

## Effect of extraction temperature on physicochemical constituents and antioxidant potentials of Pu-erh tea

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**Abstract** Of all the various types of tea that are available, Pu-erh tea has attracted much attention because of its health-promoting effects. The objective of this study was to investigate the physicochemical and antioxidant properties of two types of Pu-erh tea, Gushu Pu-erh and typical Pu-erh, extracted at 80 and 100°C, respectively. The Gushu Pu-erh extracts showed lower pH but higher antioxidant potential at both the temperatures, as compared to the typical Pu-erh. The mineral content of the tea extracts was greater at 100°C than at 80°C. Among the seven categories of the flavor compounds that were detected, the peak area of esters was the highest in the Gushu Pu-erh extracts. The type of tea and the extraction temperature significantly affected the physicochemical and functional properties of these tea extracts. This study revealed that Gushu Pu-erh extracted at 100°C for 3 min with 30 s of shaking provides better results in terms of the antioxidant potential and mineral content.

**Keywords:** Antioxidant potential, Typical Pu-erh, Flavor compound, Gushu Pu-erh

### Introduction

As an ancient beverage, tea is one of the most popular drinks in the world. The tea products have been categorized into six groups (green tea, yellow tea, white tea, oolong tea, black tea, and dark tea) based on the processing methods applied (Hilal, 2017). Three types of tea green, yellow, and white undergo minimal processing, other two oolong and black tea products are subjected to oxidizing while the other dark tea, such as Pu-erh tea is fermented. Pu-erh tea is typically prepared via two methods. In the first method, the raw Pu-erh tea is produced by pressing large and unoxidized tea leaves which are then fermented for several years at room temperature. In the other way of Pu-erh tea preparation, teas are ripened for several months using microbes under optimum conditions before subjected for being pressed (Chen et al., 2009). Pu-erh tea has got 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).

Pu-erh tea has already been established as a favorite drink in China and other Southeast Asian countries, and it has also been popular in Japan, USA, Britain and other countries. Pu-erh tea, which is originally produced in the Yunnan Province of China, has attracted much attention because of its unique flavor and potential

health benefits (Ahmed et al., 2010). The multiple health-promoting effects of this functional beverage 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 are also rich in several mineral elements compared with black tea, green tea, Oolong tea and white tea (McKenzie et al., 2010). In a previous study (Gao et al., 2017), 55 volatile compounds in the essential oil extracted from Pu-erh tea by fully automatic headspace-solid phase microextraction method were identified which were higher than the compounds extracted by other four extraction methods, Soxhlet, ultrasonic-assisted, simultaneous distillation, and steam distillation.

The health benefits of Pu-erh tea also vary with the raw material, processing method, extraction solvent and time and so on. In Chinese ‘Gushu’ means ancient tea trees, and the Pu-erh tea prepared from these trees is highly demanded and expensive as well. Gushu Pu-erh are known to be prepared from the tea leaves obtained from trees of several hundred years old. Pu-erh teas are also prepared from the leaves of cultivated young tea trees, which are, in this study, referred to as typical Pu-erh. There have various studies on Pu-erh tea been published, however, a detailed comparative study on quality and functional properties of Gushu Pu-erh and typical Pu-erh extracted in different temperatures is not well documented. A previous study of our research team (Choi et al., 2018) investigated some of the quality characteristics and antioxidant potential of the two tea types, in which the pH value of Gushu Pu-erh tea was lower than typical Pu-erh. However, Gushu Pu-erh showed higher antioxidant potentials and free amino acid content than the typical Pu-erh. The volatile aroma compounds are important criteria in the evaluation of tea quality (Ho et al.,

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2015). Considering the effect of extracting temperature (Hajiaghaalipour et al., 2016) and age of tea tree on their quality and antioxidant properties, this study was designed to compare the functional property and quality characteristics, including the volatile aromas of Gushu Pu-erh and typical Pu-erh teas. The finding of study could provide useful information for further study on the effect of extraction temperature and source of tea leave on the quality and functional properties of Pu-erh teas.

## Materials and Methods

### Chemicals and materials

Folin-Ciocalteu phenol reagent, DPPH, ABTS, and pyrogallol were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used were analytical grade. The tea samples, typical Pu-erh and Gushu Pu-erh manufactured in Yunnan province of China, were obtained for this study. There are mainly two types of Pu-erh teas: raw and ripe. In the present, ripe type of both typical Pu-erh and Gushu Pu-erh tea was considered. Ripe pu-erh leaves are made from black tea leaves. During processing, ripe Pu-erh tea is subjected to controlled post-fermentation, which helps reduce the bitter astringent taste, eliminate the musty odor, and shorten the aging period.

