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Comparison of Quality Characteristic and Antioxidant Potential of Cultivated Pu-erh and Gushu Pu-erh Tea Extracts at Two Temperatures

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Pu-erh, a type of post-fermented dark tea, has attracted much attention of food scientists because of its health-promoting effects. pH, titratable acidity, color value, antioxidant potential and free amino acid contents of cultivated Pu-erh and Gushu Pu-erh teas extracted at 90 and 100°C were compared in this study. The pH of Gushu Pu-erh tea extract was slightly acidic than cultivated Pu-erh. Gushu Pu-erh contained higher antioxidant potentials and free amino acid content at the both extraction temperatures than the cultivated Pu-erh. The antioxidant potentials and free amino acid content were high in the tea extracted at 100°C. The results of this study showed that Gushu Pu-erh tea extracted at 100°C for 3 min with 30 s of shaking yields high amounts of phenolics and free amino acids.

Keywords: Antioxidant potential, Cultivated Pu-erh, Extraction temperature, Gushu Pu-erh.

Tea is one of the most popular ancient beverages in the world and is prepared in various forms as green tea (non-fermented), oolong tea (semi-fermented), black tea (fully fermented by oxidising enzymes) and dark tea (post-fermented by microbes). Pu-erh tea, originally produced in the Yunnan Province of China, is categorized as a type of post-fermented dark tea as opposed to the fermented tea, such as black tea. Pu-erh teas are typically prepared via two methods. Firstly, the raw pu-erh tea is produced by pressing large and unoxidized tea leaves which are then fermented for several years at room temperature. Secondly, the pu-erh teas are ripened for several months using microbes under optimum conditions before subjected for being pressed [1]. 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[2].

Recently, Pu-erh tea has attracted much attention because of its health benefits. Nowadays, in addition to being a favorite drink in China and other Southeast Asian countries, it has also been popular in Japan, USA, Britain and other countries. Studies have shown a multiple health-promoting effects of Pu-erh tea as a functional beverage, including anti-oxidative[3-5], anti-mutagenic[6], antibacterial[6,7], antiviral[8,9], anti-tumor[10], cholesterol-lowering [11], anti-obesity[12,13], hypoglycemic[14] and anti-allergic[15] activities.

Pu-erh tea made from 'Gushu', meaning ancient tea trees in Chinese, is highly demanded and expensive as well. Gushu Pu-erh are prepared from the tea leaves obtained from trees of several hundred years old. Pu-erh teas are also prepared from cultivated tea trees, which are, in this study,

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referred to as Pu-erh cultivated. The market value of Pu-erh teas greatly varies with the age of the tea tree which the leaves were obtained from. Although there have various reports on Pu-erh tea been published, a comparative study considering the effect of extracting temperatures and types of Pu-erh tea is not found documented. Considering the effect of extracting temperature[16] and age of tea tree[17] on their quality and functional properties, the objective of this study was to compare the quality characteristics and antioxidant properties of Gushu Pu-erh and cultivated Pu-erh teas. This study will provide an insight into 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 and DPPH were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used were analytical grade.

Two commercial tea samples, cultivated Pu-erh and Gushu Pu-erh manufactured in Yunnan province of China were used in this study.

Preparation of tea extracts

Two kinds of extract were prepared, one at 90°C and the other at 100°C with the two tea samples and were named as follows.

CP-90: a 1.5-g of dried sample of cultivated Pu-erh tea was extracted with 150 mL of boiling water and incubated at 90°C for 3 min with shaking for 30 s; GP-90: a 1.5-g of dried sample of Gushu Pu-erh was extracted with 150 mL of boiling water and incubated at 90°C for 3 min with shaking for 30 s; CP-100: a dried sample of cultivated Pu-erh tea (1.5 g) extracted with boiling water (150 mL) and incubated at 100°C for 3 min with shaking for 30 s; GP-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 shaking for 30 s. The extraction conditions were designed to get a close similarity to an actual tea brewing.

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[18].

DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the methods described by Dhungana *et al*[19] with some modifications. A 0.8-mL of 0.2 mM DPPH ethanol solution 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 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 varieties were estimated according to the Folin-Ciocalteau method[20]. A 250-µL of undiluted Folin-Ciocalteau reagent was added to 50 µL of the tea extracts. After 1 min, 750 µL of 20% (w/v) aqueous Na_2CO_3 was added to the mixture and the 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). Gallic acid was used to prepare a calibration curve and 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*. [21]. The method is based on the ability of an antioxidant to reduce ABTS cation radical into its colorless form. The ABTS cation radical was generated by reacting 2.4 mM potassium persulfate with 7 mM ABTS solution. The reaction mixture

was left in the dark at room temperature for 12 16 h. The reaction mixture was diluted with double distilled water to achieve an absorbance value of 0.7 ± 0.02 at 734 nm. The tea extracts and ABTS reagent were mixed 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.

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

where *AC*= absorbance of ABTS radical cation,

AS= absorbance of a mixture of ABTS radical solution and tea extracts.

SOD (superoxide dismutase)-like activity

The SOD-like activity was measured according to the method described by Debnath *et al*[22]. A reaction mixture prepared by adding 1.3 mL of Tris-HCl buffer (50 mM Tris, 10 mM EDTA, pH 8.5) and 100 µL of 7.2 mM pyrogallol was added to an aliquot (100 µL) of sample extracted and reacted at 25°C for 10 min. The reaction was stopped by adding 50 µL of 1 N HCl. The amount of pyrogallol oxidised during the reaction was measured at an absorbance 420 nm using a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific). The SOD-like activity was determined using the following equation.

$$\text{SOD-like activity } (\%) = [1 - (\frac{Absorbance\ of\ solution\ with\ sample}{Absorbance\ of\ solution\ without\ sample}) \times 100]$$

Flavonoid content

The flavonoid content was measured following the method described by Mohdaly *et al*[23]. A 100-µL of tea extract was mixed with 500 µL of methanol. A 50-µL of $AlCl_3$, 50 µL of 1 M NaOH and 300 µL of double distilled water was added to the methanol-sample mixture. The reaction mixture was allowed to react in dark for 30 min and the absorbance value of the mixture was measured 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 (µg QE/mL extract).

Free amino acid composition

The free amino acid content of tea extracts was determined following the procedure described by Je *et al*[24]. 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 HC1 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-µm membrane filter (Millipore, Billerica, MA, USA). The amino acid profile was determined using an automatic amino acid analyzer (Biochrom-20, Pharacia Biotech Co., Stockholm, Sweden). All of the samples were run in duplicate and expressed as µg/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 were reported, unless otherwise stated.

RESULTS AND DISCUSSION

General chemical characteristics

pH and titratable acidity (TA) were considered to measure the general chemical composition of the tea extracts. The pH value of tea extracts was significantly varied with tea type and extraction temperature, whereas the difference in TA values was not significant (Table 1). The tea extracts were slightly acidic and the highest pH value was found in CP-90 (5.72) and the lowest in GP-90 (5.01). The TA ranged from 0.09% (CP-90) to 0.12% (GP-100). The pH value of cultivated Pu-erh tea extracts was higher than that of the Gushu Pu-erh at both temperatures. TA is a predictor of the impact of acid content on flavor of food, whereas the pH indicates the ability of a microorganism to grow in a specific food[25]. The variation in pH can influence on flavor and shelf-life[26].

Color measurement

A color value of food plays a vital role in consumers' acceptability towards the food. Hunter's color values of the tea extracts were significantly different (Table 2). The highest and lowest lightness values were found in CP-90 (48.07) and 46.53 (GP-100), respectively. In contrary the highest and lowest redness values were found in GP-100 (0.20)

and CP-90 (0.07), respectively. The higher redness value in CP-100 and GP-100 might be due to more extraction of coloring agents at 100°C than at 90°C. The yellowness value of cultivated Pu-erh (CP-90 and CP-100) was higher than that of Gushu Pu-erh tea extracts. The variation in color value among the sample extracts might be due to the effect of extracting temperature[16] and/or polyphenol Table 2 content [27].

