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Characteristic amino acids in tea leaves as quality indicator for the evaluation of Wuyi Rock Tea in different culturing regions

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Summary

Free amino acid compositions in Wuyi Rock Tea leaves from Yu (authentic rock region), Guiyan (semi-authentic rock region) and Qishan (ordinary region) tea plantations were analyzed. Results showed that contents of 18 free amino acids were 1.6-2.0 times higher in Yu and Guiyan than that in Qishan. The theanine contents reached to 17-20 mg g^{-1} in Yu and Guiyan, while it was less than 10 mg g^{-1} in Qishan. Hierarchical cluster analysis and principal component analysis were effective in distinguishing Rock Tea from different regions. The ratios of theanine, sweet and umami amino acids were 8%, 5% and 6% higher, respectively in Yu than that in Qishan. Sensory evaluation score were positively correlated with the ratios of theanine, sweet and umami amino acids ($P < 0.05$ or $P < 0.01$). Our results highlight that the favourite characteristic amino acids are dominant contributors to sweet aftertaste of Rock Tea.

Keywords: Authentic rock region; sweet amino acids; umami amino acids; theanine; bitter amino acids; sweet aftertaste

Introduction

Wuyi Rock Tea is one of China's top ten teas, named specially after its origin from the rocky mountain region of Wuyishan County, Fujian Province of China. The authentic Wuyi Rock Tea has a unique flavour termed 'Rock Flavour' (Yanyun in Chinese) (GB/T 14487-2008) – a rock characteristic aroma and lasting sweet aftertaste, which should be significantly different from other teas. Rock Flavour is also approved by government as a geographical indication of Wuyi Rock Tea. Traditionally, local farmers use the valley, coves, pits, curve and flat slopes, making staircases with stone, masonry stone blocks, embankments, and constructing "potted style" tea plantations along mountains. These tea plantations are known as 'the authentic rock regions' because the tea products from these regions are the high-grade Rock Tea. However, Rock Tea produced from the authentic rock regions could far from satisfy consumer demand, and so farmers reclaimed a large number of tea plantations in the non-rocky regions, and even planted tea in the plains farmland. To distinguish different quality of Rock Tea, in the folk custom of Wuyi County, the tea producing from the core rock regions with high-grade quality is called as 'authentic Rock Tea' (Zhengyan Cha in Chinese) (Fig. 1), those from the regions located around the core rock regions with middle-grade quality as 'semi-authentic Rock Tea' (Banyan Cha in Chinese), and those from the ordinary regions as 'continent tea' (Zhou Cha in Chinese) (Ye et al., 2004; Chen et al., 2016). The fresh leaves of 'authentic Rock Tea' sells for > 400 yuan (RMB) kg^{-1}, while of 'continent tea' sells only for < 20 yuan (RMB) kg^{-1}, less than 5% of the authentic Rock Tea.

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Amino acids, as an most important contributor to tea infusion taste, are usually used as evaluation index for tea quality (Nakagawa, 1975; Nagata et al., 1986; Nishimuraa et al., 1988; Harbowy et al., 1997; Hayashi et al., 2008; Akitomi et al., 2013; Chen et al., 2016), characters of tea cultivars and types (Horanni et al., 2013; Huo et al., 2014), quality control of tea processes and growth conditions (Tan et al., 2016; Zhu et al., 2016), levels of tea leaves with age, plucking seasons and positions (Nakagawa, 1970; Xu et al., 2012; Liu et al., 2016), as well as identification of tea geographical origins (He et al., 2009; Song et al., 2012; Lee et al., 2015). Alcazar et al. (2007) used the differentiation of free amino acids content as chemometric descriptor for classification of green, white, black, Oolong, and Pu-erh teas. Miyauchi et al. (2014) reported that in high-quality Japanese green tea (Tencha), glutamine, arginine, and theanine are the main constituent amino acids, and could be as an indicator to evaluate the quality of indoor-planted tea. Feng et al. (2014) showed that in albino tea leaves, an increase in total amino acid content combined with a decrease in catechins and caffeine reduced its astringency and bitterness, thus enhancing the umami taste, which is one of the indicators of high quality in tea. The amino acid content has a positive correlation with the quality of green tea, Oolong tea, and Pu-erh tea (Nakagawa, 1970; Liang

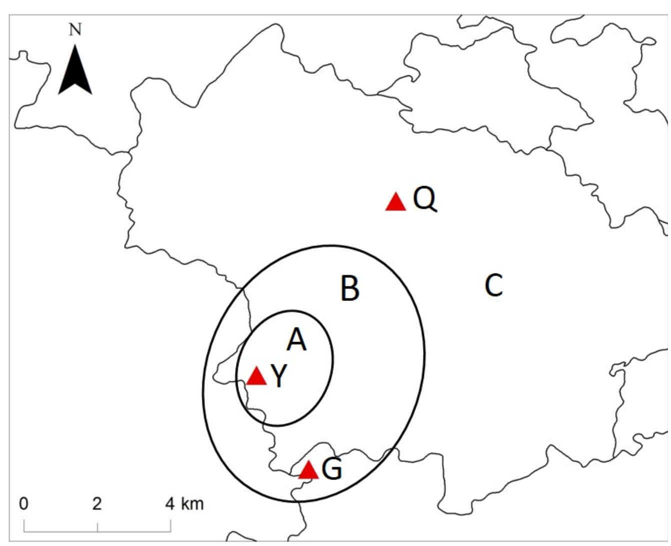

Fig. 1: Classification of rock tea region and the selected tea plantations. A – authentic rock region; B – semi-authentic rock region; C – ordinary region; Y –Yu, an authentic rock tea plantation located in latitude 27°38′42″–45″ and longitude 117°56′38″–44″; G – Guiyan, a semi-authentic rock tea plantation located in latitude 27°36′26″–34″ and longitude 117°57′52″–58′1″; Q – Qishan, an ordinary tea plantation, located in latitude 27°17′32″–39″ and longitude 117°54′41″–56″.

et al., 2005; WANG et al., 2009, 2010; XU et al., 2012). But so far, few study refers to the differentiation of free amino acids content for Wuyi Rock Tea cultured in different regions. No report refers to the relationship between sweet aftertaste and characteristic amino acids in Wuyi Rock Tea. In this paper, we selected three representative tea cultivars of Wuyi Rock Tea which planted in the authentic rock region, semi-authentic rock region, and ordinary region, respectively. Contents of free amino acids in fresh tea leaves were analyzed, and the contribution of some characteristic amino acids to taste feature of Wuyi Rock Tea were further revealed.

