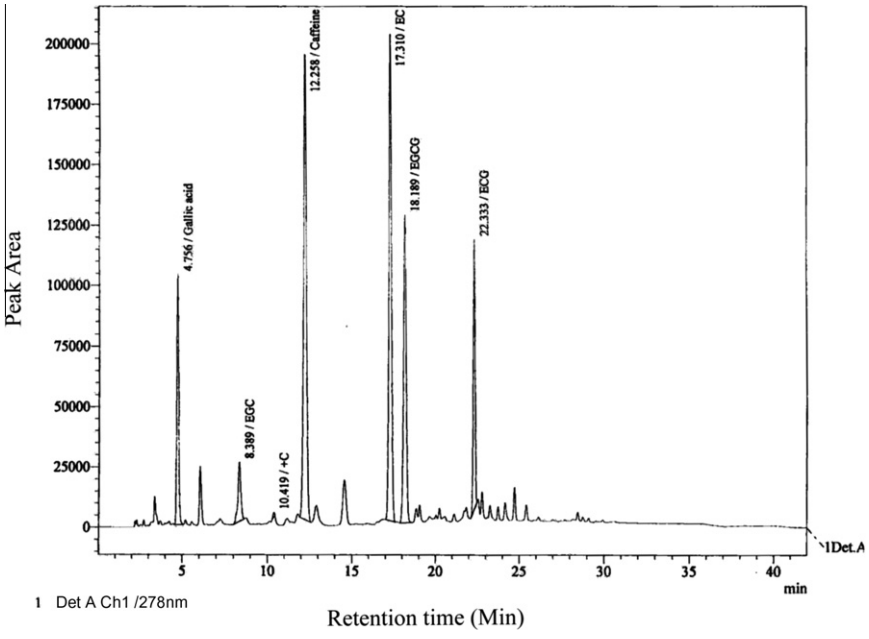

Fig. 1. HPLC chromatogram of unaerated tea from green leaf coloured clone TRFK 6/8.

Table 2
Catechin fractions (%), caffeine (%) and gallic acid (%) contents in unaerated (green) teas processed from 30 green leaf and purple leaf coloured tea cultivars.

Clones	EGC	+C	EC	EGCG	ECG	Caffeine	Gallic acid
Green leaf coloured							
GW Ejulu-L	2.35	1.22	2.38	4.04	5.69	4.03	0.91
AHP S15/10	3.07	0.51	0.88	5.61	1.76	3.78	0.85
TRFK 31/8	4.48	0.51	0.97	7.02	1.95	3.74	0.77
TRFK 31/11	2.18	0.43	0.82	4.48	1.86	3.48	0.89
TRFK 6/8	3.72	0.50	1.82	6.45	2.19	2.74	0.75
TRFK 301/2	1.43	0.37	0.83	2.38	1.09	3.79	0.65
EPK 14–3	4.20	0.30	1.05	4.24	1.39	3.57	0.64
TRFK 303/577	4.04	0.37	1.63	5.80	1.86	3.36	0.88
TRFK 303/216	3.93	0.30	1.02	3.17	1.16	3.35	0.65
Yutakamidori[a]	3.29	0.44	1.25	5.30	1.52	2.67	0.61
Yabukita[a]	2.31	0.31	1.10	2.85	0.91	2.40	0.46
TRFK 301/1	1.30	0.24	1.05	1.99	1.91	2.22	0.39
Mean	**3.03**	**0.46**	**1.23**	**4.44**	**1.94**	**3.26**	**0.70**
Purple leaf coloured							
TRFK 306/1	0.92	0.49	0.73	2.15	3.06	1.95	0.89
TRFK 306/2	0.72	0.48	0.56	1.58	3.76	1.75	0.68
TRFK 306/3	1.03	0.63	0.82	2.23	5.14	2.26	1.05
TRFK 306/4	1.00	0.68	0.76	2.34	6.34	2.48	1.15
TRFK 73/1	3.00	0.46	1.35	5.80	1.94	2.56	0.64
TRFK 73/2	2.92	0.40	1.54	5.51	1.99	2.79	0.67
TRFK 73/3	1.68	0.70	0.89	6.20	2.76	3.15	0.99
TRFK 73/4	2.58	0.56	1.10	5.69	2.13	2.55	0.64
TRFK 73/5	1.50	0.47	0.84	4.30	2.03	2.32	0.51
TRFK 73/7	3.56	0.59	1.92	4.26	3.84	2.70	0.50
TRFK K-Purple	1.03	0.79	1.27	3.13	3.65	2.99	0.79
TRFK KS1	1.80	0.77	0.88	5.35	2.09	2.15	0.57
TRFK KS2	2.86	0.38	1.27	6.40	2.07	2.66	0.68
TRFK KS3	1.15	0.56	0.87	1.34	1.44	3.16	0.90
TRFK 91/1	1.49	0.57	0.72	1.27	5.03	1.75	1.47
TRFK 91/2	1.39	0.67	0.74	3.57	3.46	2.40	0.91
TRFK 83/1	2.57	0.39	0.86	3.72	1.61	2.40	0.62
TRFK 14/1	2.31	0.32	1.68	3.63	1.54	2.38	0.51
Mean	**1.86**	**0.55**	**1.04**	**3.80**	**2.99**	**2.47**	**0.79**
Pooled Mean	2.33	0.51	1.12	4.06	2.57	2.78	0.75
LSD ($p \leqslant 0.05$)	1.24	0.34	0.56	1.86	1.96	0.76	0.23

