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Antioxidant capacity and major polyphenol composition of teas as affected by geographical location, plantation elevation and leaf grade

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ABSTRACT

Tea polyphenols have been a topic of discussion due to their health benefits. Nevertheless, detailed studies on the antioxidant capacity and polyphenol contents of teas in relation to factors including geographical locations, plantation elevations and leaf grades have been limited. In this study, 53 tea samples were analysed to determine the individual and total catechin and theaflavin contents by HPLC and the total antioxidant capacity by Oxygen Radical Absorbance Capacity (ORAC) methods. Results show that the polyphenol (catechins and theaflavins) contents were significantly influenced by plantation location. Black tea from low plantation elevation contained 22–28% more polyphenols than those from high elevation. Small tea leaves had up to 15% more polyphenols than larger leaves from similar elevation. The results were further confirmed by Principal Composition Analysis (PCA), which grouped the black and green tea samples into 3 different clusters, respectively.

1. Introduction

Tea consumption has a long history and tea is popular in some Asian, South American and European countries, as a beverage and as herbal medicine. Since the last decades, tea has emerged as a popular source of dietary antioxidants and its effects are being investigated by *in vitro* and *in vivo* methods (Yang, Du, & Yang, 2016). Black tea and green tea are two main types of tea, and their antioxidant effects are thought to be contributed by polyphenols (Vinson & Dabbagh, 1998). Tea polyphenols include flavonols (quercetein, kaempferol, myricetin), flavan-3-ols (catechins and theaflavins), and a small amount of purine alkaloids (caffeine and theobromine), gallic acid derivatives (gallic acid, 5-galloylquinic acid), and hydroxycinammate quinic esters (caffeoylquinic acids) (Del Rio et al., 2004). Among them, catechins and theaflavins are the two common indices used to determine the antioxidant ability of tea.

Antioxidants are compounds that can reduce, slow down or prevent oxidation process (Kaur & Kapoor, 2001). Antioxidant compounds can reduce radicals produced from oxidation reactions in human body, thus contributing to its anti-carcinogenic properties (Rodrigo, Miranda, & Vergara, 2011). Many studies have found that tea antioxidant compounds have a promising effect on various cancer cells *in vitro*, as well as protect DNA from being damaged by free radicals (Friedman et al., 2007; Ježovičová et al., 2016; Yang et al., 2016).

Tea catechins have two geometrical isomers (*trans*-catechins and *cis*-epicatechins), and each isomer has two optical isomers: (+)-catechin and (−)-catechin; (+)-epicatechin and (−)-epicatechin. (−)-catechin can be turned into (−)-catechin-3-gallate, epicatechin-3-gallate, (−)-epigallocatechin-3-gallate, and (−)-gallocatechin-3-gallate by esterification with gallic acid. By oxidative coupling, different catechins can form four types of theaflavins including theaflavin (TF), theaflavin-3-gallate (TF3G), theaflavin-3′-gallate (TF3′G) and theaflavin-3, 3′-digallate (TF33′G) (Friedman et al., 2005).

Catechins are the primary polyphenols present in fresh tea leaves, and are mostly preserved in green tea (Astill, Birch, Dacombe, Humphrey, & Martin, 2001; Graham, 1992). However, during processing of black tea, most catechins in fresh green tea leaves are oxidised to form theaflavins through an oxidative fermentation process (Graham, 1992). This process involves enzymatic browning by polyphenol oxidase (Sharma, Bari, & Singh, 2009) where the primary substrate of the enzyme is *o*-dihydric phenols, i.e., catechins in tea leaves (Graham, 1992). This is a complex process involving multi-step reaction pathway (Tanaka, Inoue, Betsumiya, Mine, & Kouno, 2001). Theaflavins contribute to the dark and reddish colour of black tea, as well as the astringent and bitter taste, which is also an important characteristic of black tea.

