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Chemical Composition of Green Teas According to Processing Methods and Extraction Conditions

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Abstract This study examined the influence of manufacturing processes and extraction conditions on the chemical compositions of green tea. Green tea samples grown in various areas (Korea, China, and Japan) and processed by 4 different methods (steaming, pan-firing, steaming and pan-firing, and heavy roasting after steaming and pan-firing) were collected for study. The chemical compositions of the green tea extracts and infusions were different according to their processing methods and extraction conditions, including catechins, caffeine, and free amino acids contents. In all samples analyzed, (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), and theanine were determined as the major catechins and free amino acid, respectively. Studies of samples grown in the same area (Jeju; Korea) showed that there were significant differences in the concentrations of catechins and caffeine in extract and infusion according to the processing methods. These results indicate that processing methods influenced the chemical compositions of the green tea extracts and infusions.

Keywords: green tea, chemical composition, processing method, extraction condition

Introduction

Green tea is an unfermented product manufactured from the fresh leaves of the tea plant (*Camellia sinensis* L.). In view of people's health enhancement or sensual enjoyment, many of the chemical components in tea leaves have attracted researchers' attention, such as catechins, caffeine, amino acids, vitamins, minerals, and volatile flavor compounds (1).

Green teas are produced and heavily consumed in certain Asian countries, such as China, Japan, and Korea. Based on cultural differences and consumers' preferences, various green tea processing methods have been developed. The pan-firing method in China and Korea, and the steaming method in Japan, are two of the major methods for fixing the polyphenol oxidase of fresh tea leave (2). Different processing procedures may directly affect the chemical compositions of green teas, and some researchers have tried to reveal their relationships (3-6). Yasuda *et al.* (6) found that catechins remained at a higher level in green tea roasted by the Japanese method could. Even though there is limited information available for the free amino acid constituents in green tea (6-9), it has been demonstrated that amino acid content is positively related to the flavor quality of green tea (7). Syu *et al.* (8) suggested that the sources of the raw materials (the tea growing and manufacturing regions) or tea processing procedures may play an important role in theanine variations. Higher theanine contents were observed in both green and *oolong* teas grown in high mountains with less sunshine.

The compositions of green tea infusions may be influenced not only by the processing methods but also by the extraction conditions. However, studies examining the effects of extraction conditions were usually concentrated only on catechin and caffeine contents (2,10,11). Sharma *et al.* (2) examined the extractability of tea catechins in tea extract with different temperatures, and higher levels of catechins [especially (−)-epigallocatechin gallate (EGCG) and (−)-epigallocatechin (EGC)] and caffeine could be extracted at 100°C compared to 80°C. Suteerapataranon *et al.* (11) reported that caffeine contents in non-ground tea samples were dependent on the water temperature and extraction time, in which a higher water temperature and longer extraction time resulted in greater caffeine concentrations in the tea infusion.

The objective of this study was to examine the effects of green tea processing methods and extraction conditions on the chemical compositions of green tea extracts and infusions, including catechins, caffeine, and free amino acids contents.

Materials and Methods

Materials Seven different loose-leaf green tea samples similar in harvest season (May 2008) and of medium grade were used. Four of them were made from tea leaves harvested from the same plantation in Jeju (Korea) but were processed with 4 different methods (steaming, pan-firing, steaming and pan-firing, and heavy roasting after steaming and pan-firing). Fresh tea leaves (*Camellia sinensis* L.) were immediately processed into commercial steamed, pan-fired and steamed, and pan-fired green teas. For the steamed green tea, fresh tea leaves were first 'fixed' by steam at 95-100°C for 30-40 sec to inactivate enzymes and then dried in four steps (70-80°C for 30-35 min, 60-70°C for 30-40 min, 80-90°C for 15-20 min, and 60-75°C for 30-40 min). The pan-fired green tea was processed by pan-firing at first for 10-15 min at 200-220°C and then also gradually dried (150-180°C for 15-20 min,

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Table 1. The information of green tea samples

Sample identification	Origin	Processing method	Roasting temperature (°C)[1)]
KJ-S	Jeju, Korea	Steaming	130
KJ-P	Jeju, Korea	Pan-firing	130
KJ-SPL	Jeju, Korea	Steaming and pan-firing	130
KJ-SPH	Jeju, Korea	Heavy roasting after steaming and pan-firing	200
KB-P	Bosung, Korea	Pan-firing	130
CH-P	Hangjou, China	Pan-firing	130
JS-S	Shizuoka, Japan	Steaming	100

[1)]Roasting time was 20 min for all the samples.