[a] Japanese tea cultivars mainly used for manufacture of green tea.

Table 3
Catechin fractions (%), caffeine (%) and gallic acid (%) contents in aerated (black) teas processed from 30 green leaf and purple leaf coloured tea cultivars.

Clones	EGC	+C	EC	EGCG	ECG	Caffeine	Gallic acid
Green leaf coloured							
GW Ejulu-L	1.69	1.31	1.19	1.62	4.30	4.31	0.97
AHP S15/10	2.19	0.31	0.39	1.28	0.72	3.00	0.59
TRFK 31/8	2.52	0.30	0.56	1.79	1.28	4.13	0.89
TRFK 31/11	0.64	0.18	0.29	0.90	0.60	3.40	1.10
TRFK 6/8	0.47	0.65	0.65	2.92	1.68	3.20	0.90
TRFK 301/2	1.38	0.23	0.28	0.93	0.50	3.46	0.51
EPK 14–3	1.89	0.25	0.49	1.53	0.81	3.19	0.42
TRFK 303/577	1.20	0.14	0.40	1.22	0.59	2.13	0.64
TRFK 303/216	1.02	0.35	0.44	1.26	0.84	3.39	0.53
Yutakamidori[a]	0.83	0.28	0.37	0.75	0.62	3.74	0.32
Yabukita[a]	0.71	0.19	0.33	0.64	0.54	2.68	0.65
TRFK 301/1	1.07	0.40	0.26	0.46	1.07	3.59	0.76
Mean	**1.30**	**0.38**	**0.47**	**1.28**	**1.13**	**3.35**	**0.69**
Purple leaf coloured							
TRFK 306/1	1.75	0.23	0.16	1.26	1.45	2.29	0.82
TRFK 306/2	1.35	0.28	0.30	0.66	1.70	2.35	0.70
TRFK 306/3	1.05	0.36	0.19	0.50	1.39	2.02	0.75
TRFK 306/4	1.27	0.27	0.24	0.59	1.48	2.11	0.36
TRFK 73/1	1.13	0.16	0.20	0.19	0.54	2.13	0.42
TRFK 73/2	1.10	0.11	0.37	1.00	0.56	2.50	0.77
TRFK 73/3	1.45	0.27	0.31	1.51	1.14	3.35	0.73
TRFK 73/4	1.38	0.36	0.40	1.78	1.33	3.06	0.60
TRFK 73/5	1.25	0.32	0.38	1.41	0.79	3.01	0.53
TRFK 73/7	1.09	0.44	0.88	1.71	1.49	3.01	0.49
TRFK K-Purple	0.91	0.36	0.46	1.00	1.39	2.93	0.50
TRFK KS1	0.89	0.35	0.50	0.85	0.70	2.45	0.67
TRFK KS2	1.25	0.31	0.43	1.58	1.03	3.20	0.44
TRFK KS3	0.84	0.49	0.52	1.11	1.25	1.70	0.44
TRFK 91/1	1.82	0.28	0.21	0.40	1.41	1.60	1.38
TRFK 91/2	0.93	0.37	0.35	1.07	2.12	3.59	0.67
TRFK 83/1	2.23	0.26	0.42	1.30	0.79	3.62	0.43
TRFK 14/1	0.84	0.17	0.26	0.74	0.64	3.17	0.67
Mean	**1.25**	**0.30**	**0.37**	**1.04**	**1.18**	**2.67**	**0.63**
Pooled Mean	1.27	0.33	0.41	1.13	1.16	2.95	0.66
LSD ($p \leqslant 0.05$)	1.51	0.23	0.31	0.80	0.92	1.47	0.33