### Preparation of tea extracts

Two types of Pu-erh tea were extracted in water at 80 and 100°C and the samples were named as follows: CPE-80: a 1.5-g of dried sample of typical Pu-erh tea was extracted with 150 mL of boiling water and incubated at 80°C for 3 min with gentle shaking for 30 s; GPE-80: a 1.5-g of dried sample of Gushu Pu-erh was extracted with 150 mL of boiling water and incubated at 80°C for 3 min with gentle shaking for 30 s; CPE-100: a dried sample of typical Pu-erh tea (1.5 g) extracted with boiling water (150 mL) and incubated at 100°C for 3 min with gentle shaking for 30 s; GPE-100: a dried sample of Gushu Pu-erh tea (1.5 g) extracted with boiling water (150 mL) and incubated at 100°C for 3 min with gentle shaking for 30 s. The extraction conditions were set to get a similarity to a normal tea brewing.

### Determination of pH and titratable acidity

The pH value of the sample extracts was determined using a pH meter (Model 250; Beckman Coulter, Inc., Fullerton, CA, USA). 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 of pH 8.2. Since the visibly detectable change in the tea extracts was observed at pH 8.2, the pH value was considered as right endpoint.

### Color measurement

Color measurements of tea extracts were carried out by a Chroma Meter (CR-300, Minolta Corp., Osaka, Japan) and the values were expressed as L\* (lightness), a\* (redness, + or greenness, -), and b\* (yellowness, + or blueness, -). The instrument was standardized using a Minolta calibration plate (YCIE=94.5, XCIE=0.3160, yCIE=0.330) and a Hunter L a b standard plate

(L\*=97.51, a\*= -0.18, b\*= +1.67) with a D65 illuminant as described earlier (Kim et al., 2014).

### DPPH radical scavenging activity

DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging activity was measured following a method described earlier (Dhungana et al., 2015) with some modifications. A 0.8-mL of freshly prepared 0.2 mM DPPH ethanol solution was mixed with 0.2 mL of the tea extracts in micro tubes (1.5 mL) using a vortexer. Similarly, 0.8 mL of the DPPH and 0.2 mL of ethanol was mixed to prepare control. The mixture (0.2 mL) was put into 96-well plate and left to stand for 30 min at room temperature under dark condition, 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 determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965). An undiluted Folin-Ciocalteu reagent (250 µL) was mixed with the tea extracts (50 µL). After 1 min, 750 µL of 20% (w/v) aqueous Na<sub>2</sub>CO<sub>3</sub> was added to the mixture and the final volume was made up to 5.0 mL with distilled water. The mixture was incubated for 30 min at room temperature under dark condition and the absorbance value was measured at 760 nm using a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific). The amount of polyphenol in the tea samples was calculated based on gallic acid (Sigma-Aldrich, St. Louis, MO, USA) which was used to prepare a calibration curve. The total polyphenol contents were reported as gallic acid equivalents (µg GAE/mL extract).

### ABTS radical scavenging activity

The ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging activity of tea extracts was analyzed according to the method described by Miller et al. (1993) with a slight modification. The ABTS cation radical was generated by mixing equal amounts of stock solutions of 2.4 mM potassium persulfate and 7 mM ABTS prepared in double distilled water. The reaction mixture was left in the dark at room temperature for 10 min and absorbance value of the reaction mixture was adjusted to 0.7±0.02 at 734 nm by diluting it with double distilled water. The tea extracts (20 µL) and the diluted reaction mixture (180 µL) were mixed in 96-well plate by pipetting and kept in dark for 30 min and the absorbance was measured at 734 nm using a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific). The ABTS radical scavenging activity was calculated using the following equation.

$$\text{ABTS radical scavenging activity (\%)} = \left[ 1 - \left( \frac{AS - AS_0}{AC} \right) \times 100 \right]$$

where AC= absorbance of ABTS radical cation, AS= absorbance of a mixture of ABTS radical solution and tea extracts.

### SOD (superoxide dismutase)-like activity

Assessment of the SOD-like activity in the sample extract was

carried out according to the method described by Debnath et al. (2011). First of all, a reaction mixture was prepared by adding 1.3 mL of Tris-HCl buffer (50 mM Tris, 10 mM EDTA, pH 8.5) and 100  $\mu$ L of 7.2 mM pyrogallol and then an aliquot (100  $\mu$ L) of sample extract was added to it and allowed to react at 25°C in dark for 10 min. After the incubation, 50  $\mu$ L of 1 N HCl was added into the mixture to terminate the reaction. The amount of pyrogallol oxidised during the reaction by tea samples in relation to a control was measured at an absorbance 420 nm using a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific). For control, Tris-HCl buffer was used instead of the tea extracts. The SOD-like activity was determined using the following equation.