120°C for 15-18 min, 100°C for 10-15 min, and 80°C for 30 min). The steamed and pan-fired green tea was fixed at first by steam at 95-100°C for 30-40 sec and following processes were the same as that for the pan-fired green tea. The other Korean sample was grown in Boseong (Korea) and manufactured by pan-firing. A steamed Japanese green tea (*sen-cha*) from Shizuoka (Japan) was selected. This sample was manufactured by the steaming process, which makes up about 77.8% of the total green tea production in Japan (12). A Chinese green tea grown and manufactured in Hangjou (China) was also included. It was processed by pan-firing, the general manufacturing method of green tea in China. All of the dried green tea products were finally roasted at 130°C for 20 min except KJ-SPL at 200°C and JS-S at 100°C. The moisture content of the final green tea samples, which are KJ-S, KJ-P, KJ-SPL, KJ-SPH, KB-P, CH-P, and JS-S, were 1.29, 1.18, 1.34, 1.74, 4.27, 5.63, and 3.18%, respectively. The detailed descriptions for each sample are shown in Table 1. The samples were stored at –24°C until use.

Preparation of green tea samples Every tea sample was prepared into 2 types of extracts, referred to as total extract and tea infusion. For the total extracts, the loose tea leaves were first ground (IKA-10; IKA-Werke GmbH & Co., KG, Staufen, Germany; grinding time, 30 sec) and were passed through a 100 mesh sieve, then 1 g of the ground green tea was put into a 100-mL volumetric flask, to which 100 mL of hot distilled water (75°C) was poured and placed in a water bath (J-PWB2; Jisico Co., Seoul, Korea) at 75°C for 30 min. The extract was cooled to room temperature and filtered through a 0.2-μm nylon syringe (F-2513-6; National Scientific Co., Rockwood, TN, USA) for further analyses.

For comparison, the components of the tea infusions were also analyzed as consumed. For infusion of the green tea, the preparation procedure proposed by Lee *et al.* (13), which was developed for sensory descriptive analysis, was adopted. Aliquots (17 g) of the green tea leaves were weighed in a 1-L Erlenmeyer flask, to which 1 L of hot distilled water (70°C) was poured, and then the flask was sealed and placed in a 70°C water bath (J-PWB2; Jisico Co.). After 2 min, the green tea infusions were separated from the tea leaves with a tea strainer (mesh size <1-mm), cooled to room temperature, and filtered through a 0.2-μm nylon syringe filter (F-2513-6; National Scientific Co.) before further analyses.

Analysis of catechins and caffeine by high performance liquid chromatography (HPLC) The extracted and infused samples were subjected to analyses. The HPLC method described by Dalluge *et al.* (14) was modified to determine the catechins and caffeine contents of the prepared samples. Aliquots (20 μL) of the filtered green tea samples were directly injected into the analytical equipment. The HPLC system (2695 XE; Waters, Milford, MA, USA) equipped with a photodiode array detector (2996; Waters), and a Zorbax XDB-C_{18} column [150×4.6 mm i.d., 5 μm (Agilent, Palo Alto, CA, USA)] were used for the analysis of catechins and caffeine. The mobile phase consisted of 0.05% trifluoroacetic acid (TFA) in H_2O (mobile phase A), and 0.05% TFA in acetonitrile (mobile phase B). HPLC-grade TFA and acetonitrile were purchased from J.T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA). Separation was performed at a 1 mL/min flow rate using the following gradient conditions: 0 min, 12% B; 25 min, 21% B; 30 min, 25% B; 35 min, 100% B. The detection wavelength was set at 260 nm. The analyses were conducted in 4 replications, which were carried out from the sample preparation (extraction and infusion) to HPLC analysis in each replication. The total catechins content in the green tea was calculated from the sum of (–)-gallocatechin (GC), (–)-epigallocatechin (EGC), (+)-catechin (C), (–)-epigallocatechin gallate (EGCG), (–)-epicatechin (EC), (–)-gallocatechin gallate (GCG), and (–)-epicatechin gallate (ECG). The retention times and peak areas of authentic compounds (Sigma-Aldrich, St. Louis, MO, USA) were read for identification and quantification.

