

Comparison of Quantity Characteristics and Antioxidant Potentials of Different Tea Extracts for In-flight Beverage

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Abstract: Tea is a famous non-alcoholic beverage worldwide. Various types of tea are prepared to meet the demand of different people. Among the various tea types, green tea (GTE), black tea (BTE), and Pu-erh tea (PEE) are major ones. The objective of this study was to investigate the physicochemical characteristics and antioxidant potential of three tea extracts to be considered as an in-flight beverage. The pH (5.62) of GTE was significantly highest whereas the titratable acidity (0.18 g/100 mL) was significantly highest in BTE. The highest lightness value was found in GTE (87.12) and redness and yellowness in BTE (30.15 and 85.32). The amount of total free amino acid was significantly highest in PEE (174.52 µg/ mL), followed by GTE (31.28 µg/ mL) and BTE (24.98 µg/ mL). Similarly, the antioxidant potential of PEE was significantly high among the three tea extracts. The results indicated that Pu-erh tea could be a good in-flight beverage.

Keywords: Antioxidant Potential, Black Tea, Green Tea, In-flight Beverage, Pu-erh Tea

Introduction

Tea is one of the most popular drinks in the world. Various types of tea products have been developed and are categorized mainly into six groups (green tea, yellow tea, white tea, oolong tea, black tea, and dark tea) depending upon the processing methods (Hilal 2017). Among them, three tea varieties green, yellow, and white undergo minimal processing, other two oolong tea and black tea are subjected to oxidizing while the other dark tea, such as Pu-erh tea is fermented. There are mainly two methods of Pu-erh preparation: one is by pressing large and unoxidized tea leaves, followed by fermentation for several years at room temperature and the other method is by allowing the tea leaves to ripen for several months using microbes under optimum conditions before being subjected to pressing (Chen et al. 2009). In such ways of processing, Pu-erh tea gets reddish to brownish red or gray appearance, thick and bright red infusion color, bittersweet taste, and a unique moldy odor which becomes more prominent with the fermentation and the leaves aging (Zhou et al. 2004).

The composition of tea is influenced by species, season, age of the leaf (plucking position), climate, and horticultural practices (Lin et al. 1996). Tea products, especially green tea are rich in polyphenols, including flavanols, flavadiols, flavonoids (Hertog et al. 1993), and phenolic acids, accounting for up to 30% of the dry weight. Another most abundant content of green tea is catechins, which have been known for their hypocholesterolemic effect. Report shows that the long-term feeding of green tea powder to rats could minimize the blood level of triglyceride and other lipids (Lin et al. 1998).

The processing of black tea is characterized by a high degree of fermentation that produces a series of

chemical condensations. During the fermentation process, catechins are converted into theaflavins and thearubigins, which are the major black tea polyphenol components (Lin and Liang 2000). Theaflavins possess several health benefits, including fat-reducing and glucose-lowering capabilities and lifestyle-related disease prevention related to anti-obesity, anticancer, anti-atherosclerotic, anti-inflammatory, antiviral, antibacterial, anti-osteoporotic, and anti-dental caries properties (Takemoto and Takemoto 2018). Thearubigins are reported to have various health roles, including antioxidant, antimutagenic and anticancer properties, along with the ability to reduce inflammation and improve gastrointestinal motility (Jt and Je 2020).

Pu-erh tea has already been established as a favorite drink in China and has gained increasing popularity in Southeast Asian countries, Japan, USA, Britain, and other countries. Pu-erh tea is originally produced in the Yunnan Province of China. It has attracted much attention because of its exceptional flavor and potential health benefits (Ahmed et al. 2010). The multiple health-promoting potentials of Pu-erh tea include anti-oxidative (Fan et al. 2013), antibacterial (Hu et al. 2010), antitumor (Zhao et al. 2011), cholesterol-lowering (Peng et al. 2013), anti-obesity (Oi et al. 2012), and hypoglycemic (Du et al. 2012) activities. Pu-erh tea is also rich in various mineral elements compared with black tea, green tea, Oolong tea, and white tea (McKenzie et al. 2010).

Very few studies have been conducted on the effect of drinking tea at high altitudes such as at high mountains or in-flight. A study conducted at the Mt. Everest base camp showed that drinking tea reduced fatigue and positive effect on mood (Scott et al. 2004). The finding of the present study could be



useful to serve different types of tea as an in-flight beverage.