SOD-like activity (%)

$$= \left[ 1 - \left( \frac{\text{Absorbance of solution with sample}}{\text{Absorbance of solution without sample}} \right) \times 100 \right]$$

#### Flavonoid content

The flavonoid content of sample extracts was measured following the method described by Mohdaly et al. (2010). A 100- $\mu$ L of tea extract was diluted with 500  $\mu$ L of methanol, followed by an addition of 50  $\mu$ L of AlCl<sub>3</sub>, 50  $\mu$ L of 1 M NaOH, and 300  $\mu$ L of double distilled water. The reaction mixture was allowed to stand in dark for 30 min at room temperature, and 200  $\mu$ L of mixture was put into 96-well plate to measure the absorbance value at 510 nm using a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific). Quercetin was used to plot standard curve and flavonoid content was measured as quercetin equivalent ( $\mu$ g QE/mL extract).

#### Mineral content

Mineral content was analyzed following the method described earlier (Skujins, 1998). Sample extract (0.5 mL) and HNO<sub>3</sub> (15.0 mL) were mixed. The mixture was diluted with equal volume of distilled water. Mineral concentrations were determined using inductively coupled plasma atomic emission spectrometer (ICP AES, Varian Vista, Victoria, Australia).

#### Solid phase microextraction (SPME)

SPME parameters were optimized for effective extraction and desorption for Pu-erh tea volatiles. The sample extract (10 mL) was added to a 40-mL glass vial containing a small Teflon-coated stirring bar with a screw top and Teflon-lined septum. After allowing equilibration for 20 min, the volatiles from the extracts were extracted for 30 min at 40°C using a 100 mm 50/30  $\mu$ m DVB/Carboxen/PDMS SPME fiber (Supelco, Bellefonte, PA, USA). Before each exposure, the fiber was cleaned in a 260°C injection port for 5 min.

#### Gas chromatography-mass spectrometry (GC-MS)

The GC-MS was conducted under the following conditions: Helium as the carrier gas with a constant flow mode of 2 mL/min. The source was kept at 200°C, and the transfer line and injector were kept at 220°C. Compounds were separated at 60 m, 0.25 mm

i.d., 0.5  $\mu$ m DB-Wax column (J&W Scientific, Folsom, CA, USA). The mass spectrometer was operated in the total ion chromatogram at 70 eV. GC-MS (Clarus 500 quadruple, Perkin/Elmer, Shelton, CT, USA) analysis was equipped with a software (Turbo Mass, Perkin/Elmer, Shelton, CT, USA). Data were collected from 40 to 300 m/z. Mass spectra matches were made by comparison of National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

#### Identification of volatile compounds

Initial identifications were based upon the matches made from spectra in the NIST (NIST 2002 standard spectra) library, aroma descriptors, and linear retention index matches from literature or from standards. The final confirmation was based upon the combined matching of retention indices (LRI values), full scan mass spectra values, and aroma descriptions from standards with those observed in the sample (Kim et al., 2017).

#### Statistical analysis

Data were subjected to analysis of variance using SAS (version 9.4, SAS, Cary, NC, USA). Differences between means at  $p < 0.05$  were identified using Tukey test. Average values are presented as mean  $\pm$  standard deviation (SD) unless otherwise mentioned.

## Results and Discussion

#### pH and titratable acidity

The general chemical characteristics of the tea extracts were evaluated based on the pH and titratable acidity (TA) values. The type of tea and extraction temperature both significantly affected the pH value of tea extracts. This might be due to variation in the type and amount of phytochemicals extractable at different temperatures. The TA value was significantly lower for CPE-80 (0.08) among the four sample extracts (Table 1). The tea extracts from Gushu Pu-erh were found more acidic compared to the CPEs at both temperatures. The TA value of GPE (0.11) at both temperatures and CPE-100 (0.13) were not significantly different.