Analysis of amino acids by ultra performance liquid chromatography (UPLC) Twenty-two free amino acids, including theanine and γ-aminobutyric acid (GABA), were determined by UPLC as described by Boogers *et al.* (15). The UPLC system (AQT 2996; Waters) was equipped with a photodiode array detector (2996; Waters), and an AccQ·Tag Ultra column (2.1×100 mm i.d., 1.7 μm) from Waters was connected. The flow rate and column temperature were kept at 0.7 mL/min and 60°C, respectively. A volume of 2 μL was injected and the detection wavelength was set at 260 nm. The mobile phase consisted of 2 eluents: 5%(v/v) AccQ·Tag Ultra eluent A concentrate in water (eluent A) and AccQ·Tag Ultra Eluent B (eluent B). These reagents were obtained from Waters. The samples were eluted using the following gradient condition: 0 min, 0.1% B; 5.74 min, 9.1% B; 7.74 min, 21.2% B; 8.04 min, 59.6% B; 10 min, 0.1% B. Standard H (amino acid standard solution) was purchased from Pierce Co. (Rockford, IL, USA). Empower 2 (Waters) software was used for system control and data acquisition. The analyses were conducted in 3 replications,

Table 2. Content of individual catechins, total catechins, and caffeine in the extract of green tea samples (mg/g)

Green tea sample	Catechin							Total catechins	Caffeine
	GC	EGC	C	EGCG	EC	GCG	ECG		
KJ-S	4.4±0.3$^{b1)}$	45.5±6.1^{bc}	0.7±0.0^{bc}	24.0±4.1^{ab}	9.2±0.3^{b}	0.7±0.4^{a}	2.1±0.6^{a}	86.6±11.4^{a}	21.3±1.2^{b}
KJ-P	3.6±0.3^{ab}	34.1±3.5^{ab}	0.6±0.0^{b}	31.8±5.4^{ab}	7.3±0.1^{a}	0.7±0.2^{a}	2.6±0.6^{a}	80.7±9.7^{a}	25.4±1.0^{c}
KJ-SPL	5.9±0.4^{c}	54.7±7.5^{c}	0.9±0.0^{d}	20.2±4.3^{a}	10.2±0.5^{c}	0.6±0.3^{a}	1.6±0.5^{a}	94.1±12.8^{a}	21.2±1.0^{b}
KJ-SPH	4.3±1.2^{b}	37.3±4.3^{ab}	0.6±0.0^{bc}	42.3±12.3^{b}	7.5±0.4^{a}	1.3±0.8^{a}	4.1±0.7^{b}	97.3±18.4^{a}	24.3±0.8^{c}
KB-P	3.0±0.2^{a}	27.2±2.1^{a}	0.7±0.0^{bc}	39.9±3.3^{b}	7.6±0.2^{a}	1.1±0.2^{a}	5.3±0.3^{c}	84.8±6.0^{a}	25.0±0.6^{c}
CH-P	3.1±0.1^{a}	35.2±6.5^{ab}	0.3±0.1^{a}	36.9±8.9^{b}	7.3±0.7^{a}	0.7±0.3^{a}	4.5±1.0^{bc}	87.9±17.4^{a}	18.2±1.7^{a}
JS-S	4.5±0.3^{b}	40.8±4.1^{b}	0.8±0.0^{c}	24.5±2.4^{ab}	7.6±0.2^{a}	0.7±0.2^{a}	1.8±0.3^{a}	80.5±5.4^{a}	23.7±0.9^{c}

[1)]Values represent mean±SD (n=4). Means within a column not sharing a superscript letter are significantly different (p<0.05, Tukey's HSD test).

