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Identifying key non-volatile compounds in ready-to-drink green tea and their impact on taste profile

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ABSTRACT

Thirty-nine non-volatile compounds in seven ready-to-drink (RTD) green tea samples were analysed and quantified using liquid chromatography. Taste reconstruction experiments using thirteen selected compounds were conducted to identify the key non-volatile tastants. Taste profiles of the reconstructed samples did not differ significantly from the RTD tea samples. To investigate the taste contribution and significance of individual compounds, omission experiments were carried out by removing individual or a group of compounds. Sensory evaluation revealed that the astringent- and bitter-tasting (−)-epigallocatechin gallate, bitter-tasting caffeine, and the umami-tasting L-glutamic acid were the main contributors to the taste of RTD green tea. Subsequently, the taste profile of the reduced recombinant, comprising of a combination of these three compounds and L-theanine, was found to not differ significantly from the sample recombinant and RTD tea sample. Lastly, regression models were developed to objectively predict and assess the intensities of bitterness and astringency in RTD green teas.

1. Introduction

Tea, a beverage made by infusing the leaves of *Camilla sinensis* in hot water, is the second most popular drink in the world after water (Butt & Sultan, 2009). Tea can be classified into three major categories: unfermented green tea, partially fermented oolong tea, and fermented black tea. Tea fermentation is a consequence of the enzymatic action of polyphenol oxidase (PPO) found in tea, which catalyses the oxidation and condensation of polyphenols into complex quinones and tannins (Obanda, Okinda Owuor, & Mang'oka, 2001). Unfermented green tea undergoes little fermentation due to inactivation of PPO through drying and steaming. Partially fermented oolong tea undergoes a brief fermentation period by crushing leaves and releasing PPO, followed by a heating process to inactivate enzymes and dry the leaves. Black tea undergoes a full fermentation process, and the majority of polyphenols present are condensed to form complex polyphenols, before inactivation of PPO is initiated through a "frying" (i.e., heating) process (Wang & Ruan, 2009).

Tea quality is usually evaluated by professional tea tasters based on the appearance, aroma and taste of the tea brew, as well as appearance of the dry and infused leaves (Liang et al., 2008). Due to the subjectivity and inconsistency of this evaluation method, several studies have attempted to correlate the chemical constituents and sensory characteristics to the perceived quality index evaluated by professional tea tasters (Liang et al., 2008; Pongsuwan et al., 2008). Volatile compounds contribute to the aroma profile, while non-volatile components contribute to the taste profile of green tea, which includes the characteristic bitterness and astringency. It has been generally accepted in the literature that astringency is a tactile sensation felt on the tongue caused by the interaction between tea polyphenols and salivary proteins (Brossaud, Cheynier, & Noble, 2001). However, Rossetti, Bongaerts, Wantling, Stokes, and Williamson (2009) found that astringency is not entirely a tactile perception caused by the loss of lubrication in the oral cavity, but may instead involve other mechanisms, such as the inhibition of sodium ion channels on epithelial cells, as suggested by Simon, Hall, and Schiffman (1992).

Several groups of non-volatile compounds have been found to have potential activity on the taste profile of tea, including phenolic compounds, purine alkaloids, amino acids, nucleotides, carbohydrates, organic acids, ions and others (Kaneko, Kumazawa, Masuda, Henze, & Hofmann, 2006; Liang et al., 2008; Scharbert & Hofmann, 2005; Wang & Ruan, 2009). Tea polyphenols, particularly tea catechins, have been extensively researched, and were found to have an effect on the bitterness and astringency of tea (Narukawa, Kimata, Noga, & Watanabe, 2010). Purine alkaloids, in particular caffeine, are the other major contributors to bitterness in tea. Although the bitter taste in tea may be attributed to a range of non-volatile compounds, the bitter taste transduction pathway varies between different tastants. Green tea catechins are known

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to activate the human bitter taste receptors hTAS2R14 and TAS2R39 (Roland et al., 2013; Yamazaki, Narukawa, Mochizuki, Misaka, & Watanabe, 2013) in a dose-dependent manner, with non-linear responses at low and high concentrations. On the other hand, caffeine and other methylxanthines have been suggested to induce a bitter taste without activating bitter taste receptors (Rosenzweig, Yan, Dasso, & Spielman, 1999).

On top of the commonly associated bitterness and astringency, green tea is often associated with having a unique umami taste quality, which might largely be contributed by L-glutamic acid and L-aspartic acid. L-theanine (5-*N*-ethyl-L-glutamine), a non-proteinogenic amino acid that makes up more than 50% of the free amino acids content in green tea leaves, has been reported to have sweet, brothy and umami characteristics, and has also been described by many studies to be taste-active in green tea (Ekborg-Ott, Taylor, & Armstrong, 1997; Juneja, Chu, Okubo, Nagato, & Yokogoshi, 1999).

While many of the past studies have focused on black tea, a limited number of studies have been performed on green tea with an emphasis on its taste profile (Chaturvedula & Prakash, 2011). In light of the increasing demand for ready-to-drink (RTD) green tea in East Asian countries, companies may choose to focus their attention on the younger consumers who are frequently on-the-go and may therefore prefer products offering a greater degree of convenience (Chen, Zhu, Tsang, & Huang, 2000). Hence, RTD green tea was chosen as a model beverage system in the present study to explore the relationship between non-volatile components and sensory perception.

