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A study on chemical estimation of pu-erh tea quality

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Abstract: Chemical compositions and infusion colour differences of seven pu-erh tea samples and their correlation to sensory quality were investigated. The results showed that the pu-erh tea contained 37.1 mg g^{-1} caffeine, 15.7 mg g^{-1} amino acids, 67.0 mg g^{-1} polyphenols and 8.41 mg g^{-1} total catechins, on average. Among the 17 tested volatile compounds, *n*-valeraldehyde was not detected. The most abundant volatile was β-ionone and the next was linalool oxide II. Infusion colour analysis showed that the pu-erh tea had deep hue with ΔE ranging from 66.8 to 79.2. Spearman's linear correlation analysis showed that total quality score (TQS) of the pu-erh tea was significantly correlated to concentration of amino acids, linalool oxide II and infusion colour indicator ΔE. Five components were extracted from the 34 tested indicators by principal component analysis and were regressed on the TQS to produce six Pearson's linear regression equations for estimating sensory quality of pu-erh tea, among which two were statistically significant, ie TQS = 57.47 − 0.18geraniol + 0.33polyphenols − 1.14n-caproaldehyde − 1.38linalool oxide I + 0.21caffeine ($p < 0.01$) and TQS = 57.42-0.03Citral + 0.33polyphenols − 1.14n − caproaldehyde − 1.40linalool oxide I + 0.20caffeine ($p < 0.01$).

Keywords: *Camellis sinensis*; chemical composition; infusion colour; sensory quality; correlation; principal component analysis; regression

INTRODUCTION

Pu-erh tea is a popular beverage in Asia, especially in southwestern China, owing to its special flavour properties and potential healthy benefits. Although pu-erh tea possesses lower levels of catechins than green, oolong and black teas,[1–3] it has the remarkable features of suppressing the genotoxicity induced by nitroarenes,[2] lowering the atherogenic index and increasing HDL–total cholesterol ratio.[4] The processing of pu-erh tea is quite different from that of black tea, although they are both fermented teas. During black tea processing, fresh leaves are rolled and/or cut before drying so that tea polyphenols in tea leaves are contacted with the tea polyphenol oxidases and then oxidized in the consequent fermentation process. During the pu-erh tea process, fresh leaves are fixed by heat in a drum so as to inactivate polyphenol oxidases. The fixed leaves are then rolled and partially dried. The partially dried leaves are piled up in humid conditions for a few weeks, during which the tea polyphenols are more fully oxidized by the action of microorganisms and environmental oxygen than black tea, resulting in low concentrations of tea polyphenols and tea catechins.

Tea quality is greatly influenced by the tea polyphenol, amino acid and caffeine contents in tea leaves[5,6] and the colour differences of tea infusions.[7] Many attempts have been made to estimate the quality of black tea and green teas chemically. Biswas *et al*[8] found a statistical association of liquor characteristics with value of black tea. Hilton and Ellis[9] and Cloughley[10] confirmed a close linear regressive correlation between theaflavin concentration and broker's valuation of Central African black tea. Obanda *et al*[11] and Wright *et al*[12] showed that concentrations of some tea catechins and theaflavin were significantly correlated with sensory quality or value of black tea. Liang *et al*[13] found that theaflavin made a greater contribution to the brightness of black tea infusion than theaflavin gallates. Liang *et al*[6] confirmed that the effect of gibberellins on green tea quality was based on the chemical composition of the tea leaves. Liang *et al*[7] also revealed that black tea quality could be estimated by chemical composition and colour differences of tea infusions. Pu-erh tea has a malty flavour because of its fermentation process. Little information on the relationship of the chemical composition to the quality of pu-erh tea is available. In evaluating of the pu-erh tea, the tea taster takes into consideration mainly the infusion characteristics, ie liquor colour, aroma and taste of the infused leaf as well as the appearance of the dry tea. The

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purpose of the present study was to investigate the chemical parameters and colour difference indicators of various pu-erh tea infusions and then to reveal their relationship to sensory quality. Based on these, linear regressions of sensory quality upon the chemical parameters were constructed for estimating pu-erh tea quality.

MATERIALS AND METHODS

Materials

Seven samples (1 kg each) of pu-erh tea produced by Yunnan Dali Tea Factory and Yunnan Tea Import and Export Cooperation were bought from Beijing Maliandao Tea Market (Table 1). Tea catechins and volatile compounds for HPLC and GC references were provided by Dr Tu from Department of Tea Science of Zhejiang University, China. The other chemical reagents used were of HPLC-grade bought from Tianjin Shild Biometric Technical Co Ltd (Tianjing City, China), except where stated otherwise.