Table 3. Content of individual catechins, total catechins, and caffeine in the infusion of green tea samples (mg/g)

Green tea sample	Catechin							Total catechins	Caffeine
	GC	EGC	C	EGCG	EC	GCG	ECG		
KJ-S	1.0±0.0$^{ab1)}$	18.5±0.6^{c}	0.2±0.0^{bc}	7.0±0.2^{bc}	3.3±0.1^{c}	0.1±0.0^{ab}	0.7±0.0^{a}	46.4±19.5^{ab} (53.5%)$^{2)}$	11.0±0.4^{c} (51.4%)
KJ-P	0.7±0.1^{a}	10.6±1.4^{b}	0.1±0.0^{bc}	6.9±0.9^{bc}	2.1±0.3^{ab}	0.1±0.0^{ab}	0.7±0.1^{a}	32.9±9.9^{ab} (40.7%)	10.6±1.4^{c} (41.7%)
KJ-SPL	0.8±0.0^{a}	12.4±0.2^{b}	0.2±0.0^{bc}	3.3±0.1^{ab}	2.2±0.0^{ab}	0.1±0.0^{a}	0.4±0.0^{a}	28.8±13.4^{ab} (30.6%)	5.9±0.1^{b} (27.9%)
KJ-SPH	1.0±0.1^{ab}	10.2±0.7^{b}	0.1±0.0^{b}	8.2±0.6^{c}	1.7±0.1^{a}	0.1±0.0^{ab}	0.7±0.1^{a}	33.5±12.6^{ab} (34.4%)	7.2±0.5^{bc} (29.8%)
KB-P	0.8±0.2^{a}	12.7±2.4^{b}	0.2±0.0^{c}	12.7±2.3^{d}	2.9±0.5^{bc}	0.10±0.0^{ab}	1.4±0.3^{b}	48.2±11.3^{ab} (56.8%)	11.1±2.0^{c} (44.2%)
CH-P	1.3±0.1^{bc}	1.5±0.1^{a}	0.0^{a}	1.1±0.1^{a}	4.7±0.4^{d}	0.5±0.0^{c}	0.4±0.0^{a}	14.3±6.6^{a} (16.3%)	0.8±0.1^{a} (4.4%)
JS-S	1.5±0.2^{c}	23.5±3.7^{cd}	0.3±0.1^{d}	13.6±2.1^{a}	3.8±0.6^{bcd}	0.2±0.0^{b}	1.3±0.2^{b}	68.9±22.5^{b} (85.6%)	14.9±2.4^{d} (62.9%)

[1)]Values represent mean±SD (n=4). Means within a column not sharing a superscript letter are significantly different (p<0.05, Tukey's HSD test).
[2)]Extraction efficiencies in parentheses were calculated from the total catechins and caffeine content, respectively of the extract of green tea samples.

which were carried out from the sample preparation (extraction and infusion) to UPLC analysis in each replication. The total free amino acids contents in the green tea samples were calculated from the sum of 20 amino acids, theanine, and GABA.

Statistical analyses Analysis of variance (ANOVA) was conducted to examine the effect of processing and extraction conditions on the chemical composition of green tea samples. Mean values of catechins, caffeine, and amino acid contents from the different samples were compared using Tukey's HSD test (p<0.05). Statistical analysis was conducted using SPSS software (version 12.0, SPSS, Chicago, IL, USA).

