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Estimation of the effects of major chemical components on the taste quality of green tea

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Abstract

Major chemical components of green tea significantly influence its taste quality. However, the key components that should be strengthened during tea breeding to improve its taste quality remain unknown. Here, 78 green tea samples made from breeding lines and controls were subjected to chemical and sensory analyses. The results indicate that four major amino acids (theanine, glutamine, glutamic acid, arginine) were positively and significantly correlated with taste quality according to correlation and stepwise discriminant analyses. However, the effects of catechins and caffeine were relatively low. The four major amino acids were also closely associated with the GS/GOGAT cycle. The fact that the four major amino acids had major effects on taste quality indicates their importance for tea taste. Since the four major amino acids are directly associated or derived from the products of the GS/GOGAT cycle, the genes involved in this pathway should be more strengthened for future green tea breeding.

Abbreviations

Ala, alanine; Arg, arginine; Asp, aspartic acid; C, catechin; Cys, cystine; EC, epicatechin; EGC, epigallocatechin; ECG, epigallocatechin-3-O-gallate; EGCG, epigallocatechin-3-O-gallate; GABA, γ-amino butyric acid; GCG, gallocatechin-3-O-gallate; Gln, glutamine; Glu, glutamic acid; GOGAT, glutamine 2-oxoglutarate amino transferase; GS, glutamine synthetase; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; PCA, principal component analysis; Phe, phenylalanine; Pro, proline; SDA, stepwise discriminant analysis; Ser, serine; Thr, threonine; TAA, total amino acids; TC, total catechins; TP, total polyphenols; TS, theanine synthetase; Tyr, tyrosine; Val, valine.

Introduction

Tea (*Camellia sinensis* L.) is a popular drink worldwide. The current global consumption of tea has reached approximately 5 million ton/year, which is significantly higher than many other beverages (International Tea Committee, 2015). Tea can be generally classified into three types according to the processing methods, namely black tea (fully fermented), oolong tea (partially fermented) and green tea (non-fermented) (Wang and Ho, 2009). Among them, green tea preserves most of the natural chemical components of the tea leaves, and is the most favourable type of tea in East Asia. The popularity of green tea is attributed not only to its beneficial effects on human health, but also to its fantastic taste (Khokhar and Magnusdottir, 2002; Chen *et al*., 2010). All these features are closely associated with the chemical composition of green tea (Yu *et al*., 2014).

The major chemical components in green tea include polyphenolic compounds, caffeine and amino acids, which account for 20-30% of total dry tea leaves (Wang *et al*., 2011; Wu *et al*., 2016). The main polyphenolic compounds are catechins including (-)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin (EC), (+)-catechin (C), (-)-epicatechin gallate (ECG), (-)-gallocatechin gallate (GCG) and (-)-epigallocatechin gallate (EGCG) (Liang *et al*., 2001). As compared to other catechins, EGCG and

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ECG have a more astringent taste, and can reduce more saliva lubricity (Hayashi *et al*., 2005; Rossetti *et al*., 2009). Caffeine is the main purine alkaloid in tea. It has a bitter taste and acts as a central nervous system stimulant (Nagatomo and Kubo, 2008). Moreover, caffeine was reported to enhance tea flavour better than catechins (Chen *et al*., 2006). On the other hand, amino acids are responsible for the umami taste (Hung *et al*., 2010). There are approximately 20 types of amino acids in green tea, and the predominant unique amino acid is L-theanine (Hung *et al*., 2010). The harmony of astringency, bitterness and umami taste plays a key role in determining the tea flavour (Chen *et al*., 2006).

A tea breeder has to identify the key chemical components influencing the taste quality of tea. The key components could be used as indicators for tea breeding to significantly increase the breeding efficiency. Several studies have established a relationship between chemical components and sensory characteristics. For example, early spring green tea generally tastes better than summer and autumn green teas, and this difference is considered to correlate with higher levels of free amino acids and lower levels of polyphenolic compounds in the spring tea (Dai *et al*., 2015). Additionally, Zhu *et al*. (2017) reported that green tea leaves with high pubescence had a better taste and higher contents of amino acids and lower contents of polyphenols than did the shoots. Jing *et al*. (2017) found that the taste of green tea may correlate with the composition of several polyphenolic compounds. These studies offered valuable information about the taste contributors in tea. However, few, if any of them, suggested potential components for tea breeding, and these suggestions require a comparison between various genotypes.