Equipment for the chemical analysis was a high-performance liquid chromatograph (HPLC, Model Shimadzu SCL-10A, Shimadzu Cooperation, Tokyo, Japan) and a gas chromatograph (GC, Model Shomadzu GC-14B, Shimadzu Cooperation, Tokyo, Japan) and that for tea infusion colour difference analysis was a model TC-PIIG automatic colour difference meter (Beijing Optical Instrument Factory, Beijing, China).

Methods

Sensory assessment of tea quality

The tea samples were examined and scored independently by a tea tasting panel consisting of six trained graduate students from Department of Tea Science, Zhejiang University, China. Five gram samples of tea was infused in a 250 ml tea tasting porcelain cup with 250 ml freshly boiled water for 5 min and then the liquor was poured into a 250 ml tea tasting porcelain bowl for quality assessment. The grading system was based on a maximum score of 100 for each quality attribute (appearance, aroma, liquor colour, taste and infused leaf). The mean score given by the six tea tasters for each quality attribute was used to express the corresponding attribute score of the tasted samples. The mean value of the five quality attributes was expressed as the sample total quality score (TQS).

Table 1. Sources of the tested pu-er tea samples

Sample no	Grade	Producers
1	Special grade	Yunnan Tea Import and Export Cooperation
2	First grade	Yunnan Dali Tea Factory
3	Second grade	Yunnan Tea Import and Export Cooperation
4	Second grade	Yunnan Dali Tea Factory
5	Third grade	Yunnan Dali Tea Factory
6	Fourth grade	Yunnan Tea Import and Export Cooperation
7	Fifth grade	Yunan Dali Tea Factory

HPLC analysis of caffeine and catechins

HPLC analysis of caffeine and compounds of tea catechins was carried out using the method described previously.[7] Three grams of tea sample were extracted with 150 ml freshly boiled distilled water in a boiling water bath for 10 min. The extracts were filtered through a Double-ring no 102 filter paper (Xinhua Paper Industry Co Ltd, Hangzhou, China) and a 0.2 μm Milipore filter before injection into the HPLC. The HPLC conditions were: injection volume, 10 μl; column, 5 μm Diamonsil™ C_{18}, 4.6 × 250 mm; temperature, 40 °C; mobile phase, solvent A, acetonitrile–acetic acid–water (6:1:193, v/v/v) and solvent B, acetonitrile–acetic acid–water (60:1:193, v/v/v); gradient, 100% (v) solvent A to 100% (v) solvent B by linear gradient during the first 45 min and then 100% (v) solvent B until 60 min; flow rate, 1 ml min^{-1}; detector, Shimadzu SPD ultraviolet detector, 280 nm.

Analysis of nitrogen

A ground sample (40-mesh) of 0.5 g was placed in a 100 ml Kjeldahl flask and digested with 0.12 g $CuSO_4$, 0.88 g K_2SO_4 and 10 ml H_2SO_4 (analytic grade) for 90 min, during which time the solution was heated to boiling. When the solution was cooled to room temperature, it was transferred to a 100 ml volumetric flask and diluted to 100 ml with distilled water. Nitrogen in 10 ml of the diluted solution was determined by the method described by Zhong[14] and Wilde *et al*.[15]

Determination of amino acids

A tea sample (ground to 40-mesh) of 1.5 g was placed in a 500 ml flask with 220 ml freshly boiled distilled water and extracted in boiling water bath for 45 min. The extract was filtered through a Double-ring no 102 filter paper (Xinhua Paper Industry Co Ltd, Hangzhou, China). When the filtrate was cooled to room temperature, it was transferred to a 250 ml volumetric flask and diluted to 250 ml with distilled water. Amino acid concentration was determined by the ninhydrin assay method.[14] A 2 ml sample of the diluted filtrate was transferred to a 50 ml volumetric flask with 1 ml of reagent (20 g l^{-1} of ninhydrin and 0.8 g l^{-1} of $SnCl_2 \cdot 2H_2O$) and 1 ml of buffer (6.7×10^{-2} M NaHPO4 and 6.7×10^{-2} M KH_2PO_4, pH 8.0) and reacted in boiling water bath for 15 min. The control flask contained 2 ml of distilled water, 1 ml of reagent and 1 ml of buffer. The reacted sample was then transferred to quartz cell with black aperture (1 cm light-path) and colourimetric measurement made with an HP8453E UV–vis spectrophotometer (Hewlett-Packard Company, Palo Alto, CA, USA)

at a wavelength of 570 nm. Glutamic acid (Sigma AnalaR-grade product) was used as amino acid standard to make calibration graph, and the amino acid concentration of the tea sample was determined from the calibration graph according to its absorbance at 570 nm.