Results and Discussion

Catechins and caffeine contents in green tea samples The catechins and caffeine contents in the total extract and infusion samples of the different green teas are presented in Table 2 and 3, respectively. In both the total extract and infusion samples, EGCG and EGC were the 2 major catechins, accounting for more than 50% of the total catechins. The amounts of the other catechins in the extracts and infusions were in the following order: EC, GC >ECG, C>GCG. These results are in agreement with previous studies (2,4,6,16,17). Remarkable differences were observed between the total catechins contents of the extracts and infusions. The contents of the total catechins in extracts, which varied from 97.3 to 80.5 mg/g, are shown in Table 2. The highest content of total catechins, although not statistically significant, appeared in KJ-SPH (97.3 mg/g), which was grown in Jeju (Korea) and manufactured by a method of heavy roasting after steaming and pan-firing. The lowest total catechins content was found in JS-S (80.5 mg/g), which was grown in Japan and processed by the steaming method. The catechins contents of the infused samples tended to be lower than those of the extracted samples. Table 3 shows the considerable variation in the catechins and caffeine levels of the samples dependent on the extraction condition and processing methods. Among the samples, the JS-S extract contained the lowest level of total catechins (80.5 mg/g), but the JS-S infusion had the highest level (68.9 mg/g). The KJ-S, Korean steamed green tea, also showed the highest level (46.4 mg/g) among the Korean Jeju green tea samples. On the other hand, the CH-P extract showed relatively high total catechins content (87.9 mg/g), but the CH-P infusion had the lowest total catechins content (14.3 mg/g). It has been reported that the extraction rate of green tea components could be influenced by the shape, degree of destruction, and size of the tea leaves (10,18). Usually, the steaming method causes more destruction of the leaves than pan-firing, yielding more small tea particles (19). Also, bi-directional rolling during steamed tea manufacturing makes the tea leaves flat with their leaf juice spread (2). These may explain why KJ-S and JS-S, processed by steaming, had higher extraction efficiencies of catechins (53.5 and 85.6%, respectively) and caffeine (51.4 and 62.9%, respectively) than the other pan-fired green teas.

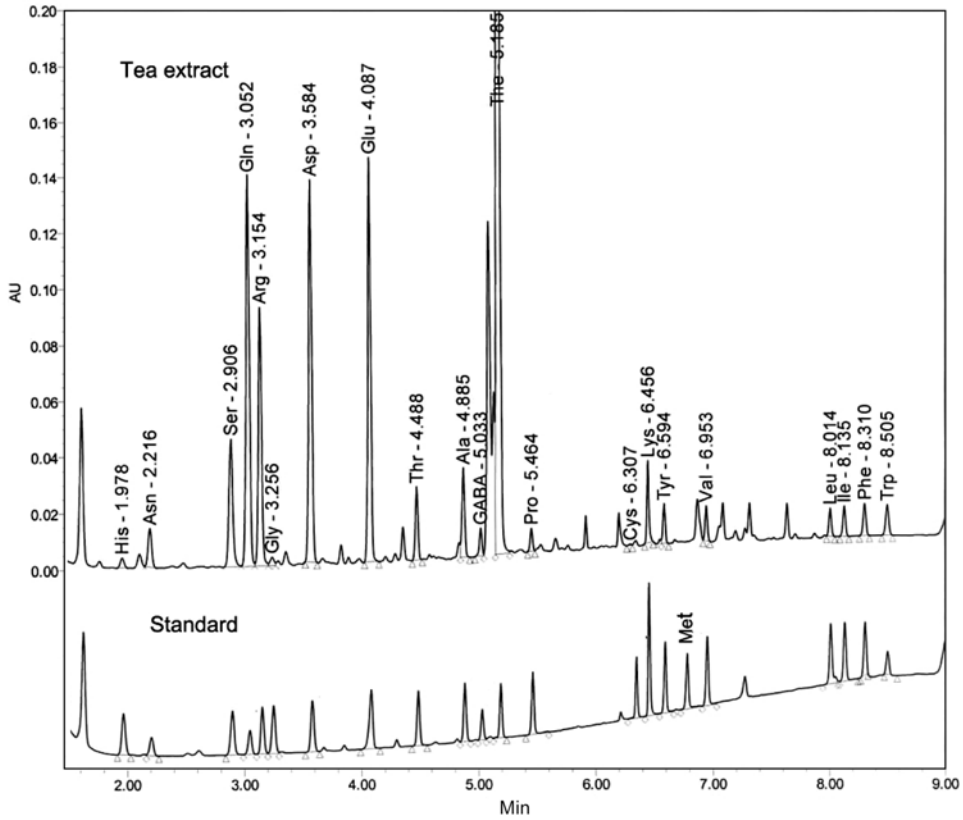

Fig 1. A representative UPLC pattern of free amino acids of green tea extract (KJ-SPL) and standard mixture.