In the present work, 78 green tea samples made from 42 lines (cultivars) for tea breeding were subjected to chemical and sensory analyses. The purpose of the present work was to investigate the association between the chemical components and taste quality in various tea lines (cultivars), and to identify the key chemical components as indicators for tea breeding.

Materials and methods

Sample preparation

A total of 42 lines (cultivars) for tea breeding were grown in the experimental tea garden of TRICAAS in Hangzhou, China. Fourteen tea lines (LJ001, LJ003, Xinxuan3, Xinxuan5, Xinxuan6, Xinxuan7, LJ192, Longqu1, SM02, XKW01, XKWN1, XKW05, XKW08 and XKW11) and three cultivars (LJ43, Zhongcha108 and Fudingdabaicha) were divided into three trial blocks. Two lines (XKW10 and XKW15) were divided into two blocks and the rest of the lines (XKW02, XKW09, Z7, Xinxuan4, Xinxuan10, 19A, 9B, 12B, 14B, 15B, 16B, 17B, Tezao, LY006, LY007, TRI003, TRI005, TRI006, LS-2013-19, LY010, LY13B, JK-1 and JK-2) had only a single block. Each block contained 42 tea plants. The block design was mainly dependent on the tea seedlings available for each line, which were clonally propagated from the original tea trees. Fresh materials (two leaves and a bud) of the first flush shoots of each block were collected in the spring of 2016. The collected samples were withered for 12 h and then used to make Chinese green tea by the method described by Dai *et al*. (2015).

The green tea samples were divided into two groups. One group was directly subjected to sensory analysis. The other group was ground into powder using a tube mill (IKA, Staufen, Germany) for chemical component analysis.

Sensory analysis

The taste quality of green teas was blindly assessed according to the method described by Jing *et al*. (2017). The grading was performed to each of five attributes; appearance, taste, aroma and colour of the infusion, and features of the infused leaves. Each tea sample (3 g) was infused in 150 mL freshly boiled water for 5 min. Tea soups were tasted twice for better comparison. As the taste quality is the main focus of the present work, other feature data were not used here.

Catechin and caffeine analysis

Standard chemicals including caffeine, EGCG, GCG, ECG, EGC, GC, EC and C were purchased from Sigma Chemical Company (St. Louis, MO, USA). The extraction method and HPLC analysis of the green tea samples were performed exactly according to the method described by Wei *et al*. (2015). The total catechin (TC) contents were calculated as the sum of seven individual catechins. The total polyphenols (TP) were determined by the photometric Folin-Ciocalteau assay according to a proposed international standard method (ISO, 1994). Three replications were taken for each sample.

Amino acid analysis

Free amino acids were purchased from Sigma–Aldrich Chemical Co. (St Louis, MO). Pre-column derivatisation of free amino acids was performed using an AccQ-Fluor Reagent Kit. Separation was performed on an HPLC system equipped with a Waters

AccQ Tag reversed-phase HPLC column (150 mm × 3.9 mm, 4 µm) exactly according to the manufacturer's specifications (Yu *et al*., 2014). Briefly, mobile phase A consisted of AccQ Tag eluent A concentrate in deionised water (1:10 v/v), and mobile phase B was 100% ACN. A gradient program was used for the separation of amino acids: 0–0.5 min, linear gradient from 0 to 1% B; 0.5–18 min, linear gradient from 1% to 5% B; 18–19 min, linear gradient from 5% to 9% B; 19–29.5 min, linear gradient from 9% to 17% B; 29.5–33 min, 60% B; 33–36 min, linear gradient from 100% to 0% B. The post-run time was 9 min. The sample injection volume was 10 µL. The flow rate was 1.0 mL/min. The column temperature was set at 37°C. The amino acids were detected at 248 nm and identified by comparison with the retention times and spectra of the standard solutions of amino acids kit and L-theanine. The quantification was done via external calibration curves.

