

Variability in hydrolyzed amino acids contents in fresh leaves of Wuyi Rock Tea from different regions of China

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ABSTRACT

Amino acids contained in leaves of Wuyi Rock Tea were determined by acid hydrolysis. Leaves of Dahongpao, Shuixian and Rougui tea varieties were selected from (i). Authentic Rock Tea region (Yu tea plantation), (ii). Semi-authentic Rock Tea region (Guiyan tea plantation) and (iii). continent tea region (Qishan tea plantation). The results showed that 11 of the 17 hydrolyzed amino acids were identified, their contents significantly differed among these 3-plantations of the same tea variety. The number of amino acids differed significantly and in Dahongpao, Shuixian and Rougui varieties these were 74 %, 70 %, 65 %, respectively. The principal component analysis showed that fresh tea leaves from Yu, Guiyan and Qishan tea plantations also varied in contents of leucine, asparagine, glutamate and arginine. In the 3-plantations, the contents of leucine, asparagine, lysine, glutamate and arginine differed in 3-tea varieties (Dahongpao, Shuixian, Rougui). This study showed the regional and inter-variety differences in the contents of hydrolyzed amino acids of Wuyi Rock Tea, which provided basis for tea quality.

Keywords : Hydrolyzed amino acids, tea leaves, tea plantations, tea quality, tea varieties, Wuyi Rock Tea.

INTRODUCTION

Wuyi rock tea is one of the top 10-famous teas in China. Its original producing area is the rock area of Wuyi Mountain. The soil of tea plantation is deep, very rich in organic matter and mineral elements and formed from the weathered rock. This soil is suitable for the growth of rock tea and imparts the unique 'Rock Flavour' in this tea. Regarding the Wuyi Mountain soil, Lu in "the Book of Tea" (14) reported that "the top layer has fertile soil, the middle layer has gravel, and the lower soil layer is loess", these are closely related to tea growth and quality. Tea growers in Wuyi county are called (i). Authentic Rock Tea (Zhengyan Cha in Chinese), (ii). Semi-authentic Rock Tea (Banyan Cha in Chinese) and (iii). Continent tea (Zhou Cha in Chinese) to differentiate the quality of Rock Tea (3,21,22,23,24). The tea quality followed the order: 'Authentic Rock Tea' > 'Semi-authentic Rock Tea' > 'Continent tea' ordinary tea (13,20).

The Authentic rock area tea has the characteristic "rocky fragrance of flowers", "rich rock flavour", "good sweet taste" etc. The planting area is small, yield is low, hence, in short supply. Therefore, the quality of 'continent tea' needs to be improved to meet the market demand. However, tea quality evaluation in different planting areas, it is still based on taste/sensory evaluation, without chemical analysis based on quality. The taste of tea is the comprehensive sensory stimulation caused by substances present in tea infusion (2). In general, there are 6-tastes of tea: Sour, Sweet, Bitter, Salty, Astringent and Fresh, among

these 3-main tastes are: Bitter, Astringent and Fresh (7,19). Amino acids are the main components in fresh taste tea, one of the common and main indicators to evaluate the tea quality. Many domestic and foreign studies have adopted the content of amino acids to evaluate the tea quality (4,9,11,12,18,).

Dahongpao, Rougui and Shuixian tea varieties of Wuyi Rock Tea are planted respectively in Authentic Rock, Semi-authentic Rock and Ordinary regions. Their fresh leaves were collected and contents of hydrolyzed amino acids were analyzed, in these fresh tea leaves to know the effects of growing region and varieties grown on tea quality in Wuyi rock tea.

MATERIALS AND METHEODS

Yu (Authentic rock tea plantation), Guiyan (Semi-authentic rock tea plantation), Qishan (Ordinary tea plantation) were chosen as sampling sites(Fig.1). These 3-tea plantations by the government are in Wuyishan County, Fujian Province, China. The Dahongpao, Rougui and Shuixian tea varieties of Wuyi Rock Tea in these 3-plantations were test research materials. The details of sampling sites are given below :

Geographical locations of 3- tea plantations sites and cultivated tea varieties

Tea plantation	Latitude	Longitude	Varieties grown
Yu	27°38'42"-45"	117°56'38"-44"	Dahongpao, Rougui, Shuixian, etc.
Guiyan	27°36'26"-34"	117°57'52"-58'1"	Dahongpao, Rougui, Shuixian, etc.
Qishan	27°17'32"-39"	117°54'41"-56"	Dahongpao, Rougui, Shuixian, etc.