Materials and methods

Sampling sites and processing

Three plantations qualifications of which have been recognized by the government were chosen as sampling sites, they are Yu plantation as a representative of authentic rock regions, Guiyan plantation as a representative of semi-authentic rock regions, and Qishan plantation as a representative of ordinary regions (Fig. 1). Three most representative cultivars of Wuyi Rock Tea – Dahongpao, Shuixian and Rougui – grown in these plantations as the study material.

The sampling time was the tea plucking season in May 2014. The tea plants were chosen from each tea cultivar in each plantation using an equidistant sampling method, in which the length of the tea plant rows were divided into 5 equal sections, and then leaf buds with 3 leaves were randomly picked from each section. The fresh tea leaves were over-dried at 105 °C for 30 min to inactivate quickly enzymes, and then dried at 80 °C for 48 h, grounded and sieved by a 60-mesh. The tea powder was used to determine contents of free amino acids subsequently. The tea products of the three tea cultivars from these plantations were used for sensory evaluation.

Determination of the free amino acids composition in tea leaves

The composition of free amino acids was measured according to the National Standards of the People's Republic of China (GB/T 5009. 124-2003), using a Hitachi L-8900 automatic amino acid analyzer and using external standards of mixed amino acids as calibrators. In brief, 3 g tea powder was placed in a 500-mL conical flask, 450 mL boiling distilled water was added, and the flask was placed in a boiling water bath for extraction for 45 min (shaking once every 5 min). After the completion of extraction, while still hot, the tea was filtered under reduced pressure, the residue was washed with a small amount of hot distilled water 3 times, and after cooling the filtrate was transferred into a 500-mL volumetric flask marked, and shaken to homogenize. There were three replicates for all the tea samples.

Theanine was assayed according to the National Standards of the People's Republic of China (GB/T 23193, 2008) using a Sykam S-433D automatic amino acid analyzer, and calibrating with a theanine external standard. Samples were prepared by taking 100 mg of tea powder, adding 10 mL deionized water, placing in a 90 °C water bath for 15 min, centrifuging at 10,000 rpm for 10 min, mixing 1:4 with a pH 2.2 dilution solution, filtering, and then loading the sample. Instrument conditions were as follows: flow rate 0.25 mL min^{-1}, pressure 35 bar, reactor temperature is 130 °C, injection volume 10 µL, and detection wavelength 338 nm. There were three replicates for all the tea samples.

Sensory evaluation

The quality of tea products from the above plantations were assessed by a tea tasting panel consisting of five professional panelists, according to standardised procedure of 'Methodology of sensory evaluation of tea', the National Standards of the People's Republic of China (GB/T 23776-2009). The total score of 100 was summed of five quality features for oolong tea, of which 20% for the appearance of dry tea, 30% for the tea aroma, 35% for the taste, 10% for the infused leaves, and 5% for the liquor colour (GB/T 23776-2009). The evaluation procedure was as follows. Five gram tea was infused in a 110 mL tea tasting porcelain cup with freshly boiled water, covered for 1 min, smelling the flavour of cup cover for tea aroma assessment, for 2 min the liquor was poured into a tea bowl for assessment of liquor colour, taste and infused leaf aroma; secondly, refilled freshly boiled water to cup, covered for 2 min for tea aroma assessment, and for 3 min the liquor was poured into a tea bowl for assessment of liquor colour, taste and infused leaf aroma again; thirdly, refilled freshly boiled water to cup, covered for 3 min for tea aroma assessment, and for 5 min the liquor was poured into a tea bowl for assessment of liquor colour, taste and infused leaf aroma. Finally, the tea leaves were poured out for assessment of infused leaf. The each tea feature was examined and scored by each of the five panelists independently. The sum total of the five quality features was expressed as the total quality score (TQS).

Data analysis

All experimental data were presented as mean ± standard error (SE). They were analysed using a one-way analysis of variance (ANOVA), and followed by the least significant difference (LSD). Hierarchical cluster analysis (HCA), Principal component analysis (PCA) and correlative analysis were all conducted with SPSS 20.0 software.

Results and discussion

The free amino acids composition in fresh tea leaves

The contents of 18 free amino acid compositions were showed in Tab. 1. There were significant differences among tea leaves from the 3 tea plantations in each of the 18 amino acid content and the total content ($P < 0.05$), except valine of Dahongpao and glycine of Rougui between Yu and Qishan. The total free amino acids content of three tea cultivars was about 20 mg g^{-1} in Qishan, while they were about 30-40 mg g^{-1} in Guiyan and Yuchayuan. It was 1.6-1.7 times higher in Yu and 1.9-2.0 times higher in Guiyan than that in Qishan. The theanine contents of three tea cultivars reached to 17-20 mg g^{-1} in Guiyan and Yu tea plantations, while it was less than 10 mg g^{-1} in Qishan, only about half of that in Yu and Guiyan (Tab. 1). High level of amino acids is essential for high quality of tea products (HARBOWY et al., 1997; MIYAUCHI et al., 2014). NAKAGAWA (1970, 1975) found that about 70% of brothy taste and sweetness in green tea was due to amino acids, and suggested that the higher the content of amino acids the better is the taste. The content of amino acids has been widely used as quality index in various teas (HARBOWY et al., 1997; ALCAZAR et al., 2007; HAYASHI et al., 2008; CHEN et al., 2011; XU et al., 2012; FENG et al., 2014; MIYAUCHI et al., 2014; CHEN et al., 2016; ZHU et al., 2016). The results indicated the quality of tea leaves was higher in Yu and Guiyan than in Qishan, based on the contents of total free amino acids and theanine.