Data analysis

The principal component analysis (PCA), stepwise discriminant analysis (SDA), correlation analysis and evaluation of normality of the taste quality data distribution were performed with SPSS 16.0 statistical software (SPSS Inc., Chicago, IL).

Results

Taste sensory evaluation of tea samples

The taste sensory scores of 78 samples ranged from 85 to 93 and the date followed a normal distribution (one-sample Kolmogorov-Smirnov test, $p = 0.007$). According to the taste scores, the tea samples were classified into three groups including normal tea (score <90, 25 samples), good tea (score ≥90 & <92.5, 47 samples) and perfect tea (score ≥92.5, six samples).

The variations of TP, catechins and caffeine contents between the tea samples

The variations of the chemical components (TP, catechins and caffeine) involved in astringent and bitter tastes are shown in Table 1. There were significant differences in the TP, catechins and caffeine contents between the 78 tea samples. TP and TC ranged from 148.7 to 238.0 mg g^{-1} and 90.5 to 159.8 mg g^{-1} respectively, with a mean of 182.2 and 125.8 mg g^{-1}. TC constituted approximately 70% of TP, consistent with the previous findings (Kerio *et al*., 2013). However, the coefficients of variation (CV) were relatively lower than those of catechin individuals. In the case of catechin individuals, EGCG was the most abundant, followed by ECG and EGC. The GCG content was the lowest of seven detected catechins. The caffeine content varied from 27.2 to 45.8 mg g^{-1}, with a mean of 34.2 mg g^{-1}. The CVs of TP, catechins and caffeine in the samples were generally negatively correlated with their contents, except for ECG.

Additional correlation analysis indicated that these chemical components did not significantly correlate with the taste quality scores. Most of the components showed negative correlations, indicating that chemical components involved in astringent and bitter tastes may not facilitate the improvement of taste quality.

Table 1. The variations of catechins, caffeine and total polyphenol (TP) contents, and their correlation with taste quality scores among tea samples.

	Max (mg g^{-1})	Min (mg g^{-1})	Mean (mg g^{-1})	CV %	Correlation coefficient with taste quality
GC	2.61	0.00	1.25	40.74	-0.118
EGC	21.07	4.56	10.22	35.92	-0.017
C	5.85	0.65	3.56	29.69	0.016
EC	12.87	0.14	7.68	36.89	-0.082
EGCG	98.81	50.17	74.75	14.27	-0.17
GCG	1.55	0.29	0.83	34.99	-0.194
ECG	36.59	18.06	27.49	12.99	-0.211
TC	159.83	90.53	125.78	11.21	-0.209
TP	238.04	148.68	182.82	10.11	-0.074
Caffeine	45.75	27.22	34.22	10.81	-0.074

The variations of free amino acid contents between the tea samples

A total of 19 free amino acids were identified by HPLC (Table 2). The total amino acid contents varied from 30.8 to 77.0 mg g^{-1}, with an average of 45.5 mg g^{-1}. Theanine was the most abundant free amino acid, accounting for nearly half of the total amino acid (TAA) contents, followed by aspartic acid (Asp) and glutamic acid (Glu). Correlation analysis showed that the total amino acid content was significantly and positively correlated with taste quality. However, different amino acids had different effects. For example, theanine, glutamine (Gln), Glu and arginine (Arg) were significantly and positively correlated with the taste quality scores. While methionine (Met), isoleucine (Ile), phenylalanine (Phe), leucine (Leu), threonine (Thr) and lysine (Lys) were significantly and negatively correlated with the taste quality scores. Therefore, specific amino acid components and not TAA should be considered as the basis of the effects on taste.

Table 2. The variations of free amino acid contents and their correlation with taste quality scores among tea samples.