**Table 1. pH and titratable acidity of typical Pu-erh extract (CPE) and Gushu Pu-erh extract (GPE)**

	Sample <sup>1)</sup>			
	CPE-80	GPE-80	CPE-100	GPE-100
pH	5.61 $\pm$ 0.01 <sup>b3)</sup>	5.00 $\pm$ 0.02 <sup>d</sup>	5.66 $\pm$ 0.02 <sup>a</sup>	5.55 $\pm$ 0.01 <sup>c</sup>
Titratable acidity <sup>2)</sup> (g/100 mL)	0.08 $\pm$ 0.01 <sup>b</sup>	0.11 $\pm$ 0.01 <sup>a</sup>	0.13 $\pm$ 0.01 <sup>a</sup>	0.11 $\pm$ 0.02 <sup>a</sup>

<sup>1)</sup>CPE-80: dried typical Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 80°C; CPE-100: dried typical Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 100°C; GPE-80: dried Gushu Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 80°C; GPE-100: dried Gushu Pu-erh tea (1.5 g) extracted with boiling water (150 mL) and incubated at 100°C. All the tea samples were incubated for 3 min with shaking for 30 s.

<sup>2)</sup>As lactic acid.

<sup>3)</sup>Values are means $\pm$ SD of triplicate measurements. Values followed by different letters in the same row are significantly different ( $p < 0.05$ ).

**Table 2. Hunter's color values of typical Pu-erh (CPE) and Gushu Pu-erh (GPE) tea extracts**

Color value <sup>2)</sup>	Sample <sup>1)</sup>			
	CPE-80	GPE-80	CPE-100	GPE-100
L*	49.00±0.21 <sup>a3)</sup>	49.11±0.09 <sup>a</sup>	45.31±0.23 <sup>b</sup>	46.11±0.12 <sup>b</sup>
a*	0.06±0.04 <sup>c</sup>	0.09±0.03 <sup>c</sup>	0.18±0.05 <sup>b</sup>	0.21±0.02 <sup>a</sup>
b*	12.31±0.08 <sup>b</sup>	9.88±0.06 <sup>d</sup>	12.66±0.05 <sup>a</sup>	10.21±0.21 <sup>c</sup>

<sup>1)</sup>CPE-80: dried typical Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 80°C; CPE-100: dried typical Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 100°C; GPE-80: dried Gushu Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 80°C; GPE-100: dried Gushu Pu-erh tea (1.5 g) extracted with boiling water (150 mL) and incubated at 100°C. All the tea samples were incubated for 3 min with shaking for 30 s.

<sup>2)</sup>L\*, lightness (100, white; 0, black); a\*, redness (, green; +, red); b\*, yellowness (, blue; +, yellow).

<sup>3)</sup>Values are means±SD of triplicate measurements. Values followed by different letters in the same row are significantly different ( $p<0.05$ ).

The results of this study was in agreement with a previous report (Choi et al., 2018). The value of TA implies an impact of acid content on flavor of food, whereas that of the pH indicates to 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). The lack of a correlation of pH and TA with extraction temperature might be due to a 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).

#### Color measurement

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. Type of tea and extraction temperature significantly affected Hunter's color values of the tea extracts (Table 2). The lightness value was significantly higher for the tea extracted at 80°C (49.00 and 49.11) than those extracted at 100°C (45.31 and 46.11). On the other hand, the highest and lowest redness values were measured in GPE-100

(0.21) and CPE-80 (0.06), respectively. The higher redness value of the tea extracted at 100 than at 80°C might be due to more extraction of coloring agents at higher temperature. CPE (12.31 and 12.66) showed greater yellowness value than GPE (9.88 and 10.21) at both temperatures (80 and 100°C). The substantial variation in color value of tea extracts might be due to the effect of extracting temperature (Hajiaghaalipour et al., 2016) that affect extraction efficiency and/or difference in the polyphenol content (Harbowy and Balentine, 1997) of two types of tea.

#### Antioxidant potential

Antioxidant potential of tea extracts were evaluated through DPPH and ABTS radical scavenging activities, SOD-like activity, and total polyphenol and flavonoid contents. The value of antioxidant potentials observed at 100°C was significantly greater compared to that found at 80°C (Table 3). Similar results with the higher phenolics in the tea extracted at higher temperatures were also observed in previous studies (Choi et al., 2018). Results of this study also showed that GPE possessed higher antioxidant potentials compared to the CPE (Table 3). The higher antioxidant potential obtained with GPE was possibly due to the higher catechin content of the tea leaves obtained from older tea trees (Ahmed et al., 2010). The tea leaves harvested from older trees tastes much bitter, and the bitter taste of the tea leaves is more related to catechin, a group of polyphenol, content (Ahmed et al., 2010). The tea catechins demonstrate a range of cellular mechanisms that have antioxidative, anti-inflammatory, neuro-protective, anti-cancer, anti-microbial, and anti-atherosclerotic activities (Clement, 2009). Phenolic compounds in tea play vital role in physicochemical properties of tea. About 20-40% dry matter of young tea shoots comprises of polyphenols which are responsible for the color, flavor and brightness of tea (Turkmen et al., 2009).