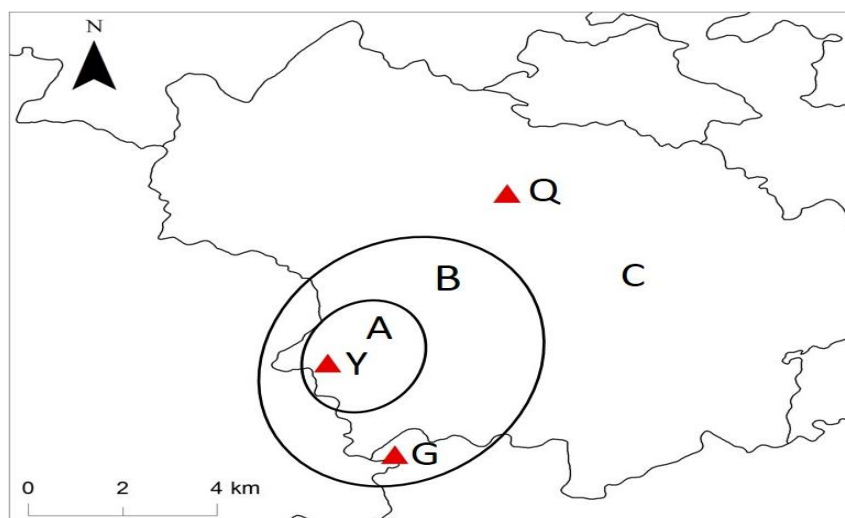


Figure 1. Division of rock tea area and the sites of tea research plantations
 A : Authentic rock area; B : Semi-authentic rock area; C : Ordinary area;
 Y : Yu tea plantation; G : Guiyan tea plantation; Q : Qishan tea plantation.

The tea plants sampling was done during April and May (Tea picking season). The new shoots of tea plants were collected using the equidistant sampling method, described briefly. The tea planting areas were divided into 5-equal sections. Three tea plants in the

center of each section were considered as one sample, and 3-such samples were collected from each section. Soil around the tea plant radius of 15-25 cm and depth of 25-35 cm was collected. Each tea sample was dried for 60 min at 105 °C and thereafter dried to constant weight at 80 °C. The dried samples were grounded to powder and sieved through 60 mesh. This tea powder was used to determine the contents of hydrolyzed amino acids.

Hydrolyzed amino acids

The powdered tea leaves samples were subjected to acid hydrolysis following the protocol in "Determination of amino acids in food" (GB5009.124-2016). The 2 g of above tea powder was taken in hydrolysis tube, added 10 mL of 6 mol/L hydrochloric acid and also added and 3~4 drops of phenol were added. Refrigerate the hydrolysis tube for 3~5 min, connect it to the suction pipe of vacuum pump, vacuum it - fill nitrogen repeatedly thrice and then seal the screw cap after filling the nitrogen. The sealed hydrolysis tube was placed in an electric blast thermostat at 110 °C. After hydrolysis for 22 h, it was taken out and cooled to room temperature. Open the hydrolysis tube, filter the hydrolysate into a volumetric flask, rinse the hydrolysis tube with small amount of water several times. Finally, made the volume of water to 50 mL and shake. Take 1.0 mL of filtrate and dry under pressure at 40 °C~ 50 °C. After drying, the residue was dissolved in 1~2 mL water, then dried under reduced pressure and finally evaporated to dryness. The solution was added with 1.0~2.0 mL of sodium citrate buffer solution (pH 2.2) and dissolve in dry test tube. After oscillating and mixing, the solution was passed through 0.22 µm filter membrane. The filtrate was subjected to Hitachi L-8900 automatic amino acid analyzer.

Statistical analysis

Data are presented as mean ± standard error (SE). Excel software 2010 was used to conduct statistics and variance analysis. Principal component analysis (PCA) and correlative analysis were all conducted with DPS 7.05 software.