[a] Japanese tea cultivars mainly used for manufacture of green tea.

($p \leqslant 0.05$) differences between processed teas from the assayed cultivars. However, no significant ($p > 0.05$) difference was noted between the types of manufacture (aerated and unaerated tea). In the category of unaerated teas from the ordinary green leafed coloured cultivars, the highest levels of caffeine was exhibited by GW Ejulu-L (4.03%), with cultivars TRFK 31/8 and AHP S15/10 having comparable levels of caffeine at 3.74% and 3.78%, respectively. Unaerated tea from the popular and widely cultivated TRFK 6/8 had lower caffeine levels (2.74%). Overall, the unaerated teas processed from the two Japanese tea cultivars, Yabukita and Yutakamidori, had much lower caffeine contents (2.67% and 2.40%, respectively) than had the Kenyan cultivars. The Japanese varieties are mostly used for processing green teas and are preferred due to their lower astringency which may be attributed to the low levels of polyphenols and caffeine. Unaerated teas processed from the purple leaf coloured cultivars TRFK 306/1, TRFK 306/2, TRFK 306/3 and TRFK 306/4 were very low in their caffeine contents (1.95%, 1.75%, 2.26% and 2.48%, respectively) which may be indicative of the potential of purple leaf coloured tea cultivars to provide suitable raw material for processing of less astringent unaerated tea.

3.5. Anthocyanin concentration

The anthocyanin content and profiles of the assayed teas is as reported earlier by the same authors (Kerio et al., 2012).

3.6. Antioxidant activity

The results of the *in vitro* antioxidant activity of the tea products processed from the 30 tea cultivars are as presented in Table 4. The results showed high levels of antiradical properties of the tea products from both the green leaf coloured and purple leaf coloured tea cultivars. Generally, unaerated tea products had significantly higher ($p \leqslant 0.05$) antioxidant activity than had the aerated products. The tea products from the purple leaf coloured cultivars had antioxidant activity comparable to the products processed from the widely cultivated high quality Kenyan tea cultivars, such as GW Ejulu-L, TRFK 31/8 and TRFK 6/8. The percent radical inhibition of the unaerated teas ranged from 86.9% for cultivar Yabukita to 94.4% for GW Ejulu-L. For the aerated teas, % inhibition ranged from 62.6% (TRFK 301/1) to 91.7% (GW Ejulu-L). Teas from the Japanese cultivars, Yabukita and Yutakamidori, were shown to have lower antioxidant activities when compared to the Kenyan tea cultivars.

3.7. Relationship between catechins, anthocyanins and antioxidant activity in unaerated and aerated tea products

Correlation analysis showed that, in both unaerated and aerated tea products, total polyphenols correlated most significantly with antioxidant activity ($r = 0.844^{**}$, $p \leqslant 0.01$ and $r = 0.797^{**}$, $p \leqslant 0.01$, respectively). Anthocyanins seemed to have a significant role in

Table 4
DPPH radical-scavenging activities (%) of aerated and unaerated teas processed from 30 green leaf and purple leaf coloured tea cultivars.