Analysis of tea polyphenols

Tea polyphenols were determined by spectrophotometric method described by Zhong[15] and Liang *et al*.[7] A 1 ml sample of filtered tea extract, which was obtained in sample preparation for HPLC, was transferred to a 25 ml volumetric flask with 5 ml reagent (containing 3.6×10^{-3} M $FeSO_4$ and 3.5×10^{-3} M $KNaC_4H_4O_6$), 4 ml distilled water and 15 ml buffer (6.7×10^{-2} M NaHPO4 and 6.7×10^{-2} M KH_2PO_4, pH 7.5). The solution in the volumetric flask was mixed well and stood at room temperature for 1 min before its absorbance at 540 nm (E_1) was measured in a 1 cm light-path quartz cell of the HP8453E UV–vis spectrophotometer. Absorbance at 540 nm (E_2) of a control solution (5 ml distilled water, 5 ml reagent and 15 ml buffer) was determined as described earlier. Concentration of the tea sample was calculated by the following equation:

$$\text{polyphenols (mg g}^{-1}) = (E_1 - E_2) \times 3.9133 \times 150/3$$

In the equation, the 3.9133 is a constant, meaning that polyphenol concentration was 3.9133 mg ml^{-1} when absorbance at 540 nm was 1.0 under the earlier conditions, while the 150/3 means that 3 g of tea were extracted in 150 ml water.

Tea infusion colour difference analysis

Five grams of the tea sample were extracted in 240 ml of freshly boiled distilled water for 5 min. Tea infusion was filtered on Double-ring no 102 filter paper (Xinhua Paper Industry Co Ltd, Hangzhou, China) when cooled to room temperature. The filtrate obtained was then diluted to 250 ml with distilled water. The white plate supplied by the TC-PIIG automatic colour difference meter was used as background. To diminish the errors arising from different determination conditions such as various equipment and temperatures, distilled water was used as control. Infusion colour difference indicators of ΔL, Δa, Δb and ΔE, which represent the light–dark, red–green, yellow–blue and total colour difference in the three dimensional colour coordinate system between the tea infusion and the distilled water, were given by the TC-PIIG automatic colour difference meter.

Gas chromatograph analysis of volatile constituents

An successive distilation extraction (SDE) apparatus was used to extract volatile constituents of tea samples (Fig 1). Fifteen grams of the tea sample and 350 ml freshly boiled distilled water were placed in flask A of the SDE apparatus, which was held in the boiling water bath. The sample was extracted for 1 h, during which time the volatile compounds were evaporated and absorbed in 30 ml of ethyl ether in flask B held in a 50 °C water bath. The ethyl ether phase was then transferred into a 50 ml glass tube and dehydrated with 5 g of Na_2SO_4 overnight. The dehydrated ethyl ether phase was concentrated to about 0.2 ml under reduced pressure at 42 °C. The concentrate was used for gas chromatograph (GC) analysis.

A Shimadzu GC-14B gas chromatograph equipped with HP-FFAP fused capillary column (30 m × 0.22 mm id) was used for the GC analysis. The GC conditions were as follows: the injector was held at a constant 200 °C and the detector at 250 °C during the analysis; oven temperature was maintained at 50 °C for 5 min, followed by a linear programming from 50 to 210 °C at a rate of 3 °C min^{-1}. The carrier gas helium was at 100 kPa.

Data analysis and statistics

The tests in the present paper were carried out in two replications except for the sensory quality evaluation and the mean values of the two replications presented. Spearman's linear correlation, principal component analysis and pearson's linear regression were carried out by software of SPSS 10.0 for Windows (SPSS Inc., Cary, NC, USA, 1999).

