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Effects of infusion conditions and decaffeination on free amino acid profiles of green and black tea

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ABSTRACT

This study aimed to investigate free amino acid profiles of black and green tea infusions. Hydrophilic interaction liquid chromatography (HILIC) coupled to tandem mass spectrometry was used to analyze free amino acids in tea without any pre-column or post-column derivatization. A total 20 amino acids (18 proteinogenic and 2 non-proteinogenic) were determined in black and green tea samples. Sum of the concentrations of free amino acids in tea infusion after 2 min of brewing process was 220.53 mg/l and 211.21 mg/l for black and green tea, respectively. It linearly increased reaching to 311.17 mg/l and 277.43 mg/l for black and green tea, respectively, within 15 min of brewing at 85 °C. Leaching rates were differed significantly for different individual amino acids. In general, hydrophilic amino acids leached into infusion faster than hydrophobic amino acids. Approximately 30% of total free amino acids in tea infusions after 15 min of brewing was theanine. Partial decaffeination (>50%) of tea by means of supercritical carbon dioxide extraction resulted in 22% of reduction in total free amino acids in tea when water was used as co-solvent.

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1. Introduction

Tea is the most widely consumed beverage in the world (Harbowy & Balentine, 1997). Tea consumption is beneficial to health and longevity due to its physiologically active components including phenolic compounds, amino acids, caffeine and other purine alkaloids, and vitamins (Horie & Kohata, 2000; Syu, Lin, Huang, & Lin, 2008).

Composition of a tea beverage depends on several factors such as brewing temperature, time, size of tea leaves and stirring. Diffusion of polyphenols, caffeine and theanine into beverage has been extensively studied for years by several researchers (Astill, Birch, Dacombe, Humphrey, & Martin, 2001; Friedman et al., 2005; Keenan, Finnie, Jones, Rogers, & Priestley, 2011; Khokhar & Magnusdottir, 2002; Kyle, Morrice, McNeill, & Duthie, 2007). A typical black or green tea beverage contains approximately 6% of free amino acids in dry mass basis (Harbowy & Balentine, 1997). They contribute to the taste and quality of tea infusions (Alaşalvar, Topal, Serpen, Bahar, Pelvan, & Gökmen, 2012; Hsieh & Chen, 2007).

The umami taste of green tea is due to amino acids, especially glutamic acid and theanine (Kaneko, Kumazawa, Masuda, Henze, & Hofmann, 2006). Theanine (5-N-ethyl glutamine), a non-proteinogenic amino acid, constitutes the major part of free amino acids in tea leaves and takes part in the biosynthesis of polyphenols (Alcazar et al., 2007; EkborgOtt, Taylor, & Armstrong, 1997). Theanine and γ-aminobutyric acid (GABA) have especially been taking attention in tea infusions because of their biological activities. Theanine has many biological effects including anti-hypertensive effect in rats (Yokogoshi et al., 1995), anti-obesity effect in mice (Zheng, Sayama, Okubo, Juneja, & Oguni, 2004), neuroprotective effect in rats (Kakuda, 2012), inhibition of caffeine stimulation in rats and anti-carcinogenic effects in human cancer cells in vitro (Friedman et al., 2007). GABA acts as a major inhibitory neurotransmitter in the mammalian central nervous system (Macdonald & Olsen, 1994). In addition to that lowering of high blood pressure in rats (Abe et al., 1995; Hayakawa, Kimura, & Kamata, 2002) and in human (Inoue et al., 2003) has been shown.

Considering its worldwide consumption in large quantities, tea might be a substantial dietary source of certain free amino acids. This study aimed to investigate the effects of various infusion conditions and decaffeination on individual free amino acid profiles of black and green tea. A rapid analytical method based on hydrophilic interaction liquid chromatography coupled to tandem mass spectrometry was used to determine amino acids in the samples.

2. Material and methods

2.1. Chemicals and consumables

Acetonitrile (HPLC grade) was obtained from Merck Co. (Darmstadt, Germany). Formic acid (98%) was purchased from J.T. Baker (Deventer, Holland). High-purity (>98%) alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), caffeine, cysteine (Cys), glutamic acid (Glu), glutamine (Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline

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(Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), valine (Val), and theanine (Thea) were purchased from Merck Co. (Darmstadt, Germany). Gama-aminobutyric acid (GABA) was supplied by Fluka Bio Chemika AG (Buchs, Switzerland). Syringe filters (nylon, 0.45 μm) and Atlantis dC18 column (4.6 mm × 250 mm, i.d., 5 μm) were supplied by Waters (Millford, MA). SeQuant ZIC-HILIC (150 × 4.6 mm id., 3.5 μm particle size) column was supplied by Merck Co. (Darmstadt, Germany). Ultra-pure water was used throughout the experiments (Milli Q-System, Millipore, Millford, MA, USA).

2.2. Infusion

Black and green tea samples were obtained from a local market. To monitor diffusion of amino acids during the brewing process, 3 g of black or green tea was put into a glass flask with 100 ml of hot water (85 °C) and tightly closed. The flask was placed in a water bath at 85 °C (Infusion-I) or left in a room at 25 °C (Infusion-II) without agitation. One milliliter of infusion was withdrawn from the flask after 2, 5, 10, and 15 min of incubation for the analysis of free amino acids. The flask was shaken gently before sampling the infusion. The pH values of black and green tea infusions were 5.01 ± 0.02 and 5.20 ± 0.07, respectively. These values remained constant throughout the infusion process.