Hierarchical cluster analysis (HCA)

HCA was applied on the 18 free amino acids compositions to group the cultured regions. The results showed that the 9 tea samples could be classified 3 types correctly according to their cultured regions (Fig. 2). The tea samples between Guiyan and Yu tea plantations had similar characteristic based on the 18 free amino acids compositions. The results indicated the free amino acids compositions combined with HCA could distinguish clearly Wuyi Rock Tea from different cultured regions.

Tab. 1: Contents of free amino acids in tea leaves of the different plantations (mg g^{-1})

Amino acid	Dahongpao			Shuixian			Rougui		
	Qishan	Guiyan	Yu	Qishan	Guiyan	Yu	Qishan	Guiyan	Yu
Threonine	0.51±0.01c	0.88±0.02a	0.63±0.02b	0.42±0.01c	0.87±0.03a	0.58±0.02b	0.47±0.02c	0.94±0.03a	0.64±0.03b
Valine	0.80±0.01b	1.29±0.02a	0.73±0.02b	0.69±0.02c	1.25±0.04a	0.95±0.02b	0.77±0.04c	1.28±0.07a	0.99±0.05b
Methionine	0.29±0.01c	0.70±0.02a	0.45±0.03b	0.22±0.01c	0.68±0.04a	0.40±0.03b	0.25±0.03c	0.73±0.05a	0.44±0.02b
Isoleucine	0.72±0.02c	1.21±0.03a	0.95±0.08b	0.62±0.02c	1.20±0.05a	0.89±0.02b	0.69±0.07c	1.26±0.04a	1.00±0.03b
Leucine	0.63±0.02c	1.09±0.02a	0.86±0.01b	0.50±0.02c	1.03±0.05a	0.69±0.09b	0.60±0.03c	1.08±0.05a	0.82±0.02b
Phenylalanine	0.72±0.02c	1.22±0.01a	0.94±0.01b	0.64±0.03c	1.14±0.04a	0.82±0.02b	0.65±0.07c	1.19±0.06a	0.87±0.04b
Lysine	0.53±0.01c	1.01±0.01a	0.74±0.02b	0.49±0.02c	0.98±0.04a	0.68±0.03b	0.54±0.02c	1.05±0.04a	0.74±0.04b
Asparagine	0.89±0.02c	1.39±0.02a	1.12±0.02b	0.84±0.02c	1.35±0.09a	1.08±0.03b	0.90±0.04c	1.34±0.12a	1.09±0.05b
Serine	0.75±0.03c	1.27±0.03a	1.03±0.02b	0.69±0.02c	1.21±0.04a	0.92±0.01b	0.75±0.04c	1.28±0.10a	0.98±0.06b
Glutamic acid	1.16±0.04c	1.72±0.01a	1.42±0.03b	1.04±0.03c	1.58±0.06a	1.27±0.04b	1.08±0.06c	1.63±0.10a	1.38±0.04b
Glycine	0.61±0.03c	1.03±0.02a	0.75±0.01b	0.50±0.03c	0.94±0.08a	0.66±0.02b	0.61±0.07b	1.04±0.05a	0.74±0.03b
Alanine	0.63±0.03c	1.26±0.03a	0.98±0.01b	0.65±0.04c	1.19±0.03a	0.89±0.02b	0.71±0.04c	1.24±0.06a	0.93±0.05b
Cysteine	0.44±0.03c	0.89±0.02a	0.60±0.01b	0.36±0.04c	0.85±0.03a	0.54±0.02b	0.43±0.03c	0.90±0.04a	0.60±0.04b
Tyrosine	0.65±0.03c	1.21±0.02a	0.93±0.02b	0.57±0.04c	1.18±0.03a	0.88±0.01b	0.63±0.04c	1.20±0.05a	0.90±0.04b
Histidine	0.26±0.02c	0.82±0.01a	0.55±0.02b	0.22±0.02c	0.73±0.03a	0.43±0.02b	0.27±0.02c	0.80±0.05a	0.51±0.03b
Arginine	0.82±0.03c	1.39±0.02a	1.09±0.02b	0.79±0.01c	1.32±0.04a	1.01±0.02b	0.81±0.03c	1.37±0.06a	1.05±0.04b
Proline	0.33±0.01c	0.81±0.02a	0.55±0.02b	0.28±0.03c	0.78±0.03a	0.46±0.01b	0.32±0.04c	0.84±0.06a	0.53±0.05b
Theanine	9.19±0.20c	17.85±0.46a	16.84±0.17b	9.62±0.71c	20.01±0.11a	18.60±0.34b	9.95±0.32c	20.57±0.33a	18.26±0.35b
Total	19.92±0.47c	37.03±0.73a	31.16±0.44b	19.13±1.07c	38.29±0.77a	31.74±0.67b	20.41±0.88c	39.74±1.32a	32.46±0.95b

Means standard error (±SE) from three replications for each determination is shown. The lowercase letters represent a significant level at $P < 0.05$.