RESULTS AND DISCUSSION

Amino acids in fresh tea leaves

Table 1 showed significant differences in the contents of 11 of the 17 amino acids detected in the leaves of 3-tea cultivars (Dahongpao, Shuixian, Rougui) investigated in 3-plantations (Yu, Guiyan and Qishan) of tea production. The 11 amino acids were: threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, glutamic acid, glycine, serine and arginine. Proportions of the number of amino acids with significant difference in Yu, Guiyan and Qishan tea plantations were about 74 %, 70 %, 65 %, respectively. Among the 17 components, only phenylalanine significantly differed between the Shuixian and Dahongpao or Rougui tea varieties, while other components were similar in these varieties.

Principal component analyses

Graphical analysis of principal component analysis based on the total contents of hydrolyzed amino acids in the tea leaf collected from different plantations showed that Shuixian located in Qishan, Guiyan and Yu were in the first quadrant. While, Rougui and Dahongpao planted in these 3-tea zones were distributed in the second quadrant (Fig. 2) Rougui and Dahongpao varieties contained similar total hydrolyzed amino acid contents,

which differed from contents in Shuixian. An additional principal component analysis of total hydrolyzed amino acid contents set according to the cultivars indicated Dahongpao, Shuixian and Rougui in the first quadrant when planted in Qishan, while the three tea cultivars were positioned in the second quadrant, when planted in Guiyan and Yu (Fig. 3).

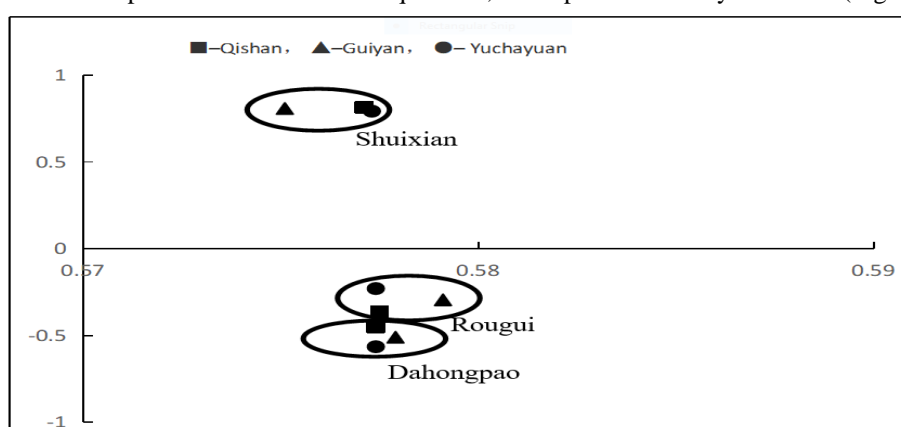


Figure 2. Principal components analysis of different regions based on hydrolyzed amino acids

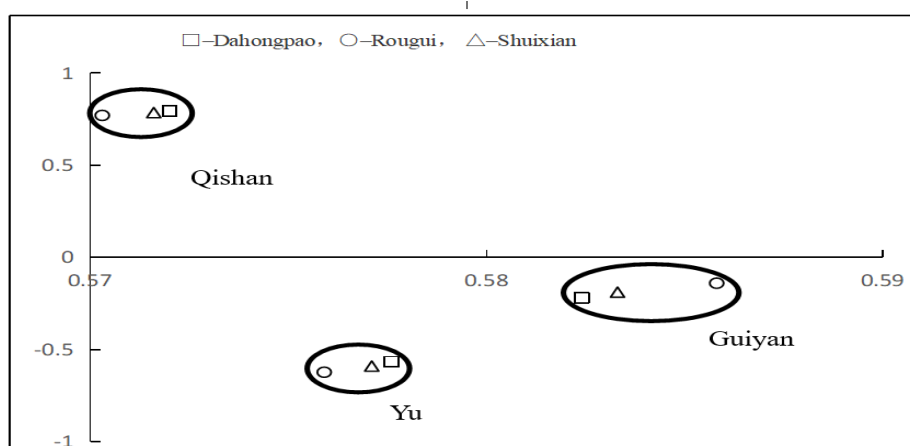


Figure 3. Principal components analysis of different varieties based on hydrolyzed amino acids

Tea varieties : Principal components analysis of 17-hydrolyzed amino acids in fresh leaves of 3-tea plantations found that valine, leucine, asparagine, lysine, glutamic acid, arginine differed in Dahongpao in 3-tea plantation (Fig. 4). The leucine, asparagine, glutamic acid, arginine differed in Shuixian. While, leucine, asparagine, lysine, glutamate, arginine differed in Rougui (Fig. 4). In brief, leucine, asparagine, glutamate and arginine were consistent in all 3 tea varieties.

