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Studies on quality of orthodox teas made from anthocyanin-rich tea clones growing in Kangra valley, India

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ABSTRACT

Recently anthocyanin-rich purple tea varieties have been developed. The quality of these new purple tea varieties developed in Kangra valley was assessed, and compared with the quality of tea from standard Kangra clone. Purple tea shoots (PL) recorded higher amount of polyphenols compared to standard green tea shoot (GL) while epigallocatechin gallate (EGCG) recorded higher levels in GL. Higher levels of theaflavins were recorded in orthodox black tea from purple shoots (BTP) compared to black tea (BT) made from green shoots. Both theanine and caffeine recorded higher levels in GL. Volatile flavour profiles of these teas showed qualitative and quantitative differences. Aroma extract dilution assay showed higher dilution factors in BTP than BT. Orthodox teas from purple shoots exhibited higher antioxidant activity compared to standard black tea. Strong correlation of total quality scores with aroma and infusion colour was observed. Tea from anthocyanin-rich cultivars can become specialty teas with high antioxidant activity.

1. Introduction

The parameters for quality determination of black and green tea differ due to variations in their processing. In 2009, 61% of global production was black tea with 38% CTC, 23% orthodox while 31% was green tea (Anonymous, 2010). Tea contains up to 30% phenols, mainly catechins, that play important role in determining quality and characteristic taste of different teas (Robertson, 1992). When processing green tea, endogenous enzymes are inactivated to prevent oxidation of leaf catechins, thereby retaining natural catechins, all in higher concentrations than other types of teas (Hung, Chen, Chen, & Cheng, 2010). Catechins are responsible for taste and astringency of green tea. Black tea processing, on the other hand, involves oxidation of catechins by endogenous leaf polyphenol oxidase and peroxidases to produce coloured theaflavins and thearubigins. These coloured compounds are responsible for characteristic taste, brightness and colour of black tea liquors. Orthodox black teas possess fine, distinct and complex aroma compared to CTC black and green teas. Darjeeling region and Kangra valley in India and Keemun Maofeng in China predominantly manufacture and export orthodox black teas. Apart from the non-volatile compounds, more than 600 volatile flavour compounds belonging to different class of compounds viz. hydrocarbons, alcohols, aldehydes, esters and lactones have also been detected in different teas to date.

Recently, breeders in different tea growing countries have developed anthocyanin-rich purple tea varieties (Hsu, Shih, Lin, Chiu, & Lin, 2012). Profiles of phenols and catechins of various anthocyanin-rich purple and normal green tea cultivars growing in Kenya have been compared (Kerio, Wachira, Wanyoko, & Rotich, 2013). Caffeine content was lower in purple tea leaves compared to green tea leaves (Kerio et al., 2013). Other studies conducted on anthocyanin-rich tea shoots laid emphasis on isolation and characterisation of anthocyanins from tea shoots and flowers, and studied the cytoprotective effects of tea anthocyanins on oxidative stress induced in HEK 293 WT cells by *t*-butyl hydroperoxide (Kerio, Bend, Wachira, Wanyoko, & Rotich, 2011). However, no systematic chemical analysis in relation to tea manufacture and quality has been reported for anthocyanin-rich tea. In Kangra valley, anthocyanin-rich tea bushes are marked with purple apical shoots which are stable and uniform under different growth flushes. The present study proposes to quantify quality-related chemicals in anthocyanin-rich tea shoots, green and black tea made from them and compare the quality with teas from normal shoots.

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2. Material and methods

2.1. Reagents and standards

HPLC-grade solvents acetonitrile, methanol and dichloromethane were purchased from Merck India, Mumbai. Catechins and flavonoid standards were purchased from Sigma–Aldrich, Bangalore. Amino acid standards and estimation kit was supplied by Waters India, Mumbai. All other chemicals and reagents used were of analytical grade.

2.2. Plant material and green tea manufacture

Fresh tea leaves (two and a bud) for the study were plucked from tea bushes undergoing 7 days regular plucking at the Institute's Tea Experimental Farm at Banuri (36° N and 78.18° E and 1290 m above mean sea level) during the main flush (July to September). The purple-leaf coloured tea clones under study are IHBT-269, IHBT-270, IHBT-271, IHBT-272, IHBT-273, IHBT-274 and IHBT-275. Clones IHBT-182 and IHBT-002 were used as reference standard tea varieties because of their good quality. Fresh plucked anthocyanin-rich purple shoots (PL) and standard green (GL) shoots were divided into three sets. The shoots of the first set were immediately dried to constant weight at power level 7 in an IFB microwave-cum-convection domestic oven (30-L capacity, 1400 W) and kept in desiccators till further use.