Materials and Methods

Chemicals and materials

Folin-Ciocalteu phenol reagent and DPPH were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used were analytical grade. Three commercial tea samples (green tea, black tea, and Pu-erh tea) were purchased from a local market in Deagu, Korea.

Preparation of tea extracts

Three kinds of tea extracts were prepared with three tea samples and were named as follows: GTE, 1.5 g of dried green tea was extracted with 150 mL of boiling water and incubated at 90°C for 3 min with shaking for 30 s; BTE, 1.5 g of dried black tea was extracted with 150 mL of boiling water and incubated at 90°C for 3 min with shaking for 30 s; PEE, 1.5 g of dried Pu-erh tea was extracted with 150 mL of boiling water and incubated at 90°C for 3 min with shaking for 30 s. The extraction conditions were designed to get a close similarity to an actual tea brewing (Choi et al. 2018).

Determination of pH and titratable acidity

A pH Meter (Model 250; Beckman Coulter, Inc., Fullerton, CA, USA) was used to measure the pH value of tea extracts. Titratable acidity (lactic acid in g/L) was measured by mixing 5 mL of the extracts and 125 mL of deionized water followed by titration with 0.1 N sodium hydroxide to an endpoint pH of 8.2.

Color measurement

The L* (lightness), a* (redness, + or greenness, -), and b* (yellowness, + or blueness, -) values of the extracts were determined using a Chroma Meter (CR-300; Minolta Corp., Osaka, Japan). A Minolta calibration plate (YCIE=94.5, XCIE=0.3160, YCIE=0.330) and a Hunter Lab standard plate (L*=97.51, a*= -0.18, b*= +1.67) were used to standardize the instrument using a D65 illuminant as described earlier (Kim et al. 2014).

DPPH radical scavenging activity

The DPPH radical scavenging activity was measured according to the methods described earlier (Dhungana et al. 2019) with some modifications. A 0.8-mL of 0.2 mM ethanolic solution of DPPH was mixed with 0.2 mL of the tea extracts. The mixture was thoroughly mixed using a vortexer and left to stand for 30 min at room temperature under dark conditions, and then the absorbance value was measured at 517 nm using a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Vantaa, Finland).

Determination of the total polyphenol content

The total polyphenol contents of tea extracts were estimated according to the Folin-Ciocalteu method (Dhungana et al. 2016) with some modifications. Fifty microliters of the sample extracts and 1 mL of 2% (w/v) aqueous Na₂CO₃ were mixed in microtubes and allowed to react at room temperature for 3 min. After 3 min, fifty microliters of 1 N Folin-Ciocalteu reagent was mixed into the mixture and incubated at room temperature for 30 min under dark conditions. The absorbance value of the reaction mixtures was measured at 750 nm using a microplate spectrophotometer (Multiskan GO; Thermo Fisher Scientific). The total polyphenol content of the samples was calculated using the calibration curve drawn using gallic acid (GA) as standard.

Free amino acid composition

The free amino acid content of tea extracts was determined following a procedure described earlier (Je et al. 2005). An aliquot of the tea extract (1 mL) was hydrolyzed with 6 N HCl (10 mL) in a sealed-vacuum ampoule at 110°C for 24 h. The HCl was removed from the hydrolyzed sample using a rotary evaporator and a known volume (5 mL) of the reaction mixture was prepared with 0.2 M sodium citrate buffer (pH 2.2). The mixture was passed through a Sep-Pak C18 cartridge (Waters Co., Milford, MA, USA) and filtered through a 0.22- μ m membrane filter (Millipore, Billerica, MA, USA). The amino acid profile was determined using an automatic amino acid analyzer (Biochrom-20, Pharmacia Biotech Co., Stockholm, Sweden). All of the samples were run in duplicate and expressed as μ g/mL of tea extract.

Statistical analysis

All the data were subjected to analysis of variance (ANOVA) using SAS (SAS Institute, Cary, NC, USA). A significant difference between means was determined at $p < 0.05$ using Tukey test. A mean value of three replicates was reported unless otherwise stated.