Antioxidant potential

DPPH and ABTS radical scavenging activities, SOD-like activity, and total polyphenol and flavonoid contents were considered to evaluate the antioxidant potentials of tea extracts. Value of antioxidant potentials was high for the extraction at 100°C compared to 90°C and significantly different among the sample extracts (Table 3). Higher contents of phenolics in the tea extracted at higher temperatures were also observed in previous studies [28,29]. The results also showed that Gushu Pu-erh tea extract possessed high antioxidant potentials in terms of ABTS radical scavenging activity and total polyphenol and flavonoid contents compared to the cultivated Pu-erh (Table 3). The higher antioxidant potential of Gushu Pu-erh tea extracts was possibly due to the tea leaves obtained from old tea trees. Ahmed *et al*[30] implied that the tea leaves harvested from older trees tastes much bitter, and the bitter taste of Pu-erh tea is more related to catechin, a group of polyphenol, content. It has been reported that the tea catechins demonstrate a range of cellular mechanisms that have anti-oxidative, anti-inflammatory, neuro-protective, anti-cancer, anti-microbial, and anti-atherosclerotic activities[31].

Free amino acid composition

The content of individual free amino acids and their total amount in the four tea extracts are listed in Table 4. A total of 37 free amino acids were analyzed, out of which seven, seven and nine were detected at least in one sample extract as essential, non-essential, other free amino acids, respectively. Essential L-methionine and non-essential proline were not detected in any of the four tea extracts. Similarly, taurine, urea, L-á-amino asipic acid, L-á-amino-n-butyric acid, L-cystine, cystathionine, hydroxylysine, L-ornithine, 3-methyl-L-histidine, L-anserine, L-carnosine and hydroxy proline were the other free amino acids which were not detected in any sample extract. The highest amount of total free amino acids was found in GP-100 (691.56 ug/mL) followed by CP-100 (566.68 ug/mL) and GP-90 (557.69 ug/mL). Gushu Pu-erh contained higher amount of total free amino acid than cultivated Pu-erh in the both extraction temperatures. L-phenylalanine was the most abundant essential and L-asparitic acid and L-glutamic acid were

Table 2. Hunter's color values of cultivated Pu-erh (CP) and Gushu Pu-erh (GP) tea extracts

Sample[1)]	Color value[2)] L*	a*	b*
CP-90	48.07±0.33a[3)]	0.07±0.02d	10.04±0.12b
GP-90	47.46±0.10b	0.12±0.03c	8.91±0.07d
CP-100	46.54±0.32c	0.16±0.04b	10.64±0.23a
GP-100	46.53±0.23c	0.20±0.04a	9.64±0.31c

[1)]Samples are defined in Materials and Methods (Preparation of tea extracts).
[2)]L*, lightness (100, white; 0, black); a*, redness (, green; +, red); b*, yellowness (, blue; +, yellow).
[3)]Quoted values are means±SD of triplicate measurements. Values followed by different letters (a-d) in the same column are significantly different ($p<0.05$).

Table 1. pH and titratable acidity of cultivated Pu-erh (CP) and Gushu Pu-erh (GP) tea extracts

	Sample[1)] CP-90	GP-90	CP-100	GP-100
pH	5.72±0.02a[3)]	5.01±.001d	5.62±0.01b	5.56±0.01c
Titratableacidity[2)] (g/100 mL)	0.09±0.01a	0.10±0.02a	0.11±0.02a	0.12±0.01a

[1)]Samples are defined in Materials and Methods (Preparation of tea extracts).
[2)]As lactic acid.
[3)]Quoted values are means±SD of triplicate measurements. Values followed by different letters (a-d) in the same row are significantly different ($p<0.05$).