Table 1. Comparison of hydrolytic amino acids contents fresh tea leaves in different plantations

Items	Dahongpao			Shuixian			Rougui		
	Qishan	Guiyan	Yu	Qishan	Guiyan	Yu	Qishan	Guiyan	Yu
Threonine	4.97±0.11c	6.68±0.15b	8.12±0.12a	4.11±0.12c	6.15±0.19b	7.62±0.23a	4.23±0.10c	6.08±0.15b	7.85±0.24a
Valine	5.15±0.08c	7.12±0.12b	8.31±0.22a	4.28±0.19c	6.46±0.21b	8.04±0.11a	4.26±0.14c	6.62±0.09b	7.85±0.37a
Methionine	0.50±0.02c	0.69±0.02b	0.80±0.02a	0.42±0.03c	0.51±0.02b	0.68±0.03a	0.41±0.01c	0.51±0.02b	0.70±0.02a
Isoleucine	4.77±0.12c	6.41±0.12b	8.00±0.19a	4.09±0.15c	6.03±0.10b	7.74±0.13a	4.04±0.08c	5.91±0.10b	7.64±0.24a
Leucine	8.27±0.15c	10.39±0.25b	12.5±0.35a	6.9±0.17c	10.06±0.21b	12.28±0.30a	7.20±0.26c	9.72±0.37b	12.61±0.51a
Phenylalanine	3.81±0.11c	4.82±0.12b	5.75±0.13a	3.10±0.13b	1.36±0.11c	5.50±0.10a	3.13±0.12c	4.26±0.15b	5.50±0.24a
Lysine	5.80±0.17c	7.02±0.12b	8.00±0.14a	5.05±0.15c	6.23±0.22b	7.26±0.21a	5.35±0.12c	6.49±0.24b	7.72±0.12a
Serine	5.14±0.11c	6.06±0.10b	7.21±0.16a	4.15±0.19c	5.61±0.13b	6.83±0.12a	4.19±0.20c	5.47±0.30b	6.94±0.31a
Glutamate	12.04±0.19c	14.99±0.24b	17.96±0.75a	10.80±0.29c	14.64±0.10b	17.85±0.31a	10.63±0.58c	14.23±0.41b	17.87±0.67a
Glycine	4.86±0.12c	6.18±0.13b	7.08±0.16a	4.95±0.12c	5.74±0.07b	6.84±0.11a	4.17±0.23c	5.53±0.26b	6.91±0.15a
Arginine	9.21±0.20c	11.76±0.09b	13.39±0.19a	8.52±0.42c	11.37±0.34b	13.09±0.34a	9.38±0.53c	11.17±0.15b	13.19±0.29a
Cysteine	2.03±0.05a	2.06±0.08a	2.07±0.07a	1.66±0.06a	1.63±0.09a	1.75±0.05a	1.64±0.05a	1.72±0.05a	1.78±0.07a
Tyrosine	3.00±0.17a	3.00±0.12a	3.23±0.17a	2.92±0.08a	2.86±0.08a	2.95±0.10a	2.84±0.05a	2.84±0.03a	2.92±0.17a
Histidine	4.03±0.11a	4.02±0.08a	4.08±0.09a	3.71±0.09a	3.66±0.05a	3.77±0.09a	3.61±0.17a	3.53±0.26a	3.73±0.13a
Proline	5.19±0.16a	4.29±1.56a	5.36±0.24a	4.97±0.15a	5.11±0.19a	5.37±0.46a	4.90±0.15a	4.99±0.14a	5.17±0.15a
Alanine	5.82±0.17a	5.88±0.37a	6.19±0.26a	5.84±0.11a	5.84±0.13a	5.99±0.13a	5.71±0.36a	5.83±0.13a	5.93±0.22a
Asparagine	12.38±0.27a	12.27±0.32a	12.89±0.22a	11.45±0.44a	11.52±0.36a	12.51±0.24a	11.59±0.40a	11.64±0.34a	12.34±0.42a
Total	96.97±0.73c	113.63±2.16b	130.94±3.16a	86.93±1.25c	104.77±0.63b	126.07±1.58a	87.26±1.17c	106.54±1.60b	126.66±2.65a