Determination of total catechins and theaflavins has been normally used to describe the quality of green and black teas. However, individual catechin and theaflavin especially (−)-epigallocatechin gallate (EGCG) and TF33′G are being used to evaluate the quality of green and black teas (El-Shahawi, Hamza, Bahaffi, Al-Sibaai, & Abduljabbar, 2012). EGCG is the main catechin in green tea and has been found to

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have anticarcinogenic property (Chowdhury, Sarkar, Chakraborti, Pramanik, & Chakraborti, 2016). The advantages of EGCG as anti-cancer remedy are due to its safety, bioavailability and low cost (Singh, Shankar, & Srivastava, 2011). It has been reported to be a powerful polyphenolic antioxidant, which is proposed to prevent oxidative damage of healthy cells and inhibit the inflammatory procedures that cause transformation, hyperproliferation and suppression of cancer (Thawonsuwan, Kiron, Satoh, Panigrahi, & Verlhac, 2010; Wang, Zhang, Zhong, Perera, & Shahidi, 2016; Yang et al., 2016). EGCG has also been reported to have other beneficial effects on diabetes, stroke and obesity (Oršolić et al., 2013; Sergent, Vanderstraeten, Winand, Beguin, & Schneider, 2012; Zhang et al., 2016).

TF3′G, one of the theaflavins, is a polymerized and oxidized product from catechins in black tea. Studies have shown that TF3′G has powerful anti-cancer and antioxidant properties (Gao, Rankin, Tu, & Chen, 2016; Kimutai et al., 2016). In a recent study, Ying, Rankin, Youying, and Charlie Chen (2016) verified that TF3′G has even more potent anti-tumour activity compared to EGCG in inhibiting woman ovarian carcinoma OVCAR-3 cell-induced angiogenesis via Akt and Notch-1 pathways. A positive and significantly high correlation between TF3′G and antioxidant activity has also been found in black tea (Kimutai et al., 2016).

In addition to derivatization during tea processing, the concentrations of catechins and theaflavins in green and black teas may be influenced by other factors such as tea tree variety, growing environment, manufacturing conditions, *etc.* Many studies have been conducted on the antioxidants in tea (Damiani, Bacchetti, Padella, Tiano, & Carloni, 2014; Koczka, Ombódi, Móczár, & Stefanovits-Bányai, 2016), but few studies aimed to analyse the diversity of tea antioxidant activity affected by different geographical locations, plantation elevation, leaf size and especially leaf grade. New Zealand, has diversified tea-consuming ethnics, with various tea types and brands from different parts of the world. This provides a convenient platform to study tea properties from a wide range of samples.

This study is therefore aimed to investigate factors (geographical and plantation locations, plantation elevations and leaf grades) that affect antioxidant capacity. It also aims to study the contents of the major individual catechins and theaflavins of the 53 samples sourced from different countries and areas.

2. Materials and methods

2.1. Materials

A total of 53 black tea and green tea samples were analysed in this study. Among which, 48 of them were kindly given by Bell Tea and coffee Company Ltd. (Auckland, New Zealand), 4 Longjing tea samples were obtained from Meichun tea estate (Hangzhou, China) and one additional sample was obtained from Aaah Tea (Auckland, New Zealand). All the standards used were of HPLC grade including, (−)-Catechin (C); (−)-catechin gallate (CG); (−)-epicatechin gallate (ECG); (−)-gallocatechin (GC); (−)-epigallocatechin (EGC); (−)-gallocatechin gallate (GCG); (−)-EGCG which were purchased from Sigma-Aldrich (St. Louis, USA). The mixed theaflavins were purchased from Sigma-Aldrich (St. Louis, USA) which contains TF, TF3G, TF3′G, TF33′G. Other HPLC grade chemicals used are as follows: formic acid from BDH Chemical Ltd., Co. (Poole, England); acetonitrile from Romil Ltd., Co. (Cambridge, England); acetone, ethanol and methanol from Burdick and Jackson Ltd., Co. (Muskegon, USA). Milli-Q water was used in the experiments quality.