Because small tea particles from the steaming process are usually not removed in Japan, JS-S showed higher extraction efficiencies than KJ-S whose broken particles had been sieved out. CH-P, produced by the pan-firing method in a metal roaster and using a unidirectional rotator roller, had twisted and compact leaves (2). Consequently, CH-P's extraction efficiencies for catechins and caffeine were quite low (16.3 and 4.4%, respectively). These variations might be attributed to the flatness and small particles of steamed green tea, having more surface area that leads to faster extraction of catechins and caffeine, while compactly rolled leaves of pan-fired green teas would require a longer extraction time. Table 3 shows the significant differences in EGC, EGCG, EC and caffeine levels among 4 samples (KJ-S, KJ-P, KJ-SPL, and KJ-SPH) harvested from the same area (Jeju; Korea) but processed by different methods. This data proves that the processing methods are one of the key factors determining the compositional variations of green tea.

UPLC analysis of free amino acids in green tea samples

Figure 1 is a UPLC chromatogram showing the free amino acids composition of a green tea (KJ-SPL) extract and amino acids standard mixture. The calculated results are presented in Table 4 and 5. The UPLC method is a simple, reproducible, and accurate procedure for the quantitative analysis of free amino acids (15). As shown in Table 4, the total free amino acids contents in the green tea extracts varied from 6,787.2 to 33,793.5 μg/g, and they decreased in the following order: KB-P>JS-S>KJ-P, KJ-SPL, KJ-S> CH-P>KJ-SPH. This result is in agreement with previous studies (3,4) such that tender and younger tea leaves may have higher total amino acids content. Theanine (5-*N*-ethyl glutamine), which is the only free form (nonprotein) amino acid occurring in green tea, is considered to be very valuable due to its pharmaceutical effects (20,21). In addition, its sensory properties, providing a brothy sweet umami taste to the green tea, are important for the quality determination of green tea (22,23). Theanine was the most abundant amino acid and accounted for more than 50% of the amino acid fractions in all the extract and infusion samples, and the theanine contents of the extract samples ranged from 3,729.6 to 18,442.9 μg/g. The other major amino acids beside theanine were glutamic acid, aspartic acid, glutamine, and arginine. These results agree with previous reports (4,6,9,24). The total amino acids contents of the infusions varied from 310.0 to 2,079.9 μg/g (Table 5) and they decreased in the following order: JS-S>KB-P>KJ-S, KJ-SPL, KJ-P>CH-P >KJ-SPH. It was observed that the quantitative patterns of the amino acids in the extract and infusion samples were quite similar among the green teas grown in the same area (Jeju; Korea), with the exception of KJ-SPH. KJ-SPH contained the lowest total amino acids in both its extract (6,787.2 μg/g) and infusion (310.0 μg/g). This may be due to the degradation of free amino acids caused by the heavy roasting of the green tea leaves. Similar to those of catechins and caffeine, the extraction efficiencies of the total amino acids were higher in the steamed teas (JS-S and KJ-S) than the pan-fired teas (KJ-P, CH-P, and KB-P). Therefore, even though the extract of KB-P contained the highest level of total amino acids (33,793.5 μg/g), the amino acid content of its infusion was lower (1,357.2 μg/g) than that of the JS-

Table 4. Content of individual free amino acids and total amino acids in the extract of green tea samples