The second set of shoots was processed for green tea manufacture following the method of Gulati, Rawat, Singh, and Ravindranath (2003). Tea shoots were inactivated by heating at power level 6 for 4 min in a covered glass container in an IFB microwave-cum-convection domestic oven (30-L capacity, 1400 W) so that enough steam is generated from the moisture trapped within the shoots and was sufficient to inactivate the endogenous polyphenol oxidase enzyme. The inactivated shoots were cooled and rolled in an orthodox piezy roller wherein the shoots were twisted under pressure. The rolled shoots were then dried using microwave-cum-convection domestic oven for 7 min at power level 6. The teas were labelled as green tea (GT) made from normal shoots and green tea purple (GTP) from anthocyanin-rich shoots.

2.3. Orthodox black tea manufacture

A third set of shoots was subjected to withering under ambient air at constant flow for 10 h; rolling, using an orthodox piezy roller for 0.5 h, fermentation for 90 min, and drying in a microwave-cum-convection domestic oven for 4–5 min. The orthodox black teas were labelled as orthodox black tea (BT) from normal shoots and orthodox black tea purple (BTP) from anthocyanin-rich shoots.

2.4. Tea extracts

Different tea samples (GL, PL, GT, BT, GTP and BTP) were dried to constant weight and extracted following the method of Sharma, Gulati, and Ravindranath (2005). Dried tea samples (0.1 g) were extracted sequentially with 2 mL, 1.5 mL and 1.5 mL of methanol (70%), followed by 30 s vortex mixing and centrifuging at 4000 rpm for 10 min at 4 °C. The final volume was made up to 5 mL with methanol (70%).

Dried tea samples (0.1 g) were also extracted in a similar way with acetone (70%) followed by vortex mixing and centrifuging. The final volume was made up to 5 mL with acetone (70%). The experiment was repeated thrice.

2.5. Estimation of total polyphenols

Polyphenols were estimated in various tea extracts using Folin–Ciocalteu reagent. Acetone extracts prepared above were dispensed into triplicate sets of 25-mL volumetric flasks. Folin–Ciocalteu reagent (500 μL, 1 N) was added, followed by saturated Na_2CO_3 (100 μL). The volume was made up to 25 mL with distilled water. Absorbance was taken at 730 nm on a Shimadzu UV–Vis spectrophotometer after incubation of 30 min at room temperature. Total polyphenols were expressed as per cent polyphenols equivalent to gallic acid. The experiment was repeated thrice.

2.6. Estimation of total flavonoids

Total flavonoid contents of the tea extracts were measured according to Zhishen, Mengcheng, and Jianming (1999). The acetone extract (1 ml) was diluted with distilled water and $NaNO_2$ (5%) followed by $AlCl_3$ (10%) were added. The mixture was incubated for 5 min before adding NaOH (1 M). Finally, the volume was made up to 10 mL with distilled water and absorbance read at 510 nm. Total flavonoid was expressed as per cent flavonoid equivalent to quercetin. The experiment was repeated thrice.

2.7. Flavonoids profile

Different flavonoids were separated and estimated following the method of Sharma et al. (2008) in methanolic extracts prepared above. Analysis was performed on a Waters HPLC system equipped with 600 quaternary gradient pump, 2998-PDA and 717 autosampler. Separation was achieved at 25 °C on a RP-18 Lichrocart column (250 mm × 4.0 mm, 5 μm), fitted with suitable guard column using 0.05% trifluoroacetic acid in water (**A**) and acetonitrile (**B**) as mobile phase. Elution of standards and samples was performed at a flow rate of 1.0 ml/min and detection at 355 nm. Identification of compounds was performed on the basis of the retention time, co-injections, and spectral matching with standards. Three replicates were considered.

2.8. Extraction and estimation of total catechins

Catechins were estimated by the method of Singh, Ravindranath, and Singh (1999). The procedure is specific for catechins and is based on the ability of diazotised arylamine to form coloured complexes with the A-ring of catechins. Acetone extract (40 μL) reacts with diazotised sulphanilamide reagent (1 mL) in the presence of HCl (30%). The mixture was incubated for 1 h at ambient temperature before reading the absorbance at 425 nm using a Shimadzu UV–Vis spectrophotometer. Catechins expressed as per cent catechins equivalent to d-catechin. The experiment was repeated thrice.

2.9. HPLC analysis of catechins

The methanolic extracts prepared above were filtered through a 0.45-μm nylon filter (Millipore, Billerica, MA) before analysis on a Waters HPLC system following the method of Sharma, Gulati, Ravindranath, and Kumar (2005). Separation was achieved using an RP-18 Lichrocart column (250 mm × 4.0 mm, 5 mm). The mobile phase employed was 0.1% ortho-phosphoric acid in water (*w*/*v*) (**A**) and acetonitrile (**B**) with a flow rate of 1 mL/min. The HPLC elution program consisted of ratios of solvents **A**:**B** of 90:10 (0–10 min), 90:10 to 80:20 (10–12 min), 80:20 to 65:35 (12–15 min), 65:35 to 64:36 (15–18 min), 64:36 (18–20 min), 64:36 to 70:30 (20–24 min), 70:30 to 80:20 (24–28 min) and 80: 20 to 90:10 (28–30 min). Injection volume was 20 μL. Identification of compounds was performed by matching retention time, co-injec-