2.2. Extraction and preparation of tea samples

Extraction methods were based on the experiment performed by Friedman, Levin, Choi, Kozukue, and Kozukue (2006). A total of 1.5 g of dried tea leaves was weighed into a conical flask. Fifty mL of 80% ethanol was added and incubated for 15 min capped with cold finger condensers to prevent solvent evaporation in a 60 °C water bath (Ratek Shaking Water bath SWB, Australia). The extracted solvents were placed into another collection conical flask. The residue tea leaves were further extracted for the second time under the same condition. The total 100 mL extraction supernatants were then mixed and centrifuged at 2000 rpm, 4 °C for 20 min to subside solid remains.

One mL of supernatant was then transferred into a 1.5 mL Eppendorf snap-cap microcentrifuge vial to evaporate the solvent using an Eppendorf vacuum concentrator (Eppendorf 5301, Germany) at 30 °C. The dried extract was then sealed and stored in the freezer at −20 °C (Fisher & Paykel, NZ). All tests were performed in triplicate. Prior to HPLC analysis, extracts from each Eppendorf vial were reconstituted with 1 mL of 80% methanol and then sonicated in an ultrasonic bath (Elma Transsonic T460, Germany) until all solids were dissolved. The sample was then filtered with a 0.45 μm Millipore hydrophobic syringe filter (USA). An aliquot of 200 μL was diluted to 1000 μL with 80% methanol in a 2 mL HPLC glass vial for a well-prepared use of HPLC analysis.

2.3. HPLC protocol

Catechin and theaflavin were determined by HPLC according to Del Rio et al. (2004). Briefly, chromatographic separation was performed on a HP 1100 HPLC (Agilent Technologies, Wilmington, DE, USA) equipped with a C12 reverse phase column (4.6 mm × 250 mm; 4 μm, Agilent Technologies, Wilmington, DE, USA) with auto-sampler and photodiode array detector. Two mobile phases: A, acetonitrile, and B, 1% formic acid in water (v/v), were used and elution was at a flow rate of 1.0 mL min^{-1}. The mobile phase was programmed consecutively in linear gradients as follows: 0 min, 5% A (95% B); 5 min, 10% A (90% B); 40 min, 25% A (75% B); 45 min, 30% A (70% B); 55 min, 30% A (70% B); 60 min: 5% A (95% B); 65 min: 5% A (95% B). The UV λ_{max} of 280 nm was used for catechins, and 365 nm was used for theaflavin detection. The injection volume was 20 μL. Individual catechin and theaflavin compound were determined using standards as mentioned in Section 2.1.

2.4. Oxygen-radical absorbance capacity (ORAC) assay

The ORAC assay was adapted from the methods of Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002) and Bisby, Brooke, and Navaratnam (2008). The reaction was performed in phosphate buffer (75 mM, pH 7.4) in 96 well plates. Twenty-five μL of tea extract and 150 μL fluorescein (8.4×10^{-5} mM) were mixed in the 96 well plate and pre-incubated for 5 min at 37 °C, followed by addition of 25 μL of AAPH solution (0.3072 M). The plate was then placed in the plate reader (PerkinElmer 2300 EnSpire multilabel reader) and measured at 485 nm and 520 nm respectively. The control consisted of 25 μL phosphate buffer. Trolox solutions of 0, 10, 20, 30, 40, 50, 100 mg/L were used to construct the standard curve for the ORAC assay ($R^2 = 0.999$). The ORAC values were expressed as μmoL Trolox equivalents/g (TE/g) of dried tea using the standard curve established previously. All samples were analysed in triplicate.

2.5. Statistical analysis

Data were reported as mean value ± standard deviation as calculated by Microsoft Excel. ANOVA, *post hoc* tests, Bivariate correlation and PCA of the data were conducted using SPSS Statistics 23. Data were considered significantly different when $p < 0.05$.