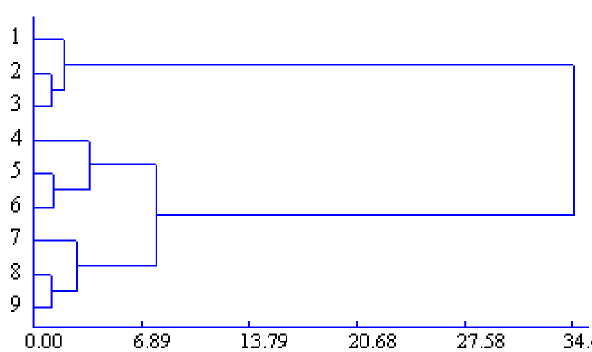

Fig. 2: Dendrogram of the 9 tea samples in different tea plantations by Hierarchical cluster analysis (HCA) according to the 18 free amino acids compositions. The 1, 2 and 3 represent Dahongpao, Shuixian, and Rougui cultivars, respectively in Qishan plantations, 4, 5 and 6 represent Dahongpao, Shuixian, and Rougui cultivars, respectively in Guiyan plantations, and 7, 8 and 9 represent Dahongpao, Shuixian, and Rougui cultivars, respectively in Yu plantations.

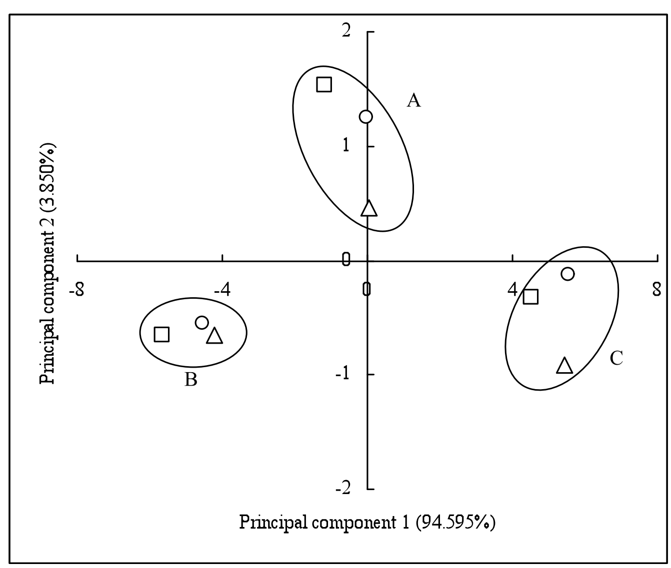

Fig. 3: Loadings for principal component analysis (PCA) derived from the 18 free amino acid contents of tea leaves from the different plantations. A: Yu. B: Qishan. C: Guiyan. Δ: Dahongpao. □: Shuixiang. ○: Rougui.

Principal component analysis (PCA)

The contents of 18 amino acids components were imported to soft ware for PCA analysis (Fig. 3). The results showed that three sampling plantations dispersed in different quadrants and each group represented independently one type of the 3 tea plantations. The first two principal components, PC1 (94.595%) and PC2 (3.850%), explained 98.445% of the total system variance, which means that the first two principal components can presents most of discrimination of Wuyi Rock Tea in the three plantations. The results indicated the amino acids components could be used to distinguish from tea leaves quality of different tea plantations.

Characteristic amino acids profile in tea fresh leaves

Each amino acid has a different taste. For example, glycine and alanine, because of a small molecular chain, can bind taste receptors and produce considerably strong sweetness, while amino acids with a branched chain, such as leucine, isoleucine, and valine, are particularly bitter taste (NISHIMURAA et al., 1988; SCHARBERT et al., 2005; AKITOMI et al., 2013). MATOBA et al. (1972) study indicated that the more hydrophobic amino acids, the stronger the bitterness. The dissolution of hydrophilic amino acids in tea infusion is much faster than that of hydrophobic amino acids, and within 15 min theanine comprises about 30% of the free amino acids in tea infusion (KOCADAĞLI et al., 2012). It was suggested that theanine could be synthesized from glutamic acid and ethylamine, and theanine and glutamate are the major contributors to the umami taste of green tea (FELDHEIM et al., 1986; SCHARBERT et al., 2005; KANEKO et al., 2006; DENG et al., 2008).

On in-depth analysis of these special characteristics of amino acids, we found that the percentage of hydrophilic amino acids (threonine, lysine, serine, glycine, cysteine, tyrosine, histidine, arginine, asparagine, glutamic acid, and theanine) to the total free amino acids in tea leaves were 82-84% in Yu tea plantation, and 79-81% in Guiyan and Qishan, respectively, while the percentage of hydropholic amino acids (alanine, valine, leucine, isoleucine, proline, phenylalanine, and methionine) were 16.0-17.5% in Yu tea plantation, and 19.0-20.7% in Guiyan and Qishan, respectively (Fig. 4). Further, the percentage of sweet amino acids (glutamic acid, alanine, glycine, and theanine) were 64.2-67.5% in Yu tea plantation, 58.2-62.0% in Guiyan and Qishan, respectively, was about 5% higher in the authentic rock region (Yuchayuan) than that in the ordinary region (Qishan). While the percentage of bitter amino acids (valine, leucine, and isoleucine) were 8.0-8.7% in Yu tea plantation, 9.1-9.7% in Guiyan, and 9.4-10.8% in Qishan, respectively, was about 2% lower in the authentic rock region (Yuchayuan) than that in the ordinary region (Qishan) (Fig. 4). And more, the proportion of umami amino acids (glutamic acid and theanine) were 58.6-62.6% in Yu tea plantation, 52.8-56.4% in Guiyan, and 51.9-55.7% in Qishan, respectively, was about 6% higher in the authentic rock region (Yu) than that in the ordinary region (Qishan) (Fig. 4). All proportions of those favourite amino acids in tea leaves was with the order of Yu > Guiyan ≈ Qishan. These data further support the sweet aftertaste is the characteristic of high-grade Rock Tea.