Clones	Unaerated (Green) tea	Aerated (Black) tea
Green leaf coloured		
GW Ejulu-L	94.4	91.7
AHP S15/10	93.4	90.9
TRFK 31/8	94.3	91.4
TRFK 31/11	90.9	86.7
TRFK 6/8	94.1	90.8
TRFK 301/2	92.5	90.4
EPK 14–3	92.5	84.9
TRFK 303/577	92.7	77.0
TRFK 303/216	92.4	79.1
Yutakamidori[a]	88.6	56.3
Yabukita[a]	86.9	70.8
TRFK 301/1	89.5	62.6
Mean	**91.9**	**81.1**
Purple leaf coloured		
TRFK 306/1	92.6	91.2
TRFK 306/2	92.0	90.9
TRFK 306/3	92.8	90.3
TRFK 306/4	92.7	90.8
TRFK 73/1	93.3	90.8
TRFK 73/2	92.6	89.4
TRFK 73/3	93.1	91.6
TRFK 73/4	92.6	90.8
TRFK 73/5	92.8	90.8
TRFK 73/7	93.6	91.8
TRFK K-Purple	92.5	91.1
TRFK KS1	89.6	90.7
TRFK KS2	92.8	90.6
TRFK KS3	89.5	85.8
TRFK 91/1	92.9	91.2
TRFK 91/2	89.5	90.2
TRFK 83/1	89.1	65.7
TRFK 14/1	89.9	82.6
Mean	91.9	88.7
Mean	91.9	85.6
CV%	0.85	3.38
LSD ($P \leqslant 0.05$)	1.28	4.73

[a] Japanese tea cultivars mainly used for manufacture of green tea.

the antioxidant activity of the tea products, as shown by higher and significant correlations in both tea products (Table 5). The most potent anthocyanins were Cyanidin-3-*O*-glucoside ($r = 0.843^{**}$, $p \leqslant 0.01$), cyanidin-3-*O*-galactoside ($r = 0.801^{**}$, $p \leqslant 0.01$) and malvidin ($r = 0.783^{**}$, $p \leqslant 0.01$) in unaerated tea products. In addition to peonidin, the above three anthocyanins were also strongly correlated with antioxidant capacity of aerated tea.

3.8. Extractability of tea anthocyanins in water

The maximum extraction efficiency of anthocyanins in water was achieved after 5 min for aerated tea and 7 min for unaerated tea (Table 6.). After 4 h, methanol extraction achieved maximum extraction efficiency, equivalent to that of the water extract after only 2 min. This showed that anthocyanins were highly soluble in water.

Table 5
Correlation coefficients between anthocyanins, catechins and antioxidant activity (* and ** represent significant correlations at $p \leqslant 0.05$ and $p \leqslant 0.01$ levels, respectively).

	ANTIOXIDANT ACTIVITY	
	Green tea (unaerated)	Black tea (aerated)
Total polyphenols (TP)	0.844**	0.797**
Total catechins (TC)	0.818**	0.031
Total anthocyanins	0.789**	0.700*
Anthocyanin Conc.	0.760*	0.711*
Cyandin-3-*O*-galactoside	0.801**	0.748**
Cyanidin-3-*O*-glucoside	0.843**	0.764*
Delphinidin	0.478	0.570
Cyanidin	0.598	0.416*
Pelargonidin	0.411	0.696*
Peonidin	0.661*	0.732*

Table 6
Total monomeric anthocyanins (mg/l) extracted after brewing tea for different time intervals in water compared with methanol extract.

Brewing time in boiling water		Type of purple tea (green)	
		Aerated (black)	Unaerated (green)
	2 min	28.2	99.9
	5 min	53.1	134
	7 min	47.6	139
	10 min	36.5	99.5
Methanol extract	4 h	24.0	98.9