Table 3. DPPH and ABTS radical scavenging activities, SOD-like activity, and total polyphenol and flavonoid contents of cultivated Pu-erh (CP) and Gushu Pu-erh (GP) tea extracts

Sample[1)]	DPPH (% Inhibition)	ABTS (%)	SOD (%)	Total polyphenlol (μg GAE[2)]/mL)	Flavonoid (μg QE[3)]/mL)
CP-90	81.79±0.05c[4)]	83.83±0.51d	23.36±0.30c	1621.67±1.31d	300.50±1.27d
GP-90	88.76±0.10b	85.11±0.11c	24.64±0.52b	1639.71±3.12c	357.67±3.66c
CP-100	90.47±0.20a	94.62±0.10b	27.34±0.09a	1948.43±7.23b	453.67±8.12b
GP-100	90.34±0.31a	95.02±0.02a	27.05±0.37a	1997.99±8.99a	573.67±6.91a

[1)]Samples are defined in Materials and Methods (Preparation of tea extracts).
[2)]Gallic acid equivalent.
[3)]Quercetin equivalent.
[4)]Quoted values are means±SD of triplicate measurements. Values followed by different letters (a-d) in the same column are significantly different ($p<0.05$)

Table 4. Free amino acid composition of cultivated Pu-erh (CP) and Gushu Pu-erh (GP) tea extracts

Amino acid	Sample[1)]			
	CP-90 (ug/mL)	GP-90 (ug/mL)	CP-100 (ug/mL)	GP-100 (ug/mL)
Essential				
L-Threonine	2.60[2)]	4.32	4.08	5.48
L-Valine	3.42	3.98	4.56	4.25
L-Isoleucine	2.06	2.57	2.94	2.87
L-Leucine	2.38	3.17	3.29	3.51
L-Phenylalanine	6.94	7.45	8.67	8.78
L-Lysine	1.59	2.30	2.56	2.76
L-Histidine	0.44	0.55	0.63	0.76
Sub-total	19.43	24.34	26.73	28.42
Non-essential				
L-Asparitic acid	19.44	20.11	26.47	25.22
L-Serine	5.13	8.09	7.86	9.67
L-Glutamic acid	20.99	24.62	31.54	29.67
Glycine	0.81	0.95	1.04	1.13
L-Alanine	4.63	4.72	6.94	6.09
L-Tyrosine	2.84	4.48	4.07	5.37
L-Arginine	0.91	7.95	1.37	7.57
Sub-total	54.74	70.91	79.29	84.72
Other				
O-Phospho-L-serine	3.72	5.34	5.37	6.40
O-Phospho ethanol amine	2.15	2.96	3.07	3.70
L-Sarcosine	279.33	449.52	443.45	561.08
L-Citrulline	ND[3)]	0.87	ND	ND
â-Alanine	2.08	2.23	3.52	3.45
D,L-â-Amino isobutyric acid	ND	ND	1.07	1.02
ã-Amino-n-butyric acid	1.38	1.23	2.31	1.46
Ethanolamin	ND	0.27	1.21	1.16
1-Methyl-L-histidine	0.42	ND	0.67	0.15
Sub-total	289.07	462.43	460.66	578.42
Total free amino acids	363.24	557.69	566.68	691.56

[1)]Samples are defined in Materials and Methods (Preparation of tea extracts).
[2)]Quoted values are means±SD of duplicate meaurements.
[3)]Non-detectable.

the most abundant non-essential amino acids detected in the tea extracts. ã-Amino-n-butyric acid (GABA) is primarily synthesized in plant tissues by decarboxylation of glutamic acid in the presence of glutamate decarboxylase[32]. GABA and glycine are related to learning and memory, stroke and neurodegenerative diseases; relieving anxiety, sedation, Table 4 anticonvulsant, and muscle relaxation function[33-35].

CONCLUSION

This study compared the pH, titratable acidity, color value, antioxidant potential and free amino acid contents of cultivated Pu-erh and Gushu Pu-erh tea extracted at 90 and 100°C. The results of the present study showed that extraction temperature significantly affect the chemical and functional values of tea. Gushu Pu-erh showed lower pH but higher antioxidant potentials and free amino acid content at the both extraction temperatures. The antioxidant potentials and free amino acid content were high in the tea extracted at 100°C compared to at 90°C. Based on the nutritional and functional parameters investigated in the present study, Gushu Pu-erh tea extracted at 100°C for 3 min with 30 s of shaking provides high amounts of total polyphenol, flavonoid and free amino acid.