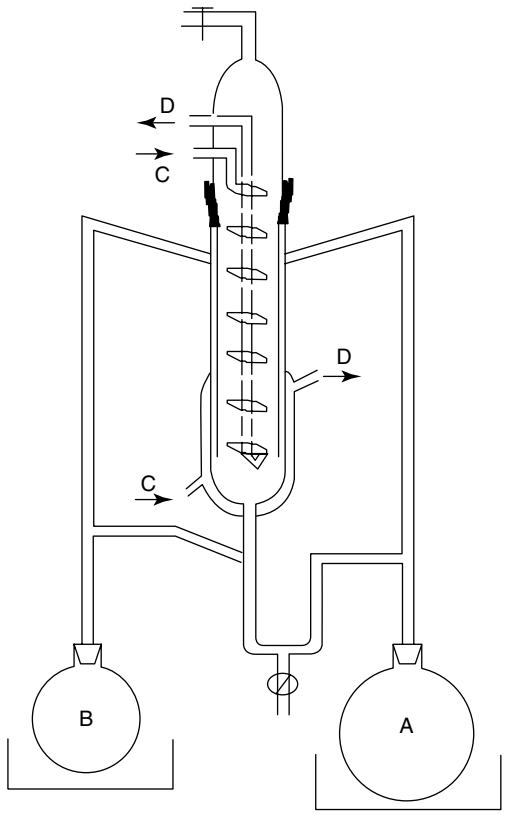

Figure 1. SDE apparatus for extracting volatile constituents. A, 1000 ml glass flask containing tea and water; B, 250 ml glass flask containing ethyl ether; C, cooling water inlet; D, cooling water outlet.

Table 2. Sensory quality score of the tested Pu-er tea samples

Sample no	Grade	Appearance	Aroma	Liquor color	Taste	Infused leaf	Total quality (TQS)[a]
1	Special grade	90.3 ± 1.9	93.2 ± 1.8	90.5 ± 1.5	93.5 ± 1.8	90.3 ± 1.5	91.6 ± 1.8^{A}
2	First grade	88.8 ± 1.8	85.2 ± 0.9	88.0 ± 1.6	88.0 ± 0.9	88.0 ± 0.9	87.6 ± 1.2^{B}
3	Second grade	87.8 ± 1.6	81.0 ± 1.4	86.8 ± 1.8	87.2 ± 0.8	86.5 ± 0.8	85.9 ± 1.4^{C}
4	Second grade	85.3 ± 2.5	81.2 ± 1.8	87.2 ± 1.8	86.8 ± 0.8	85.3 ± 0.7	85.2 ± 1.6^{C}
5	Third grade	81.8 ± 2.4	80.3 ± 1.8	81.3 ± 1.2	80.3 ± 1.5	82.2 ± 0.6	81.2 ± 1.6^{D}
6	Fourth grade	77.2 ± 2.2	81.2 ± 2.3	81.5 ± 1.0	78.0 ± 1.6	78.3 ± 0.8	79.2 ± 1.7^{DE}
7	Fifth grade	74.8 ± 1.7	77.5 ± 1.9	80.7 ± 1.5	76.7 ± 1.5	77.5 ± 0.9	77.4 ± 1.6^{E}

Means ± SD, $n = 6$.
[a] Mean values with different letters were significantly different at the $p = 0.05$ level.

RESULTS AND DISCUSSION

Correlation of concentration of nitrogen, amino acids, caffeine and polyphenols to sensory quality

Table 2 shows that the TQS decreased with the decrease in grade of the pu-erh tea, ranging from 91.6 ± 1.8 to 77.4 ± 1.6. The interval of the sensory quality scores between grades varied from grade to grade. The difference between the special grade (sample 1) and the first grade (sample 2) and between the second grade (samples 3 and 4) and the third grade (sample 5) was about 4 points while that between the other grades was 2 points or less (Table 2). Statistical analysis showed that the TQS of the special grade was significantly higher than the other grades and that of the first grade was significantly higher than those of the second grade and below. However, samples 3 and 4, the two samples of the second-grade pu-erh tea, had similar TQS, although they were produced by different producers (Tables 1 2). There were no significant differences in TQS between third and fourth grades and between fourth and fifth grades (Table 2).

Table 3 showed that caffeine concentration of sample 6 was 7.39 ± 0.2 mg g^{-1}, which was one-fifth the mean value of the seven samples. It may be processed from fresh leaves of a low-caffeine tea cultivar, but the exact reason remains to be determined. The mean concentrations of amino acids and polyphenols of the seven samples were 15.7 ± 3.6 and 67.0 ± 11.4 mg g^{-1} respectively, which were lower than those of black tea. The average concentration of amino acids and polyphenols in black tea was 28.6 and 89.6 mg g^{-1}.[7] The difference may result from the long and full fermentation of the pu-erh tea. Table 3 also shows that the mean concentration of nitrogen in the pu-erh tea samples was 51.7 ± 4.1 mg g^{-1}, which was higher than that of black tea (39.0 mg g^{-1}) described in our previous paper.[7]

Spearman's linear correlation analysis showed that the amino acid concentration was significantly correlated to individual quality attributes and TQS, whereas correlations of nitrogen, caffeine and polyphenols to individual quality attributes and TQS were not statistically significant (Table 4).