Amino acids	Green tea sample (μg/g)						
	KJ-S	KJ-P	KJ-SPL	KJ-SPH	KB-P	CH-P	JS-S
His	6.7±3.0[1)]	7.6±5.8	1.9±0.7	2.5±0.7	44.4±9.9	22.1±0.6	15.0±5.0
Asn	78.2±0.1	64.6±9.3	41.0±0.3	46.5±7.2	377.8±38.3	65.9±39.4	142.7±2.9
Ser	208.4±61.2	258.2±31.2	224.8±24.9	88.9±14.1	461.7±38.2	366.0±174.0	237.5±24.7
Gln	809.7±47.0	766.6±43.2	196.4±10.6	155.7±37.9	2,021.3±211.5	113.0±69.3	2,063.3±98.4
Arg	332.5±39.9	553.7±47.7	123.8±26.9	145.3±25.2	3,802.4±264.1	23.5±27.6	867.0±42.8
Gly	12.0±4.8	16.6±1.8	10.0±0.7	11.7±1.4	38.5±11.9	23.5±14.9	19.3±4.0
Asp	1,725.8±111.4	1,995.6±125.3	1,698.7±161.8	870.5±139.9	2,459.6±87.4	874.1±188.2	2,360.9±88.1
Glu	2,241.6±293.3	2,503.0±123.4	2,182.9±177.5	863.0±191.1	3,328.4±97.9	1,728.9±128.3	3,227.8±168.7
Thr	212.1±2.8	207.8±11.6	214.9±14.6	91.1±3.8	339.2±1.8	235.5±11.4	301.6±1.7
Ala	239.9±26.7	289.4±4.1	246.4±11.9	148.0±17.2	426.0±3.0	381.9±55.5	315.4±17.1
GABA	73.7±0.4	136.6±17.1	42.1±4.3	51.1±10.1	208.1±20.9	67.8±22.8	157.9±13.0
The	9,300.0±208.6	10,564.9±398.9	11,582.8±412.6	3,729.6±195.8	18,442.9±3,077.4	4,865.9±458.9	13,514.4±1,827.8
Pro	52.7±3.4	59.4±4.8	40.6±10.2	47.1±13.3	160.1±14.2	98.8±15.1	97.9±7.5
Cys	101.2±17.2	129.8±3.1	104.8±9.6	37.5±5.0	87.1±12.9	96.1±8.6	82.2±7.4
Lys	80.4±30.2	82.8±20.1	52.3±6.3	43.8±10.8	323.7±13.6	168.8±12.9	172.7±23.3
Tyr	162.2±4.9	137.4±19.8	180.6±2.4	103.2±31.3	158.5±23.0	218.1±95.5	201.6±70.4
Met	ND[2)]	ND	ND	ND	ND	ND	ND
Val	69.7±23.7	62.5±6.5	36.6±1.6	59.9±17.1	168.6±22.2	176.9±13.1	124.5±13.8
Ile	66.2±2.4	48.8±12.2	36.6±8.4	42.5±0.2	141.2±4.8	103.4±18.4	97.8±1.1
Leu	96.7±6.3	72.8±3.0	49.3±5.9	41.8±0.4	229.1±9.4	104.6±15.8	142.6±1.1
Phe	166.4±10.6	125.7±0.7	105.2±3.6	100.2±29.7	315.2±17.2	357.1±0.7	259.4±7.0
Trp	201.7±15.7	220.2±0.1	191.3±10.4	107.4±25.9	259.8±2.3	410.2±7.7	280.3±9.9
Total	16,238.1±1,708.3^{c}	18,304.0±405.7^{c}	17,362.9±426.4^{c}	6,787.2±637.0^{a}	33,793.5±2,717.6^{e}	10,502.2±262.5^{b}	24,681.8±347.8^{d}

[1)]Values represent mean±SD (n=3). Means within a row not sharing a superscript letter are significantly different (p<0.05, Tukey's HSD test).
[2)]Not detected.