#### Mineral content

The total mineral content of Pu-erh tea extract was higher at 100°C (11,739.37-11,996.01 mg/kg) than at 80°C (11,295.91-11,176.98 mg/kg) (Table 4). The amount of Fe and Na was significantly higher in CPE than in GPE. Element Ca content was not significantly affected by the tea type and extraction temperature. Elements like Mg, K, and Ca, are reported to have beneficial

**Table 3. DPPH and ABTS radical scavenging activities, SOD-like activity, and total polyphenol and flavonoid contents of typical Pu-erh (CPE) and Gushu Pu-erh (GPE) tea extracts**

Sample <sup>1)</sup>	DPPH (% Inhibition)	ABTS (%)	SOD (%)	Total polyphenol (µg GAE <sup>2)</sup> /mL)	Flavonoid (µg QE <sup>3)</sup> /mL)
CPE-80	77.12±0.10 <sup>4d)</sup>	80.31±0.50 <sup>d</sup>	20.30±0.51 <sup>d</sup>	1,532.61±2.19 <sup>d</sup>	237.31±2.23 <sup>d</sup>
GPE-80	83.18±0.06 <sup>c</sup>	83.29±0.14 <sup>c</sup>	22.66±0.65 <sup>c</sup>	1,599.28±1.89 <sup>c</sup>	300.15±2.92 <sup>c</sup>
CPE-100	91.41±0.21 <sup>b</sup>	94.88±0.10 <sup>b</sup>	29.66±0.15 <sup>b</sup>	1,821.31±2.31 <sup>b</sup>	572.23±5.18 <sup>b</sup>
GPE-100	92.11±0.05 <sup>a</sup>	96.90±0.03 <sup>a</sup>	31.00±0.12 <sup>a</sup>	2,022.66±5.51 <sup>a</sup>	621.33±6.00 <sup>a</sup>

<sup>1)</sup>CPE-80: dried typical Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 80°C; CPE-100: dried typical Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 100°C; GPE-80: dried Gushu Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 80°C; GPE-100: dried Gushu Pu-erh tea (1.5 g) extracted with boiling water (150 mL) and incubated at 100°C. All the tea samples were incubated for 3 min with shaking for 30 s.

<sup>2)</sup>Gallic acid equivalent.

<sup>3)</sup>Quercetin equivalent.

<sup>4)</sup>Values are means±SD of triplicate measurements. Values followed by different letters in the same column are significantly different ( $p<0.05$ ).

**Table 4. Mineral content (mg/kg) of typical Pu-erh (CPE) and Gushu Pu-erh (GPE) tea extracts**

Element	Sample <sup>1)</sup>			
	CPE-80	GPE-80	CPE-100	GPE-100
Ca	1420.68±28.11 <sup>a2)</sup>	1407.24±26.14 <sup>a</sup>	1411.39±19.11 <sup>a</sup>	1432.12±18.91 <sup>a</sup>
Cu	6.89±0.01 <sup>c</sup>	6.88±0.01 <sup>c</sup>	9.21±0.01 <sup>b</sup>	10.99±0.01 <sup>a</sup>
Fe	105.88±0.40 <sup>b</sup>	74.20±0.30 <sup>d</sup>	119.77±0.21 <sup>a</sup>	99.31±0.39 <sup>c</sup>
K	8476.40±208.32 <sup>b</sup>	8395.89±185.95 <sup>b</sup>	8862.32±180.21 <sup>a</sup>	8931.27±188.29 <sup>a</sup>
Mg	938.02±19.07 <sup>a</sup>	826.36±16.41 <sup>b</sup>	931.99±15.37 <sup>a</sup>	942.12±10.02 <sup>a</sup>
Mn	298.96±1.78 <sup>d</sup>	436.54±7.02 <sup>b</sup>	332.66±2.02 <sup>c</sup>	521.33±6.21 <sup>a</sup>
Na	34.45±0.91 <sup>b</sup>	14.58±0.68 <sup>d</sup>	39.88±0.18 <sup>a</sup>	18.99±1.12 <sup>c</sup>
Zn	14.63±0.01 <sup>d</sup>	15.29±0.05 <sup>c</sup>	32.16±0.09 <sup>b</sup>	39.88±0.16 <sup>a</sup>
Total	11295.91	11176.98	11739.37	11996.01

<sup>1)</sup>CPE-80: dried typical Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 80°C; CPE-100: dried typical Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 100°C; GPE-80: dried Gushu Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 80°C; GPE-100: dried Gushu Pu-erh tea (1.5 g) extracted with boiling water (150 mL) and incubated at 100°C. All the tea samples were incubated for 3 min with shaking for 30 s.