	Max (mg g^{-1})	Min (mg g^{-1})	Mean (mg g^{-1})	CV %	Correlation coefficient with taste quality
Asp	5.30	2.21	3.75	19.03	-0.228
Ser	7.88	0.92	2.41	38.58	0.229
Glu	7.84	1.67	3.46	33.83	0.283 *
His	0.30	0.17	0.21	11.60	-0.116
Gln	3.62	0.58	1.40	40.60	0.385 **
Arg	7.98	1.17	3.09	40.32	0.234 *
Thr	1.24	0.40	0.82	18.78	-0.279 *
Ala	1.60	0.19	0.83	19.96	0.159
Pro	0.62	0.30	0.45	11.92	-0.009
GABA	1.75	0.46	1.04	25.99	-0.104
Theanine	43.13	12.65	22.30	22.65	0.323 **
Cys	0.61	0.15	0.36	25.91	0.052
Tyr	0.40	0.12	0.22	21.01	-0.021
Val	2.56	0.56	1.38	32.62	0.013
Met	0.71	0.30	0.51	15.97	-0.332 **
Lys	2.19	0.36	0.89	30.47	-0.277 *
Ile	1.18	0.19	0.51	28.28	-0.317 **
Leu	1.76	0.27	0.77	27.47	-0.296 *
Phe	2.51	0.16	1.09	40.15	-0.314 **
TAA	77.00	30.83	45.48	15.00	0.249 *

Classification of the taste quality of the tea samples by PCA and SDA

PCA was used to further explore correlations between the chemical components in the tea samples and taste quality. A total of eight principal components (PCs) were identified explaining 76.0% of the total variance. Among the components, PC1 and PC2 explained 21.3% and 16.9% of the total variance respectively. The PC1 vs. PC2 score and loading plots are presented in Fig. 3. Loading plots generated based on PCA indicated that amino acids were mainly distributed in the first and second quadrants, while catechins and caffeine were distributed in the fourth quadrant (Fig. 3B). The score plot showed that the majority of the normal, good and perfect tea samples were overlapping (Fig. 3A), thus making any classification unsatisfactory.

Then, SDA was used to classify the taste quality of the tea samples by their chemical components (Table 3). A total of 87.2% of the tea samples were correctly classified, indicating the feasibility of the stepwise discriminant analysis. Relatively high accuracy rates were achieved in the normal (96%) and good (85.1%) tea groups, while low accuracy was observed in the perfect (66.7%) tea group. This may be due to the low number of samples in perfect tea group (only six samples).

Table 3. Classification of tea samples according to taste quality by stepwise discriminant analysis.

		Classified			Total
		Normal	Good	Perfect	
Count	Normal	24	1	0	25
	Good	5	40	2	47
	Perfect	0	2	4	6
%	Normal	96	0	4	100
	Good	10.6	85.1	4.3	100
	Perfect	0	33.3	66.7	100

A summary of the canonical discriminant analysis indicated that function 1 and function 2 explained 60.9% and 39.1% of the total variance respectively. Additional canonical discriminant functions and a structure matrix of relative function coefficients are shown in Fig. 1. Most of the tea samples can be classified into three groups according to their taste quality (Fig. 1A) and this classification was much better than that of PCA.

Structure matrix analysis of relative function coefficients showed that amino acids, including Gln, theanine, threonine (Thr), methionine (Met), phenylalanine (Phe), isoleucine (Ile), leucine (Leu) and Glu had significantly higher effects than catechins and caffeine (Fig. 1B). It is interesting to note that all amino acids that significantly and positively correlated with taste quality (theanine, Gln, Glu and Arg) were distributed in the third quadrant (Table 2, Fig. 1B), indicating that these chemical components should be strengthened in tea breeding.