4. Discussion

The extent of variation in the TP content between the cultivars is an important trait for tea breeders since it provides a basis for selection, improvement and management of tea quality. A comparison, either between aerated or unaerated teas processed from Kenyan and two Japanese tea cultivars (Yabukita and Yutakamidori), revealed that the former had higher levels of total polyphenols. It was also noted that the aerated teas from Kenyan germplasm had higher TP content than had the unaerated teas from the Japanese cultivars. This observation translated to lower antioxidant activities for teas processed from the Japanese cultivars. A previous study by Wachira and Kamunya (2005) confirmed the superiority of the Kenyan tea germplasm in their TP content when compared with germplasms from China and Japan which are traditionally used for the manufacture of unaerated teas. In their study, Karori, Wachira, Wanyoko, and Ngure (2007) also established that tea products derived from Kenyan tea cultivars were rich in total polyphenols when compared to those from Japanese and Chinese cultivars. Varieties selected for the manufacture of unaerated tea have traditionally been found to be low in total polyphenols. This ensures that tea products processed from such germplasm are low in astringency and bitterness. The converse is true for the Kenyan germplasm which has been bred for high levels of total polyphenols, principally because it is used for the manufacture of aerated tea. High levels of polyphenols have been correlated with high quality in aerated tea (Obanda, Owuor, & Mang'oka, 2004).

Several factors, including genotype, geographical origin, growing conditions (including soil composition and moisture regimes, harvesting time, post-harvest treatments and physical structure of the leaves) have been shown to influence the polyphenolic content and composition of tea (Lin, Yao-Jen, Tsay, & Lin, 2003; Kamunya et al., 2009; Cheruiyot, Mumera, Ng'etich, Hassanali, & Wachira, 2007; Cheruiyot et al., 2008). Studies have shown that anthocyanins, which are polyphenolic leaf pigments, are also much affected by environmental factors, with high temperature conditions lowering their accumulation in plant leaves, fruits and flowers. This is attributed to the decreased transcript levels of two key enzymes in the anthocyanin synthesis pathway, chalcone synthase (CHS) and dihydroflavonol reductase (DFR) (Dela et al., 2003). This could also be the reason why we have observed that, during hot/dry seasons, the leaves of anthocyanin-rich teas are not as intensely pigmented as during the cold and wet season. Owing to the above observations, we hypothesize that the results obtained in this study will vary with seasons and it is important that detailed studies on the seasonal variations of polyphenols in the tea products derived from the assayed cultivars are carried out.

Catechins are the main polyphenolic compounds (flavan-3-ols) in the fresh leaf of the tea plant (*Camellia sinensis*). In general,

the total catechin content in unaerated tea products from the green and also the purple leaf coloured tea cultivars was significantly higher ($p \leqslant 0.05$) than that in the aerated tea products from the same cultivars. This finding is similar to that of Karori et al. (2007). This demonstrates that aeration ('fermentation') during black tea manufacture significantly influences the catechin content of the final processed products. The aeration step in black tea manufacture is basically a post-harvest auto-oxidation reaction which is catalysed by polyphenol oxidase (EC 1.10.3.1). On the other hand, unaerated teas do not undergo the 'fermentation' process since the leaf is initially steamed to inactivate the enzyme. The consequence of enzymatic oxidation on the catechins, which are located in the cell vacuole, is the polymerization of the flavan-3-ol monomers to form theaflavins (TFs) and thearubigins (TRs), which are compounds that influence the quality of aerated tea products (Owuor & Obanda, 2001).

The HPLC analysis of the catechin in the processed tea revealed that the catechin fractions had different elution patterns. The non-gallated catechins, epigallocatechin (EGC) and catechin (+C), eluted before the gallated catechins, epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). This is attributed to the fact that the non-gallated catechins are polar and hence bound less tightly to the non-polar C18 column where they were eluted first upon introduction of the highly polar mobile phase A (9% acetonitrile). The gallated catechins, on the other hand, were eluted last since they bound more tightly to the column and were only eluted upon increase of the non-polar mobile phase B (80% acetonitrile).