Results and Discussion

General chemical characteristics

The general chemical characteristics of various tea extracts were examined with the pH and titratable acidity (TA) values. The pH value of GTE (5.62) was significantly high, followed by PEE (5.55) and BTE (5.21). On the other hand, the TA value was significantly high for BTE (0.18 g/100 mL) and that of the other two tea samples were statistically equal (Table 1).

The TA value implies an impact of acid content on the flavor of food, whereas that of the pH indicates an environment that affects the ability of a microorganism to grow in a specific food (Je et al.

2005). Therefore, the variation in pH of tea extracts may influence their flavor and shelf-life (Tyl and Sadler 2017). An inverse correlation of pH and TA might be due to complex acid chemistry in tea extracts, suggesting many acids which exist at an

extraction temperature are not (fully) deprotonated at the pH of the extract, and thus do not contribute to the extract's pH value, but are measured during titration with a base (Gloess et al. 2013).

Table 1. pH and titratable acidity of green tea, black tea, and Pu-erh tea extracts

	Sample ¹⁾		
	GTE	BTE	PEE
PH	5.62±0.01 ^{a2)}	5.21±0.01 ^c	5.55±0.02 ^b
titratable acidity (g/100ml)	0.12±0.02 ^b	0.18±0.01 ^a	0.13±0.01 ^b

¹⁾GTE: 1.5 g of dried green tea was extracted with 150 mL of boiling water and incubated at 90°C for 3 min with shaking for 30 s; BTE: 1.5 g of dried black tea was extracted with 150 mL of boiling water and incubated at 90°C for 3 min with shaking for 30 s; PEE: 1.5 g of dried Pu-erh tea was extracted with 150 mL of boiling water and incubated at 90°C for 3 min with shaking for 30 s.

²⁾Values are means ± SD of triplicate measurements. Values followed by different superscript letters in the same row are significantly different ($p < 0.05$).

Color measurement

The type of tea significantly affected Hunter's color values of three tea extracts (Table 2). The lightness value was significantly highest for GTE (87.12), followed by BTE (59.12) and PEE (45.29). The redness value of GTE, BTE, and PEE was -2.62, 30.15, and 0.09, respectively. On the other hand, the highest and lowest yellowness values were measured in BTE (85.32) and PEE (10.11), respectively.

The substantial variation in the color value of tea extracts might be due to the difference in the polyphenol content (Harbowy et al. 1997) of three tea samples. The color of food is a key factor in making decisions by consumers for its acceptability. Consumers also consider the color of food along with its nutritional and functional values.

Table 2. Hunter's color values of green tea, black tea, and Pu-erh tea extracts

Sample ¹⁾	Color value ²⁾		
	L* (lightness)	a* (redness)	b* (yellowness)
GTE	87.12±0.13 ^{a3)}	-2.62±0.03 ^c	18.12±0.34 ^b
BTE	59.12±0.11 ^b	30.15±0.01 ^a	85.32±0.31 ^a
PEE	45.29±0.23 ^c	0.09±0.02 ^b	10.11±0.05 ^c

¹⁾Samples are defined in Table 1.

²⁾L*, lightness (100, white; 0, black); a*, redness (-, green; +, red); b*, yellowness (-, blue; +, yellow).

³⁾Values are expressed as means ± standard deviations of three replicates. Values followed by different superscript letters in the same column are significantly different ($p < 0.05$).

Free amino acid composition

The content of individual free amino acids and their total amount in three tea extracts are shown in Table 3. A total of 19 free amino acids were detected in all three samples and one amino acid L-citrulline was not detected in the samples. Only BTE was found not to contain three amino acids L-histidine, O-phospho-L-serine, and L-ornithine. The highest amount of total free amino acid was found in PEE (174.52 µg/mL), followed by GTE (31.28 µg/mL) and BTE (24.98 µg/mL). L-phenylalanine was the most abundant essential and L-aspartic acid was the most abundant non-essential amino acid detected in the tea extracts. PEE contained the highest amount of all the

amino acids.

The amount of amino acids is one of the key factors in determining the nutritional qualities of food materials (Basarova and Janousek 2000). γ -Amino-n-butyric acid (GABA) is basically synthesized in plant tissues by decarboxylation of glutamic acid in the presence of glutamate decarboxylase (Nikmaram et al. 2017). GABA and glycine are related to learning and memory, stroke, and neurodegenerative diseases; relieving anxiety, sedation, anticonvulsant, and muscle relaxation function (Krogsgaard-Larsen 1989; Mody et al. 1994; Oh and Oh 2004).