Theanine is a unique amino acid in tea plants, which is a key dedicator to the umami and sweet tastes of green tea (NAKAGAWA, 1975; HAYASHI et al., 2008; KURIHARA, 2015). The umami taste and sweetness in green tea was highly positively correlated with theanine concentration (YIN et al., 2014). Theanine can reduce the bitterness and astringency in tea (HAYASHI et al., 2013; YIN et al., 2014). As a predominant amino acid present in high-grade tea and usually accounts for more than a half of the total amino acids content, theanine has the greatest impact of all the amino acids on

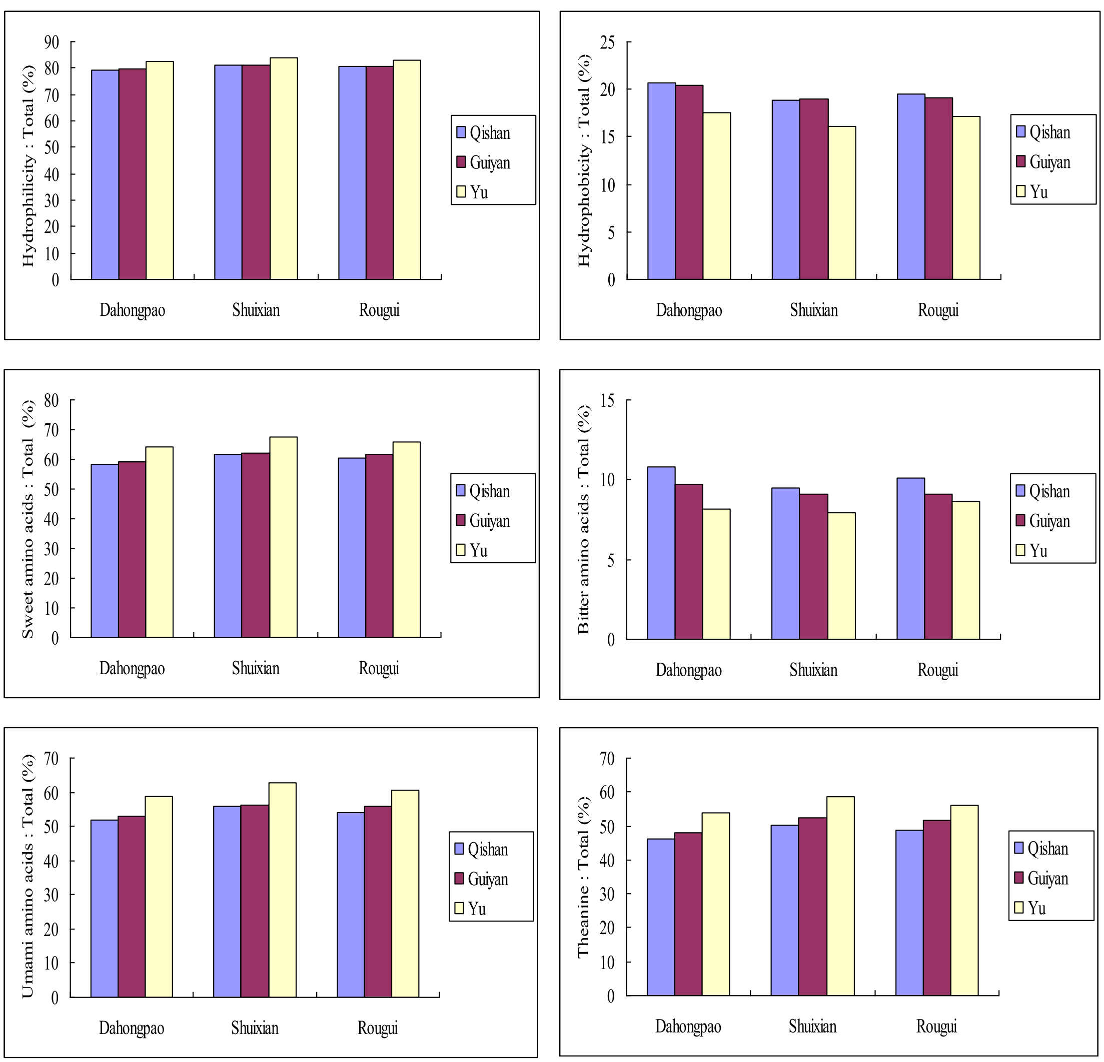

Fig. 4: The percentage of characteristic amino acids in tea leaves of the different plantations. Hydrophilic amino acids including threonine, lysine, serine, glycine, cysteine, tyrosine, histidine, arginine, asparagine, glutamic acid, and theanine. Hydropholic amino acids including alanine, valine, leucine, isoleucine, proline, phenylalanine, and methionine. Sweet amino acids including glutamic acid, alanine, glycine, and theanine. Bitter amino acids including valine, leucine, and isoleucine. Umami amino acids including glutamic acid and theanine.

the tea's natural quality (HORANNI et al., 2013; MIYAUCHI et al., 2014; KURIHARA, 2015). Our results showed that the percentage of theanine to the total amount of the 18 free amino acids in tea leaves were 54.0-58.6% in Yuchayuan, 48.2-52.3% in Guiyan, and 46.1-50.3% in Qishan (Fig. 4). The ratio of theanine to the 18 free amino acids contents in tea leaves was 8% higher in the authentic rock region (Yu) than that in the ordinary region (Qishan). This data reflects the real differential of quality between 'authentic Rock Tea' and 'continent tea'. We suggested that rather than net contents, the ratio of those favourite amino acids such as sweet amino acids and umami amino acids, as well as theanine, may a solid indicator to evaluate quality of Wuyi Rock Tea.