<sup>2)</sup>Values are means±SD of duplicate measurements. Values followed by different letters in the same column are significantly different ( $p < 0.05$ ).

health effects in the prevention and treatment of essential hypertension (Houston and Harper, 2008). On the other hand, elements like As, Pb, Cd, and Hg; which are also health hazardous; were not detected in the tea extracts. High-quality teas produced in China have been found to contain high amount of minerals like Zn, Mn, Mg, K, and Ca (Czernicka et al., 2017).

#### Volatile flavor compounds

Origin of tea and extraction temperature influenced the volatile flavor compounds of tea extracts (Table 5). A total of 80 volatile compounds, categorized under seven groups (28 alcohols, 11 aldehydes, 9 esters, 11 hydrocarbons, 10 ketones, 4 phenols, and 7 others) were detected according to their mass spectrum and retention time. Total peak area for esters was higher than that of either group. The peak areas of alcohols (6.95 and 6.69%), aldehydes (7.41 and 6.73%), hydrocarbons (3.65 and 3.36%), phenols (7.09 and 7.43%), and others (18.48 and 9.45%) for GPE at 80 and 100°C were higher than those of CPE (6.91 and 4.42%, 5.92 and 6.56%, 3.08 and 2.93%, 6.27 and 5.87%, and 3.85 and 4.21%), respectively. However, the peak areas of ester and ketones was greater for CPE than those for GPE at 80°C.

Quantitative abundance and odor thresholds of the volatile compounds account for the overall aroma of tea (Kraujalytė et al., 2016). Among others, floral aroma of alcohols, stale/musty aroma of methoxyphenolic compounds, and woody or floral aroma of ketones play an important role in the special flavor of Pu-erh ripe tea (Selli and Cayhan, 2009). Oxidative degradation of fatty acids may result into formation of alcohols. Many of the alcohols identified in the present study are also detected in orthodox black tea (Joshi and Gulati, 2015). 1-Octen-3-ol imparts a mushroom odor and has a key role in the overall aroma formation in different foods. This compound was also identified in Pu-erh and Fuzhuan teas (Lv et al., 2014). Branched chain aldehydes are reported being responsible for the malty smell odor and are recognized as key odorants contributing to the aroma of tea (Schuh and Schieberle, 2006). Other two compounds hexanal and 2-hexenal impart a green and fruity odor into tea (Schuh and Schieberle, 2006; Wang

et al., 2008) which is supposedly developed from fatty acids. Oxidative degradation of amino acids phenylalanine might be responsible for the formation of aromatic aldehydes like benzaldehyde and benzeneacetaldehyde (Kumazawa and Masuda, 2002), which contribute for an almond odor in teas (Wang et al., 2008). (E,E)-2,4-Heptadienal is found to impart a fatty odor in teas (Schuh and Schieberle, 2006). The highest amount of peak area was found for methyl salicylate, which was also detected in tea samples previously and has been known as an important compound for overall tea aroma (Lv et al., 2014; Wang et al., 2008). Many of the ketones detected in the present study have possibly been produced from the oxidation/degradation of fatty acids. 2,3-Butanedione exerts a buttery odor in black tea (Schuh and Schieberle, 2006) and green tea (Kumazawa and Masuda, 2002). Linalool and linalool oxides are developed during enzymatic hydrolysis of green tea leaves (Wang et al., 1994). Linalool is considered as one of the vital odorants in black tea leaves and tea infusions (Schuh and Schieberle, 2006), green tea (Kumazawa and Masuda, 2002). It has been reported contributing for citrus and floral odor in teas (Schuh and Schieberle, 2006; Wang et al., 2008; Wang et al., 1994). Similarly, beta-ionone is known to contribute for violet aroma and designated as a complex woody and fruity scent. It is synthesized as a result of oxidative degradation or enzymatic oxidation of carotenoids present in teas. A typical flavor in green tea and oolong tea is supposed to be contributed by beta-ionone (Joshi and Gulati, 2015).