As such, the main objectives of this study were (i) to quantify several groups of non-volatile compounds in commercially available RTD green tea samples; (ii) to investigate the taste activity of each compound; (iii) to validate the taste contribution of the key compounds *via* sensory assessments consisting of taste reconstruction and omission experiments; and lastly (iv) to perform regression analysis to obtain regression models for the objective prediction of various taste attributes of RTD green tea.

2. Materials and methods

2.1. Samples, reagents, and standards

Seven types of ready-to-drink (RTD), bottled, unsweetened green tea samples, with no other added flavours unrelated to green tea (e.g., lemon, honey), were obtained from supermarkets in China and Japan. Unopened green tea samples were stored at 25 °C away from direct sunlight in their original packaging. Samples were opened prior to analyses, and were not reused. Green tea samples were filtered through a 0.45-μm membrane prior to chromatographic separation, and used directly for sensory analyses.

The following reagents and standards were obtained commercially: *o*-phosphoric acid 85%, HPLC-grade acetonitrile (ACN) and HPLC-grade methanol (MeOH) from Merck KGaA (Darmstadt, Germany); caffeine, gallic acid, (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin gallate (GCG), (−)-epicatechin gallate (ECG), (−)-catechin gallate (CG), (−)-epigallocatechin (EGC), (−)-gallocatechin (GC), (−)-epicatechin (EC), (+)-catechin (C), L-aspartic acid (Asp), L-glutamic acid (Glu), amino acids standard, guanosine-5′-monophosphate disodium salt hydrate (GMP), inosine-5′-monophosphate disodium salt octahydrate (IMP) and sodium hydroxide solution 50% from Sigma–Aldrich Chemical Co. (St Louis, MO); L-theanine (Thea) from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan); ascorbic acid from DSM Nutritional Products Ltd. (Basel, Switzerland); AccQ-Fluor Reagent Kit and AccQ-Tag Eluent A Concentrate from Waters Corporation (Milford, MA); skimmed milk (0% fat) from CP-Meiji Co. Ltd. (Bangkok, Thailand).

2.2. Quantitative analyses

2.2.1. High-performance liquid chromatography (HPLC) system

All HPLC analyses were performed on an Agilent 1100 Series HPLC system (Santa Clara, USA), which consisted of a micro-vacuum degasser, a quaternary pump, an autosampler, a thermostated column component, a diode array detector, a variable wavelength detector, and a refractive index detector.

2.2.2. Analyses of catechins, gallic acid and caffeine

Standard solutions of catechins, gallic acid and caffeine were prepared by dissolving the required weight of each compound in deionised water. Ascorbic acid was added at a concentration of 5 mg/10 mL sample. All samples were filtered through a 0.45-μm membrane prior to HPLC analyses. Analysis was performed on the HPLC system equipped with an Agilent ZORBAX Eclipse XDB-C18 HPLC column (250 mm × 4.6 mm, 5 μm), according to Wang and Zhou (2004) with slight modifications described below. Mobile phase A consisted of 0.01% phosphoric acid in water, while mobile phase B was 100% methanol. Column temperature was set at 25 °C. Catechins, gallic acid and caffeine were detected at 230 nm and identified by comparison of retention times and spectra of standard solutions. Analytes were quantified by external calibration standards. Performance of the HPLC method was validated through accuracy and precision tests.

2.2.3. Analyses of free amino acids

Pre-column derivatisation of free amino acids was performed using the AccQ-Fluor Reagent Kit according to the manufacturer's specifications. Separation was performed on the HPLC system equipped with a Waters AccQ Tag reversed-phase HPLC column (150 mm × 3.9 mm, 4 μm), according to the manufacturer's specifications with slight modifications. Briefly, mobile phase A consisted of AccQ Tag Eluent A Concentrate in deionised water (1:10 v/v), while mobile phase B consisted of 60% ACN in deionised water. A gradient programme was used for the separation of amino acids: 0–0.5 min, linear gradient from 0 to 2% B; 0.5–15 min, linear gradient from 2% to 7% B; 15–19 min, linear gradient from 7% to 10% B; 19–32 min, linear gradient from 10% to 33% B; 32–33 min, 33% B; 33–34 min, linear gradient from 33% to 100% B; 34–40 min, 100% B; 40–42 min, linear gradient from 100% to 0% B. Post-run time was 2 min. Sample injection volume was 10 μL. Flow rate was 1.0 mL/min. Column temperature was set at 37 °C. Amino acids were detected at 248 nm, and identified by comparison of retention times and spectra of standard solutions of amino acids kit and L-theanine. Quantification was done *via* external calibration curves. Performance of the HPLC method was validated through accuracy and precision tests.

2.2.4. Analysis of 5′-nucleotides

GMP and IMP were analysed on the HPLC system equipped with an Agilent ZORBAX SB-AQ HPLC column (250 mm × 4.6 mm, 5 μm), using an isocratic elution system. Mobile phase A consisted of 20 mM potassium dihydrogen phosphate in deionised water, adjusted to pH 7 using sodium hydroxide. Total run time was 30 min. Post-run time was 2 min. Sample injection volume was 10 μL. Flow rate was 0.5 mL/min. Column temperature was set at 30 °C. GMP and IMP were detected at 254 nm, and identified by comparison of retention times and spectrum of standard solutions. Analytes were quantified through the use of external calibration curves. Performance of the HPLC method was validated through accuracy and precision tests.