Distinct metabolism in various tea samples

To further understand the important chemical components in tea, a metabolic pathway was proposed with global variations of metabolites identified in the present work. A schematic diagram (Fig. 2) was used based on the metabolic pathways reported by Ji *et al*. (2017). According to the taste quality changes, it was easier to find out the changing patterns of each metabolite. Levels of caffeine and catechins, except for ECG, in the perfect tea group were slightly lower but were not significantly different from those in the normal or good tea groups ($p > 0.05$). However, the amino acid (Phe) directly involved in catechin biosynthesis showed a clear decreasing trend from the normal to perfect tea groups. These results suggest that excessive emphasis on catechin contents may reduce the taste quality of green tea. It was interesting to find that those amino acids that positively correlated with taste quality (theanine, Gln, Glu and Arg) were closely associated with the GS/GOGAT cycle, indicating that the GS/GOGAT cycle is important for future green tea breeding.

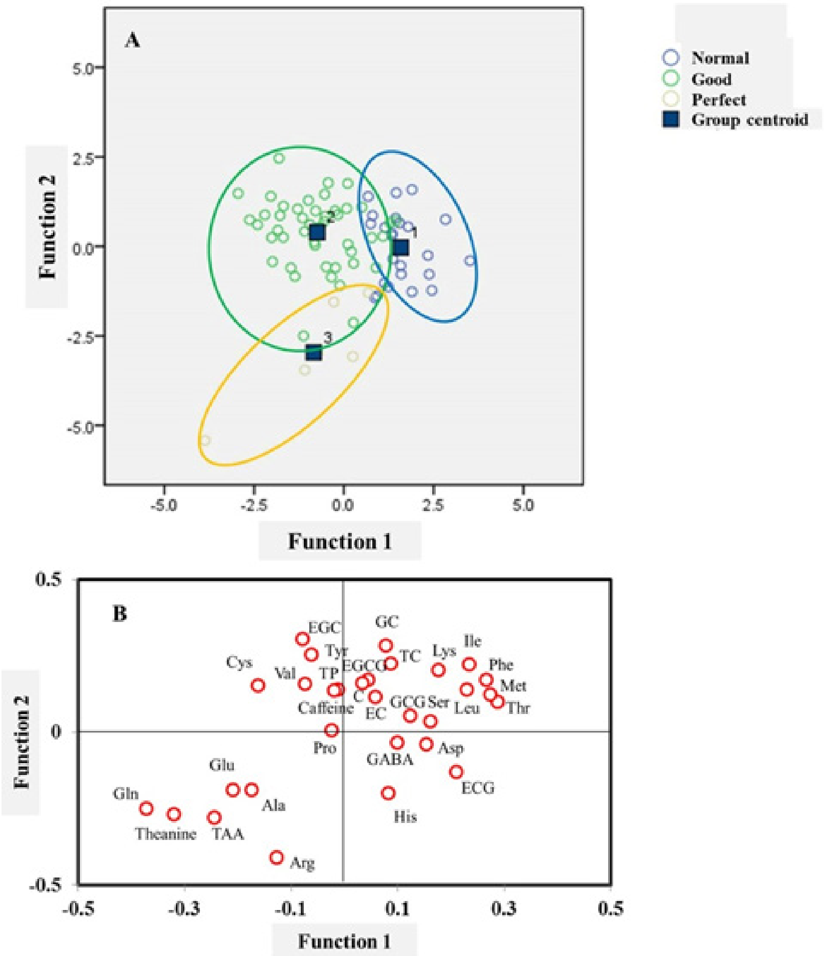

Fig. 1. Canonical discriminant functions and structure matrix of relative function coefficients of tea samples by stepwise discriminant analysis. (A) canonical discriminant functions; and (B) structure matrix of relative function coefficients.