#### Conclusion

Both origin of tea and extraction temperature significantly affected the chemical and antioxidant potentials of the tea extracts. The extracts from Gushu Pu-erh showed lower pH at both temperatures compared to typical Pu-erh. The antioxidant potentials of tea extracts measured through DPPH, ABTS, and SOD of Gushu Pu-erh was significantly higher at both temperatures compared to those of typical Pu-erh. Similarly, the total polyphenol and flavonoid contents at both extraction temperatures were

**Table 5. Volatile flavor compounds detected in the extracts of typical Pu-erh (CPE) and Gushu Pu-erh (GPE)**

Compound	Retention Time (min)	<sup>1</sup> Peak area (%)			
		CPE-80	GPE-80	CPE-100	GPE-100
<b>Alcohols</b>		<b>6.91</b>	<b>6.95</b>	<b>4.42</b>	<b>6.69</b>
pentan-2-ol	15.18	-	-	0.26	-
(E)-hex-4-en-3-ol	21.154	-	-	0.25	-
(5E)-octa-1,5-dien-3-ol	28.7	-	-	0.30	-
4-methylhexan-2-ol	30.337	-	-	-	1.36
oxocan-2-ol	35.25	0.01	-	-	-
oct-1-en-3-ol	38.465	1.00	1.80	0.52	0.58
(2S)-4-(benzenesulfonyl)-2-methylbutan-1-ol	40.603	-	-	0.18	-
2-methylidenecyclopentan-1-ol	40.606	0.27	-	-	-
2-ethylhexan-1-ol	40.974	0.67	0.69	-	0.76
2-methylcyclopentan-1-ol	41.356	0.16	-	0.49	-
octan-1-ol	45.125	-	0.81	0.30	-
4-methyl-1-propan-2-ylcyclohex-3-en-1-ol	47.719	-	0.31	-	-
(E)-dec-2-en-1-ol	48.501	0.49	-	-	-
(E)-oct-2-en-1-ol	48.506	-	0.78	0.26	-
nonan-1-ol	51.112	0.29	-	-	-
3-aminopropan-1-ol	51.201	-	-	0.23	-
2-[(1S)-4-methylcyclohex-3-en-1-yl]propan-2-ol	53.17	0.37	1.14	0.30	0.79
1-propan-2-yloxypropan-2-ol	53.797	0.08	-	-	-
hexadecan-1-ol	56.811	-	-	-	1.06
decan-1-ol	56.834	1.30	-	0.75	-
propan-2-ol	58.822	-	-	0.15	-
(2E)-3,7-dimethylocta-2,6-dien-1-ol	61.309	0.37	0.66	0.23	0.50
benzene;ethanol;hydrate	64.484	0.15	0.36	0.19	0.32
dodecan-1-ol	67.507	0.52	-	-	-
tetradecan-1-ol	67.51	-	0.40	-	0.52
1,3-diocan-5-ol	71.75	-	-	0.01	-
ethanol, 2-[2-ethoxyethoxy]ethoxy]-	77.957	0.15	-	-	-
2-[2-[2-(2-decoxyethoxy)ethoxy]ethoxy]ethanol	88.119	1.08	-	-	0.80
<b>Aldehydes</b>		<b>5.92</b>	<b>7.41</b>	<b>6.56</b>	<b>6.73</b>
hexanal	15.232	0.62	0.69	0.88	0.67
heptanal	21.315	0.18	0.26	-	-
(E)-hex-2-enal	23.338	0.14	-	-	-
octanal	27.973	-	0.24	0.18	0.24
(E)-hept-2-enal	30.173	1.24	1.39	2.35	1.46
nonanal	34.74	0.48	1.12	0.40	0.61
benzaldehyde	42.59	0.61	1.06	0.15	0.66
11-[(5Z)-5-[(2-chlorophenyl)methylidene]-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl]undecanoic acid	48.494	-	-	0.26	-
dodecan-1-ol	48.5	-	-	-	0.33
(E)-dec-2-enal	49.979	1.08	0.90	0.98	1.04
4-propylbenzaldehyde	59.986	1.57	1.75	1.36	1.72
<b>Esters</b>		<b>39.91</b>	<b>30.16</b>	<b>30.83</b>	<b>34.34</b>
methyl (2E)-2-hydroxyiminopropanoate	25.086	-	0.06	-	0.11
methoxyacetic acid, 4-tridecyl ester	25.145	0.15	-	-	-
methoxyacetic acid, 2-tridecyl ester	35.251	-	-	-	0.28
ethyl 2-trimethylsilyloxy-2-(3-trimethylsilyloxyphenyl)acetate	43.216	0.57	-	-	-
methyl 2-hydroxybenzoate	57.218	37.98	29.68	30.83	33.95
methyl 2-hydroxybenzoate	57.748	0.20	-	-	-
(z)-N-hydroxybenzenecarboximidic acid methyl ester	58.446	0.69	0.42	-	-
decyl 2-methoxyacetate	58.798	0.23	-	-	-
2-(1-ethoxyethoxy)succinic acid, diethyl ester	63.164	0.09	-	-	-