Catechin profiles varied significantly among the tea products assayed in this study with the trihydroxylated catechins (EGCG and EGC) being more abundant than the dihydroxylated catechins (+C, EC and ECG) in unaerated tea products. Aerated tea products had lower amounts of the individual catechins due to formation of TFs and TRs. Optimal TFs formation requires a correct balance of trihydroxy flavan-3-ol and dihydroxy flavan-3-ol (Wright, Mphangwe, Nyirenda, & Apostolides, 2002). The lowering of individual catechins in the aerated tea products could be attributed to the oxidation of catechins into quinones that take part in redox equilibration reactions during the auto-oxidation process, thereby causing different catechins to deplete at different rates. The B-ring trihydroxylated catechins (EGCG and EGC) have been found to oxidize at a much faster rate than the B-ring dihydroxylated catechins (+C, EC and ECG) due to their lower redox potentials (Nagle, Ferreira, & Zhou, 2006; Owuor & Obanda, 2007; Nanjo, Mori, Goto, Keiichi & Hara, 1999; Yao, Ye, Zhang, Tang, & Zhang, 2007; Ngure, Wanyoko, Mahungu, & Shitandi, 2009).

Our study has shown that the gallic acid content remained unchanged in the aerated and unaerated tea products from the different green and purple leaf coloured cultivars. In the fresh leaf of the tea plant, gallic acid is present in trace amounts though it accumulates during the auto-oxidation step of the black tea manufacturing process due to the breakdown of galloyl esters from the catechins and/or theaflavin gallates. The higher gallic acid levels in tea products from some cultivars may be ascribed to high levels of gallated catechins which lead to formation and subsequent breakdown of the TFs. Gallic acid is not a substrate for polyphenol oxidase though, during the auto-oxidation step of black tea manufacture, theaflavic acids are formed by redox equilibration, which in turn are thought to oxidize the gallocatechins (EGC and EGCG) to release gallic acid. The levels of gallic acid in teas will therefore depend on both leaf levels of gallocatechins and the extent to which auto-oxidation is carried out.

Synthesis and accumulation of caffeine in the tea plant have been found to be genotype-dependent (Obanda & Owuor, 1997; Owuor & Chavanji, 1986), a fact that is corroborated by our data. The caffeine content of the teas processed from the ordinary green leaf coloured tea cultivars was higher in both aerated and unaerated teas compared to those from the purple leaf coloured cultivars. However, this purine alkaloid is an important compound that contributes to the briskness (Bhatia, 1963) of aerated tea. It has also been proposed as a potential indicator of quality of Kenyan aerated teas (Obanda & Owuor, 1997). Caffeine complexes with the polyphenols, mainly the theaflavins, in aerated tea (Roberts, 1962). A study to compare caffeine levels in different manufactured types revealed that caffeine content was in the order: black tea < oolong tea < green tea < fresh leaf (Lin et al., 2003), showing that, the more the teas are "fermented", the lower is the caffeine content due to formation of a complex with theaflavins. The complexing of caffeine with the polyphenols in aerated tea results in formation of a coloured precipitate or 'cream' when the infusion is cooled (Roberts, 1962). The complex formed has been found to positively modify the taste characteristics of both caffeine and theaflavins (Sanderson, Berkowitz, Co, & Graham, 1972). From these results, it can be deduced that the purple leaf coloured anthocyanin-rich cultivars are likely to produce an aerated tea product that is less brisk, more palatable to the mouth and with little cream after cooling, due to lower amounts of caffeine in the cultivars. Indeed, the purple leaf coloured anthocyanin-rich cultivars would be good raw material for production of unaerated tea, as well as herbal drinks, since the resultant products would be less astringent than those from the green leaf coloured cultivars. The caffeine reduction in the purple teas may be hypothesized to be caused by biochemical mechanisms since caffeine (alkaloids), catechins and anthocyanins (flavonoids) are plant secondary metabolites, both derived from the shikimate pathway. The shikimate pathway provides aromatic amino acids that serve as precursors of natural products, such as pigments and alkaloids (Maeda & Dudareva, 2012), and it is the activities of the genes coding for caffeine synthase or anthocyanin synthase that determine their levels of caffeine and anthocyanins, respectively. Indeed, caffeine biosynthesis, just like anthocyanin biosynthesis, is also affected by seasonal variations (Mohanpuria et al., 2009). However, the mechanisms by which their levels vary warrant further studies. Despite the negative side effects of caffeine addiction, studies by Huang, Liu, Dushenkov, Ho, and Huang (2009) have shown that caffeine, in combination with black tea extract and EGCG, exhibits anti-obesity properties.