REFERENCES

1. Chen H-Y, Lin-Shiau S-Y, Lin J-K. 2009. Pu-erh tea; its manufacturing and health benefits, pp 9"15. In Ho C-T, Lin J-K, Shahidi F, (eds.), Tea and Tea Products: Chemistry and Health-Promoting Properties, CRC Press, Boca Raton, FL, USA.
2. Zhou HJ, Li JH, Zhao FL, Han J, Yang XJ, Yang W, Wu XZ. Study on main microbes on quality formation of Yunnan Puer tea during pile-fermentation process. *J Tea Sci*, 2004, **24**: 212–218 (in Chinese).
3. Jie GL, Lin Z, Zhang LZ, Lv HP, He PM, Zhao BL. Free radical scavenging effect of Pu-erh tea extracts and their protective effect on oxidative damage in human fibroblast cells. *J Agric Food Chem*, 2006, **54**: 8058–8064.
4. Duh PD, Wang BS, Liou SJ, Lin CJ. Cytoprotective effects of Pu-erh tea on hepatotoxicity in vitro and in vivo induced by tert-butyl-hydroperoxide. *Food Chem*, 2010, **119**: 580–585.
5. Fan JP, Fan C, Dong WM, Gao B, Yuan W, Gong JS. Free radical scavenging effect of Pu-erh tea extracts and their protective effect on oxidative damage in human fibroblast cells. *Food Chem Toxicol,* 2013, **59**: 527–533.
6. Wu SC, Yen GC, Wang BS, Chiu CK, Yen WJ, Chang LW, Duh PD. Antimutagenic and antimicrobial activities of Pu-erh tea. *Swiss Soc Food Sci Technol*, 2007, **40**: 506–512.
7. Hu YJ, Jia JJ, Qiao JL, Ge CR, Cao ZH. Antimicrobial activity of Pu-erh tea extracts in vitro and its effects on the preservation of cooled mutton. *J Food Saf*, 2010, **30**: 177–195.
8. Pei SB, Zhang Y, Xu H, Chen XW, Chen SY. Inhibition of the replication of hepatitis B virus in vitro by Pu-erh tea extracts. *J Agric Food Chem*, 2011, **59**: 9927–9934.
9. Huang N, Yang LM, Li XL, Zheng CB, Wang RR, Yang YP, Zheng YT. Anti-HIV activities of extracts from Pu-erh tea. *Chinese J Nat Med*, 2012, **10**: 347–352.
10. Zhao LJ, Jia ST, Tang WR, Sheng J, Luo Y. Pu-erh tea inhibits tumor cell growth by down-regulating mutant p53. *Int J Mol Sci*, 2011, **12**: 7581–7593.
11. Peng CX, Wang QP, Liu HR, Gao B, Sheng J, Gong JS. Effects of Zijuan Pu-erh tea theabrownin on metabolites in hyperlipidemic rat feces by Py-GC/MS. *J Anal Appl Pyrolysis*, 2013, **104**: 226–233.
12. Hou Y, Shao WF, Xiao R, Xu KL, Ma ZZ, Johnstone BH, Du YS. Pu-erh tea aqueous extracts lower atherosclerotic risk factors in a rat hyperlipidemia model. *Exp Gerontol*, 2009, **44**: 434–439.
13. Oi Y, Hou IC, Fujita H, Yazawa K. . Antiobesity effects of Chinese black tea (Pu-erh tea) extract and gallic acid. *Phytother Res*, 2012, **26**: 475–481.
14. Du WH, Peng SM, Liu ZH, Shi L, Tan LF, Zou XQ. Hypoglycemic effect of the water extract of Pu-erh tea. *J Agric Food Chem*, 2012, **60**: 10126-10132.
15. Yamazaki K, Yoshino K, Yagi C, Miyase T, Sano M. Inhibitory effects of Pu-erh tea leaves on mouse type IV allergy. *Food Nutr Sci*, 2012, **3**: 394–400.
16. Hajiaghaalipour F, Sanusi J, Kanthimathi MS. Temperature and time of steeping affect the antioxidant properties of white, green, and black tea infusions. *J Food Sci*, 2016, **81**: H246–H254.
17. Li JH, Nesumi A, Shimizu K, Sakata Y, Liang MZ, He QY, Zhou HJ, Hashimoto F. Chemosystematics of tea trees based on tea leaf polyphenols as phenetic markers. *Phytochemistry*, 2010, **71**: 1342–1349.
18. Kim ID, Lee JW, Kim SJ, Cho JW, Dhungana