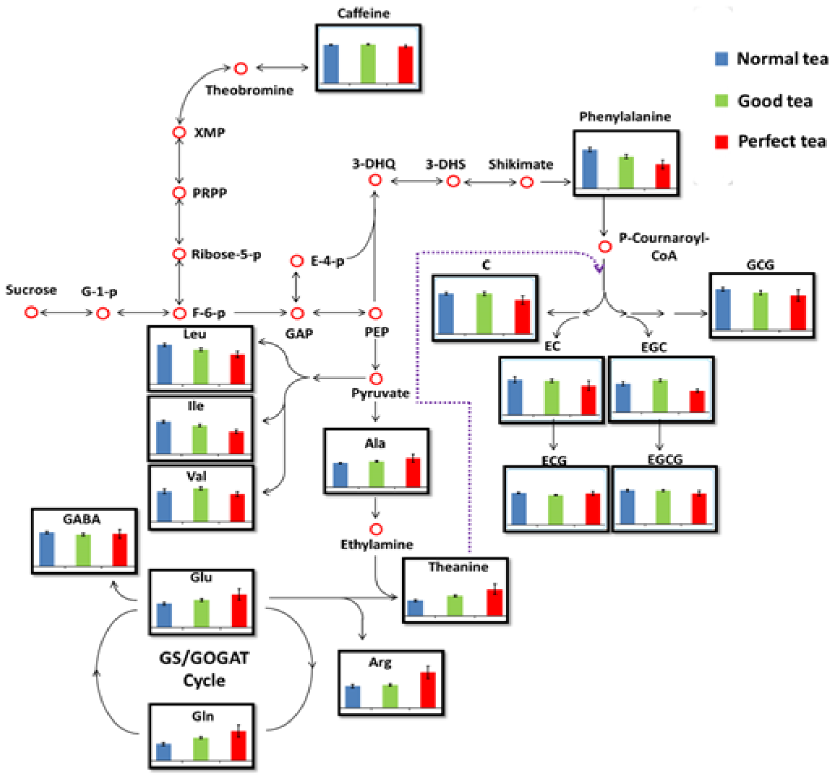

Fig. 2. Schematic diagram of the metabolic pathway based on the taste qualities of tea lines (cultivars).

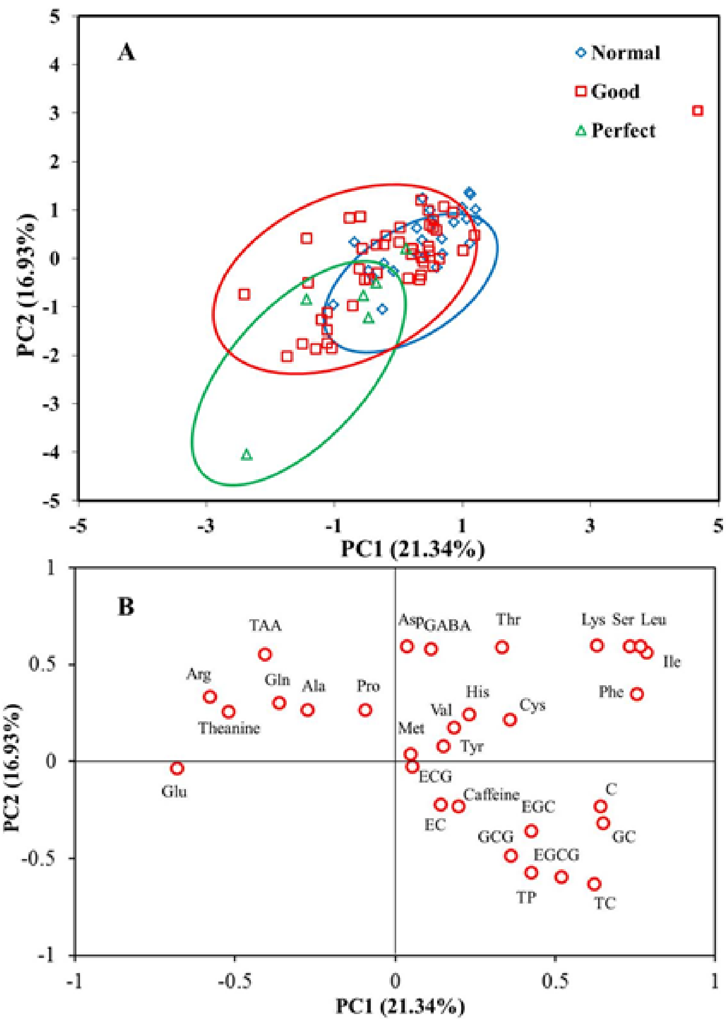

Fig. 3. PC1 vs. PC2 score and loading plots of tea samples by principal component analysis. A, PC1 vs. PC2 score plot; B, PC1 vs. PC2 loading plot.