Note: For the same tea variety, different lowercase letters in a same row represent significant differences at $P < 0.05$ ($n=3$).

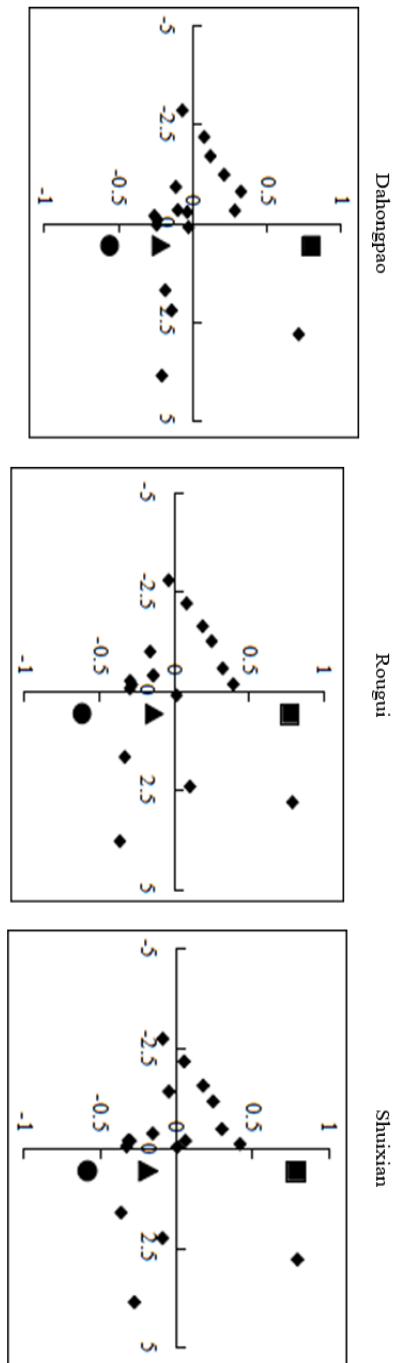


Figure 4. Principal Component analysis of hydrolyzed amino acids in fresh leaves of 3-tea cultivars. ■: Qishan; ▲: Guiyan; ●: Yuchayuan

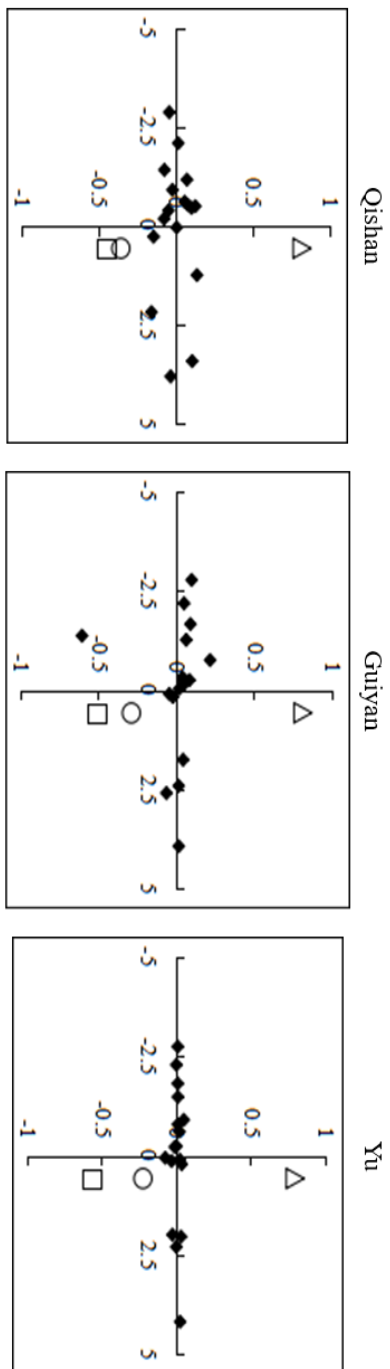


Figure 5. Principal component analysis of hydrolyzed amino acid in fresh leaves of the 3 tea plantations. □: Dahongpao; ○: Rongui; △: Shuixian