2.3. Decaffeination

Black tea leaves were partially decaffeinated by using a Thar model 1000-2-FMC supercritical fluid extraction system (Thar Instruments, Inc.) consisting of CO_2 tube, high pressure CO_2 pump, CO_2 flow-meter, temperature controllers, high pressure co-solvent pump, automated back pressure regulator (ABPR), cooler and heater. A hundred grams of black tea leaves were loaded into the extractor vessel (1 l). The temperature and the pressure of the extraction vessel were set to 80 °C and 30 MPa, respectively. The extraction process lasted in 1 h using CO_2 flow rate of 100 g/min. 10% of water (w/w) was used as a co-solvent.

2.4. Analysis of free amino acids in tea leaf and infusion

2.4.1. Tea leaves (normal or decaffeinated)

Tea leaves (normal or decaffeinated) were ground prior to extraction. One gram of ground tea was extracted with 20 ml of hot water (80 °C) in triple stage (10, 5 and 5 ml). Tubes were vortexed for 10 min in each stage of extraction. Combined extract was diluted 25 fold with a mixture of acetonitrile:water (50:50) and 1 ml of aliquot filtered through 0.45 μm nylon syringe filter into an autosampler vial.

2.4.2. Tea infusion

One milliliter of infusion was diluted 5 fold with a mixture of acetonitrile:water (50:50) and filtered through 0.45 μm nylon syringe filter into an autosampler vial.

2.4.3. Tandem LC–MS measurement

An Agilent 1200 series HPLC system coupled to Agilent 6460 triple quad mass spectrometry (Waldbronn, Germany) was used. Electrospray ionization operated in positive mode was used to analyze the samples for amino acids. The chromatographic separations were performed on a SeQuant ZIC-HILIC (150 × 4.6 mm id., 3.5 μm) column using a gradient mixture of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at flow rate of 0.75 ml/min at 40 °C. The eluent composition starting with 25% of A linearly increased to 60% in 5 min and held for 5 min. Then, it was linearly decreased to its initial conditions (25% of A) in 5 min. Doing so, the total chromatographic run was completed in 15 min. The electrospray source had the following settings: drying gas (N_2) flow of 10 l/min at 325 °C, nebulizer pressure of 30 psi, sheath gas (N_2) flow of 10 l/min at 375 °C, nozzle voltage of 1000 V, and capillary voltage positive of 4000 V. Amino acids were identified by multiple reaction monitoring (MRM) using the parameters given in Table 1. The dwell time was 0.1 s for all MRM transitions. Concentration of amino acids was calculated by means of external calibration curves built for individual amino acids in a range between 0.1 and 2.0 μg/l.

2.5. Analysis of caffeine

The caffeine contents of tea and decaffeinated tea samples were analyzed by high performance liquid chromatography (HPLC) coupled to photodiode array detector (PDA). One gram of ground sample was extracted with 20 ml of hot water (80 °C) in three stages (10, 5 and 5 ml). The extracts were diluted 25 folds with water and filtered through 0.45 μm nylon filter and injected onto an Agilent 1100 HPLC system (Waldbronn, Germany) consisting of a quaternary pump, a PDA detector and a temperature controlled column oven. The chromatographic separations were performed on Waters Atlantis dC18 column (4.6 mm × 250 mm, i.d., 5 μm) by using a gradient mixture of acetonitrile (A) and 0.1% acetic acid (B) at a flow rate of 1 ml/min. Elution program was as follows: linear increase of eluent A from 10% to 20% within 15 min, linear increase of eluent A from 20% to 40% within 10 min and linear decrease of eluent A from 40% to 10% in 5 min at 25 °C. Data acquisition was performed by recording chromatograms at 285 nm.

Table 1
MRM transitions used to detect individual free amino acids by LC–MS/MS.

Amino acid	MRM	Fragmentor Voltage (V)	Collision Energy (V)
Ala	89.8 → 44	50	6
Arg	175 → 158	60	10
	175 → 129	60	10
Asn	133 → 116	60	10
	133 → 87	60	10
Asp	134 → 88	70	6
	134 → 74	70	12
Cys	122 → 105	60	10
	122 → 76	60	10
GABA	104 → 87	60	10
	104 → 58	60	10
Glu	148 → 102	70	6
	148 → 84	70	14
Gln	147 → 130	60	10
	147 → 101	60	10
Gly	76 → 30	30	4
His	156 → 110	80	10
	156 → 93	80	10
Ile	132 → 86	60	6
	132 → 69	60	16
Leu	132 → 86	60	6
	132 → 44	60	8
Lys	147 → 130	60	10
	147 → 101	60	10
Met	150 → 133	60	10
	150 → 104	60	10
Phe	166 → 149	60	10
	166 → 120	60	10
Pro	116 → 70.1	60	16
Ser	105.9 → 88	60	6
	105.9 → 60.1	60	8
Thea	175 → 158	60	10
	175 → 129	60	10
Thr	120 → 74	60	10
	120 → 103	60	10
Trp	205 → 188	60	10
	205 → 159	60	10
Tyr	182 → 165	60	10
	182 → 136	60	10
Val	118 → 72.1	60	8
	118 → 55	60	22

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