Table 5. Content of individual free amino acids and total amino acids in the infusion of green tea samples

Amino acids	Green tea sample (μg/g)						
	KJ-S	KJ-P	KJ-SPL	KJ-SPH	KB-P	CH-P	JS-S
His	ND[2)]	ND	ND	ND	0.5±0.1[1)]	ND	1.4±0.0
Asn	5.3±0.4	3.1±0.4	3.5±0.3	1.7±0.3	15.4±1.8	3.1±0.4	11.4±0.2
Ser	5.1±0.3	3.6±0.4	9.1±0.9	2.7±0.4	6.7±0.8	15.5±2.0	8.5±0.1
Gln	33.1±2.2	21.2±2.4	14.9±1.5	1.3±0.2	64.5±7.4	5.4±0.7	96.5±1.4
Arg	11.6±0.8	10.6±1.2	2.4±0.2	ND	87.8±10.1	ND	33.5±0.5
Gly	0.7±0.1	0.6±0.1	0.3±0.2	0.4±0.1	1.0±0.1	0.2±0.1	1.5±0.1
Asp	137.7±9.3	103.1±11.8	130.1±12.8	44.0±6.9	117.8±13.5	37.2±4.8	206.3±2.9
Glu	170.3±11.5	125.5±14.4	177.7±17.5	40.5±6.4	153.3±17.6	78.6±10.1	279.8±3.9
Thr	15.2±1.0	9.6±1.1	10.7±1.1	3.5±0.5	13.7±1.6	7.5±1.0	25.2±0.4
Ala	15.9±1.1	12.0±1.4	12.9±1.3	6.0±1.0	16.1±1.8	23.6±3.0	26.0±0.4
GABA	4.7±0.3	5.0±0.6	3.6±0.4	2.4±0.4	8.5±1.0	6.3±0.8	11.6±0.2
The	669.0±45.1	638.3±73.2	539.4±53.2	152.1±23.9	773.4±88.7	171.7±22.1	1,184.0±116.6
Pro	4.4±0.3	2.8±0.3	2.4±0.2	2.7±0.4	7.5±0.9	1.9±0.2	9.4±0.1
Cys	ND	ND	ND	ND	ND	ND	ND
Lys	2.8±0.2	0.8±0.1	ND	0.2±0.1	7.3±0.8	ND	8.5±0.1
Tyr	56.6±3.8	33.2±3.8	88.7±8.8	29.2±4.6	30.2±3.5	2.1±0.3	83.7±1.2
Met	ND	ND	ND	ND	ND	ND	ND
Val	17.9±1.2	12.7±1.5	16.4±1.6	12.8±2.0	19.4±2.2	3.6±0.5	31.2±0.4
Ile	6.0±0.4	2.9±0.3	3.2±0.3	1.6±0.2	9.2±1.1	ND	12.2±0.2
Leu	4.3±0.3	1.6±0.2	1.6±0.2	1.3±0.2	5.5±0.6	ND	8.1±0.1
Phe	9.5±0.6	4.5±0.5	6.4±0.6	3.8±0.6	10.5±1.2	7.0±0.9	20.9±0.3
Trp	10.5±0.7	7.5±0.9	10.9±1.1	3.9±0.6	8.5±1.0	13.6±1.7	20.1±0.3
Total	1,180.7±79.5^{bc} (7.3%)[3)]	998.5±114.5^{b} (5.5%)	1,034.1±102.0^{b} (6.0%)	310.0±48.7^{a} (4.6%)	1,357.2±155.6^{c} (4.0%)	377.5±48.5^{a} (3.6%)	2,079.9±29.1^{d} (8.4%)

[1)]Values represent mean±SD (n=3). Means within a row not sharing a superscript letter are significantly different (p<0.05, Tukey's HSD test).
[2)]Not detected.
[3)]Extraction efficiencies in parentheses were calculated from the total amino acids content of the extract of green tea samples.

S infusion (2,079.9 μg/g). Ding *et al*. (7) indicated there is a strong relationship between green tea quality and free amino acid content, in which the higher the free amino acid content, the higher the quality of green tea. Although all the green tea samples used in this experiment were of medium grade with similar prices, their quality as determined by amino acid content appeared different.

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