Tea growing regions : Principal components analysis of 17-hydrolyzed amino acids in fresh leaves of 3-tea varieties found that leucine, asparagine, lysine, glutamic acid, alanine, arginine could differentiate the tea quality of 3-varieties in Qishan (Fig. 5) and valine, leucine, asparagine, lysine, glutamic acid, arginine discriminated the tea quality of 3-varieties in Guiyan and threonine, asparagine, valine, isoleucine, leucine, lysine, glutamic acid, arginine differentiated tea quality of 3-varieties in Yu (Fig. 5). The leucine, asparagine, lysine, glutamate, arginine existed in tea leaves in 3- tea plantations.

Amino acids contents in tea leaves affects tea colour, aroma and taste (25). Stronger hydrophobicity of amino acids provides a stronger and bitter taste (15). Theanine [is an ethylamine compound of glutamic acid, also known as glutamylphthale diamine, which is a synthetic product of glutamic acid and ethylamine (decarboxylated product of alanine) (6)] provides sweet and refreshing taste in tea (10). Nakagawa (16) found that nearly 70 % sweet taste of green tea comes from amino acids (alanine and glycine). Amino acids with branched chains (leucine, isoleucine and valine) provides bitter taste (1). In this study, the specific hydrolyzed amino acid components of tea varieties were leucine, asparagine, lysine, glutamate, arginine, these influenced the quality of different tea varieties. Based on the Principal component analyses of amino acids contents in 50 samples of Longjing tea, Zhao (25) differentiated the Xihu Longjing tea quality from teas produced in other parts of China, due to the environmental characteristic amino acids of Glutamate, Arginine, Phenylalanine and Tyrosine. We also got similar results in this study, leucine, asparagine, glutamate and arginine of 3-tea varieties showed regional differences, and Yu > Guiyan > Qishan, provided the basis for the development of 'Rock Flavour'.

During the tea leaves processing, the theanine, glutamate, aspartic acid, phenylalanine, tryptophan and tyrosine participates in the Maillard reaction, among which the interproduct, glycosamine compounds, cannot develop the baking and frying fragrance, but also the tea taste (7). Amino acids not only develops tea flavour, but also the tea aroma. During the tea processing, amino acids can also be converted into some aroma precursors (acetaldehyde, formaldehyde, methional-propanal) and other aromatic substances with low boiling points. Polyphenols and their oxides can combine with the sulfhydryl groups in glutathione and cysteine to form aromatic compounds, indicating that the content of amino acids in tea leaves is positively correlated with the tea aroma and taste (17). Jang *et al.* (8) studied the aroma substances in fresh tea leaves, and found that many aroma substances are present in the fresh tea leaves as glycosides. These glycosides were released in the process of tea production under the action of β -primrose glycosidase and β -glucosidase. Cheng *et al.* (5) studied the changes in quality indexes in oolong tea during processing, and believed that degradation of catechins and amino acids in tea produces the nitrogen-containing heterocyclic compounds. Therefore, amino acids in tea are not only related to the sweetness and taste, but also affects the tea aroma after processing. Amino acids play an important role in the quality evaluation of Wuyi rock tea.

CONCLUSIONS

There were significant differences in hydrolyzed amino acids contents in different tea varieties, especially in leucine, asparagine, lysine, glutamate, arginine. Compared with Shuixian variety, the quality of Dahongpao and Rougui varieties was similar. Significant differences existed in hydrolyzed amino acids among growing regions, and leucine,

asparagine, lysine, glutamate, arginine contents differed in 3-tea plantations. Compared with Qishan tea plantation, hydrolyzed amino acids contents of Guiyan and Yu tea plantation were similar. These research results identified the source for improving the quality of Wuyi Rock Tea.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest : The Authors declare no conflict of Interest.

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