Sensory evaluation

In China, the taste is the most important quality index in oolong tea, account for 35% of the five quality features (GB/T 23776-2009). The sensory assessment results shown that the total quality scores (TQS) were Yu > Guiyan > Qishan in all the three tea cultivars (Tab. 2), and had significant different in the three different plantations ($P < 0.05$), except the Dahongpao between Guiyan and Qishan. The highest TQS for three tea cultivars from Yu tea plantation was in good agreement with its reputation and high quality of genuine Rock Tea. Further, no significant differences were observed in the three different plantations in the appearance of dry tea, the infused leaves, and the liquor colour. However, the scores for the taste were Yu > Guiyan > Qishan in all the three tea cultivars, and had significant different in the three different plantations ($P < 0.05$). With regard to the attributes of the taste in TQS, we suggested that the taste is the key factor for Wuyi Rock Tea and is a distinct index of genuine Rock tea plantation distinguishing from other plantations.

Correlation analysis between characteristic amino acids and sensory evaluation

Correlation analysis was conducted to investigate the relationship between the each of five quality features, TQS and the characteristic amino acids. As shown in Tab. 3, most of the characteristic amino acids in the three tea leaves have no significant correlation with the appearance of dry tea, infused leaves, and liquor colour (except liquor colour of Dahongpao cultivar). However, TQS of all the three cultivars were significantly positively correlated to theanine, sweet amino acids, and umami amino acids, and were significantly negatively correlated to bitter amino acids ($P < 0.01$ or $P < 0.05$). These results revealed that the characteristic amino acids are more responsible for Wuyi Rock Tea quality than the appearance, infused leave and liquor colour. Correspondingly, the taste feature of three cultivars were significantly positively correlated to hydrophilicity amino acids, theanine, sweet amino acids, and umami amino acids, and were significantly negatively correlated to hydrophobicity amino and bitter amino acids ($P < 0.01$ or $P < 0.05$). Not coincidentally, the perceived taste score and TQS positively correlated with the concentration of amino acids and theanine in Longjing Tea ($P < 0.05$), Oolong tea ($P < 0.01$), and Pu-erh tea ($P < 0.01$) (LIANG et al., 2005; WANG et al., 2009, 2010; GAO et al., 2016). Our results further revealed that those favourite amino acids such as theanine, sweet and umami amino acids were all positively responsible for TQS and taste score, while unfavourite amino acids such as bitter amino acids were negatively responsible. As sweet aftertaste is the most notable character of Wuyi Rock Tea and is a constituent of Rock Flavour, the characteristic amino acids are dominant contributors to sweet aftertaste of Wuyi Rock Tea.

It must be pointed out that although amino acids is an important contributor for tea taste in all tea types, other chemical components such tea polyphenols, caffeines, catechins, and other soluble substances in tea infusion also contribute to perceived taste (NAKAGAWA, 1970; NAGATA et al., 1986; HARBOWY et al., 1997; LIANG et al., 2005; WANG et al., 2009, 2010; XU et al., 2012; HAYASHI et al., 2013; FENG et al., 2014; GAO et al., 2016). As the multicomponent composition resulting in tea taste, the particular feature and the coeffect of tea secondary metabolites need to further uncover the influence on the Rock Flavour of Wuyi Rock Tea. Metabonomic strategies, as an effective and powerful approach to gathering integrated information of tea metabolites (LEE et al., 2015; LIU et al., 2016; TAN et al., 2016; YOU et al., 2017), may be a helpful method for unveiling the fascinating Rock Flavour of Wuyi Rock Tea.

Conclusion

By the detailed analysis on the content of characteristic amino acids in the three cultivars of Wuyi Rock Tea sampled from the different culture regions, and their correlation with the sensory evaluation, we found that the tea taste score and TQS significantly positively correlated with the concentration of theanine, sweet amino acids,

Tab. 2: The score of five quality features and the total quality score (TQS) by sensory evaluation

Cultivar & plantation	Appearance (20%)	Aroma (30%)	Taste (35%)	Infused leaves (10%)	Liquor color (5%)	TQS (100%)
Dahongpao						
Qishan	18.20±0.84a	21.20±2.17b	25.40±0.55c	7.80±0.84a	4.20±0.84a	76.80±3.19b
Guiyan	15.60±0.55a	23.80±2.28b	27.80±0.84b	8.80±0.84a	4.40±0.55a	80.40±2.70b
Yu	18.00±0.71a	28.20±1.48a	34.40±1.14a	8.20±0.84a	4.60±0.55a	93.40±1.82a
Shuixian						
Qishan	18.20±0.84a	22.20±1.79b	23.60±0.55c	8.20±0.84a	4.20±0.45a	76.40±2.07c
Guiyan	16.80±1.48a	25.40±1.67b	26.00±0.71b	9.60±0.55a	4.80±0.45a	82.60±2.61b
Yu	18.20±0.84a	30.40±0.55a	32.20±2.17a	8.80±0.45a	4.40±0.89a	94.00±3.74a
Rougui						
Qishan	16.40±1.14a	23.20±0.84c	25.40±0.55c	9.00±1.00a	4.00±0.71a	78.00±2.65c
Guiyan	17.60±1.67a	26.20±0.84b	28.00±1.00b	8.40±0.55a	4.80±0.45a	85.00±2.55b
Yu	17.60±1.14a	29.40±0.55a	33.60±1.52a	9.00±0.71a	4.80±0.45a	94.40±1.14a

Means standard error (±SE) from the five panelists independently for each sample is shown. The lowercase letters represent a significant level at $P < 0.05$.