Table 3. Free amino acid composition ($\mu\text{g}/\text{mL}$) of green tea, black tea, and Pu-erh tea extracts

Amino acid	Sample ¹⁾		
	GTE	BTE	PEE
<u>Essential amino acid</u>			
L-Threonine	0.21 ²⁾	0.10	5.21
L-Valine	0.27	0.22	4.22
L-Methionine L-Isoleucine	0.93	0.16	2.31
L-Leucine	1.72	0.14	3.00
L-Phenylalanine	1.23	0.77	7.27
L-Lysine	0.55	1.12	2.69
L-Histidine	0.09	ND ³⁾	0.12
Sub-total	5.00	2.51	24.82
<u>Non-essential amino acid</u>			
L-Aspartic acid	2.97	1.92	24.31
L-Serine	0.27	1.21	8.12
L-Glutamic acid	0.71	1.71	25.37
Glycine	0.52	1.22	1.11
L-Alanine	1.27	1.31	6.35
L-Tyrosine	0.49	1.44	5.13
L-Arginine	1.77	1.51	6.23
Sub-total	8.00	10.32	76.62
<u>Other free amino acid</u>			
O-Phospho-L-serine	0.92	ND	6.77
O-Phospho ethanol amine	1.22	2.00	3.39
L-Sarcosine	15.77	9.99	57.22
L-Citrulline	ND	ND	ND
β -Alanine	0.09	0.02	0.22
D,L- β -Amino isobutyric acid	0.10	0.01	1.00
γ -Amino-n-butyric acid	0.12	0.06	1.31
Ethanolamine	0.02	0.07	1.60
L-Ornithine	0.04	ND	1.11
Sub-total	18.28	12.15	73.08
Total free amino acid	31.28	24.98	174.52

¹⁾Samples are defined in Table 1.

²⁾Values are means \pm SD of duplicate measurements.

³⁾ND: Non-detectable.

Antioxidant potential

The antioxidant potential of three tea extracts was evaluated by DPPH free radical scavenging activity and total polyphenol content (Table 4). The DPPH radical-scavenging potential of PEE (88.81%) was significantly highest among the tea samples. Similarly, the total polyphenol content was highest in PEE (1950.32 μg GAE/mL) and lowest in PTE (457.23 μg GAE/mL).

A similar result of higher polyphenol content in green tea than in black tea was also found in previous studies (Widowati et al. 2015; Zhao et al. 2019). The lower total polyphenol content of black tea extract

might be due to the extended fermentation by polyphenol oxidases (Atoui et al. 2005). In the present study, total polyphenol content was significantly higher in GTE than that in BTE, however, the DPPH was significantly higher in BTE than in GTE. The overall antioxidant potential of a product is a consequence of the interaction of various factors, such as the segregating nature of specific antioxidants, condition of oxidation, and/or physical state of the oxidizable substrate (Frankel and Meyer 2000). Hence, a noticeable rise in the amount of an antioxidant component, such as total polyphenol content, may not always result in elevated antioxidant potentials.

Table 4. DPPH free-radical scavenging activities and total phenol content (TPC) of green tea, black tea, and Pu-erh tea extracts

Sample ¹⁾	DPPH (% Inhibition)	TPC (μg GAE ²⁾ /mL tea extract)
GTE	74.23 \pm 1.11 ³⁾	980.31 \pm 7.23 ^b
BTE	80.23 \pm 2.05 ^b	457.23 \pm 5.34 ^c
PEE	88.81 \pm 1.23 ^a	1950.32 \pm 6.13 ^a

¹⁾Samples are defined in Table 1.

²⁾Gallic acid equivalents.

³⁾Values are means \pm SD of triplicate measurements. The Values followed by different superscript letters in the same column are significantly different ($p < 0.05$).

Conclusions

The pH, titratable acidity, color value, free amino acid contents, and antioxidant potential of green tea, black tea, and Pu-erh tea extracts were investigated as a potential in-flight beverage. The free amino acid content and antioxidant potential results showed that Pu-erh tea could be the best tea drink to serve in-flight.

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