Discussions

Polyphenolic compounds, caffeine and amino acids are the major chemical components in green tea. Polyphenolic compounds such as catechins are mainly responsible for astringent taste, while caffeine and amino acids provide bitter and umami tastes. However, bitterness and astringency in green tea are generally not preferred by the customers. For example, teas plucked in summer and autumn are more bitter and astringent than spring teas, causing a substantial decrease in their economic value (Dai *et al*., 2015). Lin *et al*. (2014) reported that green tea infusions with higher sensory ratings were less astringent and bitter. By contrast, umami taste can improve taste quality and is closely correlated with high levels of amino acids (Dai *et al*., 2015; Zhu *et al*., 2017). In the present work, only several key amino acids were positively and significantly correlated with taste quality, consistent with the previous findings, thus indicating the importance of amino acids. Moreover, the amino acid components had different or even opposite effects on taste quality, suggesting that the key amino acids rather than the total amino acids should be strengthened in the future tea breeding.

Theanine is the most abundant amino acid in the tea leaves. It is a non-proteinaceous amino acid and contributes to the favourable taste of tea according to previous studies (Vuong *et al*., 2011). Recent studies also found that theanine acts as a source of nitrogen and as a starting point for the synthesis of the carbon skeleton of compounds in the tea plants (Feldheim *et al*., 2010). Therefore, nitrogen application prior to harvest improves tea quality and is closely correlated with an increase in the theanine content in tea (Watanabe, 1995). In the present work, correlation and SDA analyses demonstrated that theanine was a key chemical component of tea taste (Table 2 and Fig. 1B) suggesting that it can be considered an indicator of good taste in future tea breeding.

Furthermore, both Gln and Glu were the key chemical components influencing taste quality (Table 2 and Fig. 1B). Yu *et al*. (2014) investigated seven types of ready-to-drink tea and considered that the contribution of Glu towards umami taste was even higher than the contribution of theanine. It should be noted that both amino acids are important precursors for theanine biosynthesis (Vuong *et al*., 2011). Theanine biosynthesis predominantly involves the so-called GS/GOGAT cycle (Liu *et al*., 2017). Glutamine synthetase (GS) fixes ammonium on a Glu molecule to form Gln, which reacts subsequently with 2-oxoglutarate by the glutamine 2-oxoglutarate amino transferase (GOGAT) to form two molecules of Glu. One Glu molecule goes back to the cycle and another molecular is converted into theanine by theanine synthetase (TS) (Okada *et al*., 2006). The results obtained in the present work reveal that the theanine biosynthetic pathway and especially GS/GOGAT cycle play an important role in the taste quality of tea. Arg is another important chemical component potentially influencing tea taste. Arg is a semi-essential amino acid in plants biosynthesised from Glu (Wu *et al*., 2012). Hence, its content is closely associated with the GS/GOGAT cycle. Arg was reported to alter taste perception including sucrose perception, umami taste, saltiness and caffeine bitterness (Melis and Tomassini Barbarossa, 2017). Therefore, Arg supplementation was considered a strategic tool to modify taste responses. In the present work, Arg was positively and significantly correlated with taste quality, although the correlation coefficient was lower than those of theanine, Gln and Glu (Table 2). The role of Arg in taste quality still requires additional research.

Conclusion

In summary, the effects of major chemical components, including TP, catechins, caffeine and amino acids on taste quality of 78 green tea samples have been investigated. A total of 87.2% of tea samples were correctly classified according to their chemical components by stepwise discriminant analysis. Four major amino acids [theanine ($r = 0.323^{**}$), Gln ($r = 0.385^{**}$), Glu ($r = 0.283^{*}$) and Arg ($r = 0.234^{*}$)] were positively and significantly correlated with taste quality, indicating their importance for tea taste. These four amino acids are directly associated with or derived from the products of the GS/GOGAT cycle (Fig. 2), indicating that the genes involved in this pathway should be strengthened for future green tea breeding.

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