The radical-scavenging activities of the tea products determined by the DPPH radical (antioxidant activity) revealed that unaerated tea had higher radical-scavenging activity than had the aerated teas, though there was no significant difference in antioxidant capacity of teas processed from the green and purple leaf coloured cultivars. The major potential antioxidant components of unaerated teas from the green leaf coloured cultivars are the catechins while the purple leaf coloured teas have both catechins and anthocyanins. The most effective radical-scavengers in unaerated teas have been found to be the catechins with 3′, 4′ and 5′-trihydroxylated substitution patterns on the B-ring and/or trihydroxyl groups at the C3 position of the catechin structures (Cabrera et al., 2003). The hydroxylation patterns of the catechins are an important feature of their antiradical potential since they confer a high degree of stability on the catechin phenoxyl radical by participating in electron delocalization, which explains the high radical-scavenging activity exhibited by the gallated catechins, EGCG and EGC in green tea (Rao, Lekh, & Takado, 2006). In the aerated tea products from both the green leaf coloured and purple leaf coloured cultivars, the major components that contribute to the antioxidant activity are the theaflavins (TFs), a mixture of theaflavin complexes with the polymerized anthocyanins for the latter type of tea, and probably some thearubigins (TRs).

Though the data obtained in this study revealed that aerated tea products had lower antioxidant capacity than had the unaerated teas, the former teas still retained significantly high radical-scavenging activity which we attribute to the presence of

theaflavins. The high percentage of inhibition of up to 91%, exhibited by the aerated teas in this study, shows that the conversion of tea catechins to TFs does not affect the radical-scavenging potency of the dimerized products. This finding is in agreement with that of Leung et al. (2001) who demonstrated that TFs in black tea possess at least the same antioxidant potency as do catechins in green tea and the conversion of the latter to TF does not significantly alter their free radical-scavenging activities. The radical-scavenging activities of aerated tea products from the Kenyan tea cultivars compared favourably with those of unaerated teas from the Japanese varieties (Yabukita and Yutakamidori), which is indicative of the superiority of the Kenyan tea cultivars in this characteristic.