Tab. 3: Correlation between the score of sensory evaluation and the proportion of characteristic amino acids

Quality feature	Theanine	Hydrophobicity amino acids	Hydrophilicity amino acids	Sweet amino acids	Bitter amino acids	Umami amino acids
Dahongpao						
Appearance	0.27	-0.45	0.45	0.38	-0.1	0.39
Aroma	0.98*	-0.93	0.93	0.95*	-1.00**	0.95
Taste	1.00**	-0.96*	0.96*	0.98*	-1.00**	0.98*
Infused leaves	-0.27	0.45	-0.45	-0.38	0.1	-0.39
Liquor color	0.97*	-1.00**	1.00**	0.99**	-0.91	0.99**
TQS	1.00**	-0.97*	0.97*	0.99*	-0.99**	0.99*
Shuixian						
Appearance	0.29	-0.55	0.55	0.47	-0.28	0.42
Aroma	0.99*	-0.91	0.91	0.94	-0.99**	0.96*
Taste	1.00**	-0.96*	0.96*	0.98*	-1.00**	0.99*
Infused leaves	0.69	-0.45	0.45	0.53	-0.69	0.58
Liquor color	-0.29	0.55	-0.55	-0.47	0.28	-0.42
TQS	0.99**	-0.92	0.92	0.95*	-0.99**	0.97*
Rougui						
Appearance	0.81	-0.62	0.62	0.67	-0.95*	0.72
Aroma	0.99**	-0.93	0.93	0.95*	-0.98*	0.97*
Taste	1.00**	-0.98*	0.98*	0.99**	-0.92*	1.00**
Infused leaves	0.11	-0.37	0.37	0.31	0.21	0.24
Liquor color	0.81	-0.62	0.62	0.67	-0.95*	0.72
TQS	1.00**	-0.96*	0.96*	0.98*	-0.95*	0.99**

TQS – total quality score. * and ** represent significant correlation at $P < 0.05$ and $P < 0.01$.

and umami amino acids, and significantly negatively correlated with the concentration of bitter amino acids ($P < 0.05$ or $P < 0.01$). And the ratios of these favourite amino acids (sweet and umami amino acids, as well as theanine) were higher, while the ratios of bitter amino acids were lower in tea leaves from the authentic rock region (Yu) than that from the ordinary region (Qishan). We suggested that the ratios of those favourite amino acids are dominant contributors to sweet aftertaste of Wuyi Rock Tea and could be as quality indicators to evaluate quality of Wuyi Rock Tea.

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References

Akitomi, H., Tahara, Y., Yasuura, M., Kobayashi, Y., Ikezaki, H., Toko, K., 2013: Quantification of tastes of amino acids using taste sensors. Sensor. Actuat. B-Chem. 179, 276-281. DOI: 10.1016/j.snb.2012.09.014

Alcazar, A., Ballesteros, O., Jurado, J.M., Pablos, F., Martin, M.J., Vilces, J.L., Navolon, A., 2007: Differentiation of green, white, black, oolong, and Pu-erh teas according to their free amino acids content. J. Agri. Food Chem. 55, 5960-5965. DOI: 10.1021/jf070601a

Chen, H., Yang, J., 2016: Research progress in quality being of Wuyi rock tea in different areas. J. Food Safety Quality 7, 257-262.

Chen, Q., Zhao, J., Chen, Z., Lin, H., Zhao, D., 2011: Discrimination of green tea quality using the electronic nose technique and the human panel test, comparison of linear and nonlinear classification tools. Sensor. Actuat. B-Chem. 159, 294-300. DOI: 10.1016/j.snb.2011.07.009

Deng, W., Ogita, S., Ashihara, H., 2008: Biosynthesis of theanine (γ-ethylamino-L-glutamic acid) in seedlings of *Camellia sinensis*. Phytochem. Lett. 1, 115-119. DOI: 10.1016/j.phytol.2008.06.002

Feldheim, W., Yongvanit, P., Cummings, P.H., 1986: Investigation of the presence and significance of theanine in the tea plant. J. Sci. Food Agri. 37, 527-534.

Feng, L., Gao, M.J., Hou, R.Y., Hu, X.Y., Zhang, L., Wan, X.C., Wei, S., 2014: Determination of quality constituents in the young leaves of albino tea cultivars. Food Chem. 155, 98-104. DOI: 10.1016/j.foodchem.2014.01.044

Gao, L., Bian, M., Mi, R., Hu, X., Wu, J., 2016: Quality identification and evaluation of Pu-erh teas of different grade levels and various ages through sensory evaluation and instrumental analysis. Int. J. Food Sci. Tech. 51, 1338-1348. DOI: 10.1111/ijfs.13103

GB/T 14487-2008, 2008: Terms of tea sensory tests. The National Standards of the People's Republic of China.

GB/T 23193-2008, 2008: Determination of theanine in tea – High performance liquid chromatography. The National Standards of the People's

Republic of China.

GB/T 23776-2009, 2009: Methodology of sensory evaluation of tea. China Standards Press.

GB/T 5009. 124-2003, 2003: Determination of amino acids in foods. The National Standards of the People's Republic of China.

Harbowy, M.E., Balentine, D.A., 1997: Tea Chemistry. Crit. Rev. Plant Sci. 16, 415-480. DOI: 10.1080/07352689709701956

Hayashi, N., Chen, R., Ikezaki, H., Ujihara, T., 2008: Evaluation of the umami taste intensity of green tea by a taste sensor. J. Agri. Food Chem. 56, 7384-7387. DOI: 10.1021/jf800933x

Hayashi, N., Ujihara, T., Chen, R., Irie, K., Ikezaki, H., 2013: Objective evaluation methods for the bitter and astringent taste intensities of black and oolong teas by a taste sensor. Food Res. Int. 53, 816-821. DOI: 10.1016/j.foodres.2013.01.017

He, W., Hu, X., Zhao, L., Liao, X., Zhang, Y., Zhang, M., Wu, J., 2009: Evaluation of Chinese tea by the electronic tongue: correlation with sensory properties and classification according to geographical origin and grade level. Food Res. Int. 42, 1462-1467. DOI: 10.1016/j.foodres.2009.08.008

Horanni, R., Engelhardt, U.H., 2013: Determination of amino acids in white, green, black, oolong, pu-erh teas and tea products. J. Food Compos. Anal. 31, 94-100. DOI: 10.1016/j.jfca.2013.03.005

