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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