The antioxidant activities of the aerated and unaerated tea products from the purple coloured tea cultivars were also high and this may largely be ascribed to the TFs and anthocyanin derivatives in the former and catechins and anthocyanins in the latter. Correlation analysis between the different biochemical parameters and antioxidant capacity revealed that some anthocyanin/anthocyanidin fractions in the purple leaf coloured cultivars were individually and collectively potent anti-radical molecules. These included; cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, peonidin and malvidin. The antioxidant efficiency of anthocyanins has been shown to be related to several parameters, e.g. the number of hydroxyl groups in the molecule, the catechol moiety in the B-ring, the oxonium ion structure in the C-ring, the hydroxylation and methylation pattern and to the acylation by phenolic acids (Prior & Wu, 2006). A study on the antioxidant activities of wines also revealed that glycosylated and methoxylated anthocyanins, e.g. cyanidin-3-*O*-glucoside and malvidin-3-*O*-glucoside (predominant anthocyanins in red wines) were responsible for the antioxidant effect of red wine (Perez-Rivero, Muniz, & Gonzalez-Sanjos, 2008). A study to determine the quenching of singlet molecular oxygen ($^1O_2$) by the flavylium cation revealed that the quenching efficiency was larger for the malvidin derivative, probably due to the electron donating methoxyl group in the B-ring of the malvidin molecule (Prior & Wu, 2006). The findings by Prior and Wu (2006) are in agreement with our results, since the most predominant anthocyanidin in the purple-leafed Kenyan tea cultivars was also found to be malvidin (Kerio et al., 2012). Further, it can also be deduced that the high antioxidant activities exhibited by the purple leaf coloured anthocyanin-rich teas could be due to the catechin–anthocyanin complexes that are believed to have additional hydroxyl groups necessary for free radical-scavenging activity. However, the lowering of the antioxidant activities in the aerated teas after the 'fermentation' process and drying can be attributed to degradation of both the coloured anthocyanins and their colourless degradation products following thermal exposure during the drying of the tea products at temperatures of 120 °C to 150 °C. The radical-scavenging activities of the purified anthocyanins showed that leaf extracts from the purple tea cultivars exhibited higher radical-scavenging activities than did those from the ordinary green leaf coloured tea cultivars whose major components are the catechins (data not shown). This can be attributed to the synergistic effect of all the anthocyanins, as well as the positively charged oxygen atom in the anthocyanin molecule that makes it a distinct hydrogen-donating compound compared to other flavonoids. The uniqueness of the anthocyanin molecule as an antioxidant has been attributed to its ability to delocalize electrons and form resonating structures after changes in pH, a characteristic feature that does not take place in other antioxidants (Bagchi, Roy, Patel, & He, 2006). The antioxidative effect of the anthocyanins could also be synergized by the presence of catechins. For example, a study on free radical-initiated peroxidation of linoleic acid on micelles revealed that the presence of catechin regenerated the highly efficient antioxidant malvidin-3-glucoside, thereby increasing its antioxidant efficiency (Rossetto et al., 2002). Catechins can therefore boost the antioxidant power of the anthocyanins and the presence of both compounds in the tea products processed from the purple leafed clones is a positive sign that the anthocyanin-rich teas can be marketed as a health drink. In addition, anthocyanins have been proven to be novel antioxidants and potent inhibitors of lipid peroxidation in comparison with other classic antioxidants (Bagchi et al., 2003). The high solubility of anthocyanins in water is also an added advantage since tea is generally consumed as a water extract liquor and this means that anthocyanin bioavailability can be guaranteed. This is evident in the maximum extraction efficiencies shown in the anthocyanin concentrations extracted by water and methanol at different time intervals. The high solubility of anthocyanins in water is attributed to the net positive charge they carry and glycosyl moieties attached to them. In this study, the extraction efficiency in water was lowered after 7 min, probably due to degradation of anthocyanins at high temperatures (Zimmerman & Gleichenhagen, 2011). During heating, the first degradation step is caused by the opening of the pyrilium ring and chalcone formation leaving colourless degradation products, such as phenolic acids and phloroglucinaldehyde (Sadilova, Stintzing, & Carle, 2006). Indeed, a study of different anthocyanin extraction methods to reduce anthocyanins in purple corn waste Jing and Giusti (2007) revealed that deionized water at 50 °C achieved the highest yield of anthocyanins with low tannins and proteins, compared to anthocyanin yield obtained by 70% acetone. The presence of water-soluble catechins in the purple tea is an added advantage since the tea will contain the two antioxidants in one brew, translating to one healthful beverage. The high solubility of tea anthocyanins in water is also good news to the food industries since the anthocyanins could be spray-dried into powder form and used to colour foodstuffs without fear of contamination from residual organic solvents used sometimes for extraction.

5. Conclusion

Numerous foods, fruits, vegetables and beverages have been found to contain polyphenols, including catechins and anthocyanins, also found in tea. These polyphenols have been reported to have higher antioxidant activities than those of vitamins C and E, as well as those of synthetic antioxidants, such as butylated hydroxyl toluene (BHT). These polyphenols form an integral part of diet and possess strong free radical and anti-radical properties (Soobrattee, Bahorun, & Aruoma, 2006; Ross & Kasum, 2002) However, synthetic antioxidants are increasingly being rejected by consumers as they are not easily metabolised by the body and can accumulate to harmful levels to become potentially carcinogenic (Kahl, 1984). Being the world's most preferred non-alcoholic beverage, tea has high contents of polyphenolic compounds which are also potent antioxidants. Tea antioxidants, including catechins and anthocyanins, are proposed as the naturally and widely available substitutes for synthetic antioxidants. Tea's antioxidants, namely catechins and especially the novel anthocyanins, are highly soluble in water, are fairly non-toxic, even when consumed at relatively high concentrations, and are therefore highly bioavailable through consumption of the tea liquor.

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