Huo, D., Wu Y., Yang, M., Fa, H., Luo, X., Hou, C., 2014: Discrimination of Chinese green tea according to varieties and grade levels using artificial nose and tongue based on colorimetric sensor arrays. Food Chem. 145, 639-645. DOI: 10.1016/j.foodchem.2013.07.142

Kaneko, S., Kumazawa, K., Masuda, H., Henze, A., Hofmann, T., 2006: Molecular and sensory studies on the umami taste of Japanese green tea. J. Agri. Food Chem. 54, 2688-2694. DOI: 10.1021/jf0525232

Kocadağli, T., Özdemir, K.S., Gökmen, V., 2012: Effects of infusion conditions and decaffeination on free amino acid profiles of green and black tea. Food Res. Int. 53, 720-725. DOI: 10.1016/j.foodres.2012.10.010

Kurihara, K., 2015: Umami the fifth basic taste: history of studies on receptor mechanisms and role as a food flavor. Biomed Res. Int. 6, 1-9. DOI: 10.1155/2015/189402

Lee, J., Lee, B., Chung, J., Kim, H., Kim, E., Jung, S., Lee, H., Lee, S., Hong, Y., 2015: Metabolomic unveiling of a diverse range of green tea (*Camellia sinensis*) metabolites dependent on geography. Food Chem. 174, 452-459. DOI: 10.1016/j.foodchem.2014.11.086

Liang, Y., Zhang, L., Lu, J., 2005: A study on chemical estimation of pu-erh tea quality. J. Sci. Food Agri. 85, 381-390. DOI: 10.1002/jsfa.1857

Liu, J., Zhang, A., Liu, M., Ma, L., Shi, Y., Ruan, J., 2016: Metabolomic analyses reveal distinct change of metabolites and quality of green tea during the short duration of a single spring season. J. Agri. Food Chem. 64, 3302-3309. DOI: 10.1021/acs.jafc.6b00404

Matoba, T., Hata, T., 1972: Relationship between bitterness of peptides and their chemical structures. Agri. Biol. Chem. 36, 1423-1431.

Miyauchi, S., Yuki, T., Fuji, H., Kojima K., Yonetani, T., Tomia, A., Bamba, T., Fukusaki, E., 2014: High-quality green tea leaf production by artificial cultivation under growth chamber conditions considering amino acids profile. J. Biosci. Bioeng. 118, 710-715. DOI: 10.1016/j.jbiosc.2014.05.008

Nagata, T., Sakai, S., 1986: Differences in caffeine, flavones, amino acids contents in leaves of cultivated species of Camellia. Jpn. J. Breeding 34, 459-467.

Nakagawa, M., 1970: Constituents in tea leaf and their contribution to the taste of green tea liquor. Jpn. Agri. Res. Q. 5, 43-47.

Nakagawa, M., 1975: Contribution of green tea constituents to the intensity of taste element of brew. J. Food Sci. Tech. 22, 59-64.

Nishimuraa, T., Katoa, H., 1988: Taste of free amino acids and peptides. Food Rev. Int. 4, 175-194. DOI: 10.1080/87559128809540828

Scharbert, S., Hofmann, T., 2005: Molecular definition of black tea taste by means of quantitative studies, taste reconstitution, and omission experiments. J. Agri. Food Chem. 53, 5377-5384. DOI: 10.1021/jf050294d

Song, M., Li, Q., Guan, X., Wang, T., Bi, K., 2012: Novel HPLC method to evaluate the quality and identify the origins of Longjing green tea. Anal. Lett. 46, 60-73. DOI: 10.1080/00032719.2012.704532

Tan, J., Dai, W., Lu, M., Lv, H., Guo, L., Zhang, Y., Zhu, Y., Peng, Q., Lin, Z., 2016: Study of the dynamic changes in the non-volatile chemical constituents of black tea during fermentation processing by a non-targeted metabolomics approach. Food Res. Int. 79, 106-113. DOI: 10.1016/j.foodres.2015.11.018

Wang, K., Liu, F., Liu, Z., Huang, Jianan., Xu, Z., Li, Y., Chen, J., Gong, Y., Yang, X., 2010: Analysis of chemical components in oolong tea in relation to perceived quality. Int. J. Food Sci. Techn. 45, 913-920. DOI: 10.1111/j.1365-2621.2010.02224.x

Wang, K., Ruan, J., 2009: Analysis of chemical components in green tea in relation with perceived quality, a case study with Longjing teas. Int. J. Food Sci. Techn. 44, 2476-2484. DOI: 10.1111/j.1365-2621.2009.02040.x

Xu, W., Song, Q., Li, D., Wan, X., 2012: Discrimination of the production season of Chinese green tea by chemical analysis in combination with supervised pattern recognition. J. Agri. Food Chem. 60, 7064-7070. DOI: 10.1021/jf301340z

Ye, N., Zheng, N., Yang, J., 2004: Original producing area protection of Fujian famous tea varieties. J. Fujian Agri. For. Univ. (Nat. Sci. Edi.) 33, 459-462.

Yin, J.F., Zhang, Y.N., Du, Q.Z., Chen, J.X., Yuan, H.B., Xu, Y.Q., 2014: Effect of Ca^{2+} concentration on the tastes from the main chemicals in green tea infusions. Food Res. Int. 62, 941-946. DOI: 10.1016/j.foodres.2014.05.016

You, R., Guan, Y., Li, L., 2017: Chinese herbal teas as a complementary therapy Metabonomics: a developing platform for better understanding. Int. J. Food Sci. Techn. 52, 13-21.

Zhu, Y., Luo, Y., Wang, P., Zhao, M., Li, L., Hu, X., Chen, F., 2016: Simultaneous determination of free amino acids in Pu-erh tea and their changes during fermentation. Food Chem. 194, 643-64. DOI: 10.1016/j.foodchem.2015.08.054

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