Table 5. Continued

Compound	Retention Time (min)	<sup>1</sup> Peak area (%)			
		CPE-80	GPE-80	CPE-100	GPE-100
<b>Hydrocarbons</b>		<b>3.08</b>	<b>3.65</b>	<b>2.93</b>	<b>3.36</b>
toluene	13.18	0.37	0.43	0.44	0.47
1,4-xylene	20.193	-	-	0.66	-
1,3-xylene	21.125	0.34	-	-	-
1,3-xylene	21.158	-	0.46	-	-
1,4-xylene	21.174	-	-	-	0.41
benzene, 1,3-bis(1,1-dimethylethyl)-	36.938	2.03	-	-	-
1,3-ditert-butylbenzene	36.942	-	2.48	1.70	2.48
3-acethylmethylthiophenol	45.266	0.14	-	-	-
2-ethoxy-1,4-dimethyl-benzene	50.07	-	0.28	-	-
1-methyl-4-propan-2-ylcyclohexa-1,4-diene	61.419	-	-	0.13	-
4-methyl-1-prop-1-en-2-ylcyclohexene	61.425	0.20	-	-	-
<b>Ketones</b>		<b>1.81</b>	<b>0.83</b>	<b>1.25</b>	<b>1.92</b>
icosane	12.967	0.35	-	-	0.48
2-methylheptan-4-one	22.301	0.16	-	-	-
4-methylheptan-2-one	22.734	0.35	0.36	0.33	0.47
4,6-dimethylheptan-2-one	24.976	-	-	0.22	-
(E)-oct-4-en-3-one	28.772	0.34	-	-	-
hept-1-en-3-one	28.78	-	-	-	0.41
2,2,6-trimethylcyclohexan-1-one	29.775	0.38	0.47	0.25	-
2-methylheptan-4-one	47.555	0.23	-	-	-
decane	21.73	-	-	0.22	-
tetracosane	47.551	-	-	0.23	0.56
<b>Phenols</b>		<b>6.27</b>	<b>7.09</b>	<b>5.87</b>	<b>7.43</b>
2-methoxy-3-methyl-phenol	45.844	0.19	-	-	-
4-(1-hydroxyprop-2-enyl)-2-methoxyphenol	74.761	-	-	0.17	-
4-methoxy-2,5-dimethyl-phenol	76.125	-	0.50	-	0.44
2,4-ditert-butylphenol	83.296	6.08	6.59	5.70	6.99
<b>Others</b>		<b>3.85</b>	<b>18.48</b>	<b>4.21</b>	<b>9.45</b>
3-(maleimido-2'-yl)-1-methyl-2-(1'-methylindol-2'-yl)indole	20.934	0.09	-	-	-
2-[(2S,5S)-5-ethenyl-5-methyloxolan-2-yl]propan-2-ol	38.008	0.25	0.47	0.20	-
2-[(2S,5S)-5-ethenyl-5-methyloxolan-2-yl]propan-2-ol	39.774	0.25	1.00	0.32	0.83
2,4,6,8-tetraoxatetrasilocan-2-ol Auramine	43.212	-	0.58	0.57	0.82
3,7-dimethylocta-1,6-dien-3-ol	44.407	3.26	15.85	3.12	6.89
tetrahydro-2-(methoxymethyl)-Furan	62.867	-	-	-	0.44
(E)-4-(2,6,6-trimethylcyclohexen-1-yl)but-3-en-2-one	65.985	-	0.58	-	0.47

<sup>1</sup>CPE-80: dried typical Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 80°C; CPE-100: dried typical Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 100°C; GPE-80: dried Gushu Pu-erh tea (1.5 g) was extracted with boiling water (150 mL) and incubated at 80°C; GPE-100: dried Gushu Pu-erh tea (1.5 g) extracted with boiling water (150 mL) and incubated at 100°C. All the tea samples were incubated for 3 min with shaking for 30 s.

significantly higher in Gushu Pu-erh compared to typical Pu-erh. Mineral content in both tea samples extracted at 100°C was higher compared to that at 80°C. Among the 80 volatile compounds, the peak area of esters was higher than that of other groups. This study indicated that production environment and extraction temperatures significantly affects the chemical properties, including polyphenol content of tea samples. Polyphenols have drawn substantial attention because of their natural abundance and remarkable biological activities. The results suggests that Gushu Pu-erh extracted at 100°C for 3 min with 30 s of shaking possesses higher antioxidant potential and mineral content.

## Conflict of Interests

The authors have declared no conflict of interest.

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