SK, Lim YS, Shin DH. Exogenous application of natural extracts of persimmon (*Diospyros kaki* Thunb.) can help in maintaining nutritional and mineral composition of dried persimmon. *Afr J Biotechnol*, 2014, **13**: 2231–2239.
19. Dhungana SK, Kim BR, Son JH, Kim HR, Shin DH. Comparative study of *CaMsrB2* gene containing drought tolerant transgenic rice (*Oryza sativa* L.) and non transgenic counterpart. *J Agron Crop Sci*, 2015, **201**: 10–16.
20. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *Am J Enol Vitic*, 1965, **16**: 144–158.
21. Miller NJ, Rice-Evans C, Davies ML, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci*, 1993, **84**: 407–412.
22. Debnath T, Park P-J, Deb Nath NC, Samad NB, Park HW, Lim BO. Antioxidant activity of *Gardenia jasminoides* Ellis fruit extracts. *Food Chem*, 2011, **128**: 697–703.
23. Mohdaly AA, Sarhan MA, Smetanska I, Mahmoud A. Antioxidant properties of various solvent extracts of potato peel, sugar beet pulp and sesame cake. *J Sci Food Agric*, 2010, **90**: 218–226.
25. Je JY, Park PJ, Jung WK, Kim SK. Amino acid changes in fermented oyster (*Crassostrea gigas*) sauce with fermentation periods. *Food Chem*, 2005, **91**: 15–18.
25. Tyl C, Sadler GC. 2017. pH and titratable acidity, pp 389–406. In Nielsen SS, (ed.), Food Analysis, Springer Science, Basel, Switzerland,
26. Aysegul K, Mehmet O, Bekir C. Effects of temperature, solid content and pH on the stability of black carrot anthocyanins. *Food Chem*, 2007, **101**: 212–218.
27. Harbowy ME, Balentine DA. Tea chemistry. *Crit Rev Plant Sci*, 1997, **16**: 415–480.
28. Castiglioni S, Damiani E, Astolfi P, Carloni P. Influence of steeping conditions (time, temperature, and particle size) on antioxidant properties and sensory attributes of some white and green teas. *Int J Food Sci Nutr*, 2015, **66**: 491–497.
29. Santos JS, Deolindo CT, Esmerino LA, Genovese MI, Fujita A, Marques MB, Rosso ND, Daguer H, Valese AC, Granato D. Effects of time and extraction temperature on phenolic composition and functional properties of red rooibos (*Aspalathus linearis*). *Food Res Int*, 2016, **89**: 476–487.
30. Ahmed S, Unachukwu U, Stepp JR, Peters CM, Long C, Kennelly E. Pu-erh tea tasting in Yunnan, China: correlation of drinkers' perceptions to phytochemistry. *J Ethnopharmacol*, 2010, **132**: 176–185.
31. Clement Y. Can green tea do that? A literature review of the clinical evidence. *Prev Med*, 2009, **49**: 83–87.
32. Nikmaram N, Dar BN, Roohinejad S, Koubaa M, Barba FJ, Ralf G, Johnson SK. Recent advances in ã-aminobutyric acid (GABA) properties in pulses: An overview. *J Sci Food Agric*, 2017, **97**: 2681–2689.
33. Krogsgaard-Larsen P. 1989. GABA receptors, p. 349383. In Williams M, Glennon RA, Timmermans PMWM, (eds.), Receptor Phamacology and Function, Marcel Dekker Inc., New York, NY, USA.
34. Mody I, De Koninck Y, Otis TS, Soltesz I. Bridging the cleft at GABA synapses in the brain. *Trend Neurosci*, 1994, **17**: 517–525.
35. Oh CH, Oh SH. Effect of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis. *J Med Food*, 2004, 7: 19–23.