Correlation of concentration of tea catechins to sensory quality

Tea catechins are the major components of polyphenols in tea and consist of at least eight compounds, ie gallocatechin (GC), epigallocatechin (EGC), catechin (C), epicatechin (EC), epigallocatechin gallate (EGCG), gallocatechin gallte (GCG), epicatechin gallate (ECG) and catechin gallate (CG) (Table 5). The concentration of GC of sample 5 was the highest among the tested catechin compounds. On average, the major constituents of the tea catechins were GC

Table 3. Content of nitrogen, amino acids, caffeine and polyphenols of various tea samples

Sample no	Caffeine	Amino acids	Polyphenols	Nitrogen
1	47.0 ± 1.2	21.0 ± 0.6	74.6 ± 2.2	57.3 ± 0.2
2	46.7 ± 1.0	19.1 ± 0.6	64.2 ± 2.0	56.4 ± 0.2
3	42.0 ± 1.4	17.1 ± 0.4	84.7 ± 2.5	47.7 ± 0.2
4	32.2 ± 0.7	15.1 ± 0.4	64.2 ± 1.9	49.7 ± 0.2
5	43.2 ± 1.0	11.0 ± 0.3	48.2 ± 0.9	53.9 ± 0.2
6	7.39 ± 0.2	14.0 ± 0.4	62.0 ± 1.1	48.3 ± 0.1
7	41.5 ± 1.4	12.3 ± 0.4	70.9 ± 1.8	48.7 ± 0.1
Mean	37.1 ± 14.0	15.7 ± 3.6	67.0 ± 11.4	51.7 ± 4.1

Mean ± SD, mg g^{-1}, $n = 2$.

Table 4. Spearman's correlation of nitrogen, amino acids, caffeine and polyphenols to sensory quality

	Appearance	Aroma	Liquor	Taste	Infused	TQS
Nitrogen	0.571	0.577	0.571	0.571	0.571	0.571
Amino acids	0.893**	0.847*	0.929**	0.893**	0.893**	0.893**
Caffeine	0.750	0.414	0.536	0.750	0.750	0.750
Polyphenols	0.432	0.136	0.342	0.432	0.432	0.432

* Correlation is significant at the 0.05 level (two-tailed).
** Correlation is significant at the 0.01 level (two-tailed).

Table 5. Concentration of catechins of various tea samples

Sample no	GC	EGC	C	EC	EGCG	GCG	ECG	CG	Total catechins
1	0.38 ± 0.02	0.98 ± 0.04	0.41 ± 0.03	0.48 ± 0.03	0.15 ± 0.01	0.02 ± 0.01	0.12 ± 0.02	0.44 ± 0.03	2.98 ± 0.14
2	3.77 ± 0.28	1.37 ± 0.08	0.45 ± 0.01	0.39 ± 0.02	0.11 ± 0.02	0.09 ± 0.02	0.53 ± 0.04	0.05 ± 0.01	6.76 ± 0.33
3	0.22 ± 0.03	1.25 ± 0.13	0.46 ± 0.02	2.36 ± 0.11	0.42 ± 0.02	0.18 ± 0.02	0.46 ± 0.08	0.89 ± 0.03	6.24 ± 0.42
4	0.39 ± 0.16	0.86 ± 0.09	1.29 ± 0.11	1.27 ± 0.06	0.29 ± 0.03	0.10 ± 0.03	0.29 ± 0.03	0.76 ± 0.04	5.24 ± 0.46
5	23.7 ± 0.28	0.58 ± 0.02	0.40 ± 0.08	0.30 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.46 ± 0.03	0.29 ± 0.01	25.8 ± 1.05
6	3.16 ± 0.08	0.13 ± 0.03	0.10 ± 0.01	0.12 ± 0.03	0	0	0.03 ± 0.01	0	3.53 ± 0.27
7	1.63 ± 0.04	0.96 ± 0.05	1.90 ± 0.02	2.19 ± 0.10	0.27 ± 0.03	0.16 ± 0.01	0.15 ± 0.06	1.05 ± 0.03	8.31 ± 0.34
Mean	4.75 ± 8.37	0.88 ± 0.42	0.72 ± 0.64	1.02 ± 0.93	0.18 ± 0.15	0.08 ± 0.07	0.29 ± 0.20	0.50 ± 0.41	8.41 ± 7.89

Mean ± SD, mg g^{-1}, $n = 2$.

Table 6. Spearman's correlation coefficient between catechins and quality attributes of pu-erh tea

	Appearance	Aroma	Liquor	Taste	Infused	TQS
GC	−0.357	−0.180	−0.357	−0.357	−0.357	−0.357
EGC	0.679	0.324	0.536	0.679	0.679	0.679
C	−0.107	−0.342	−0.107	−0.107	−0.107	−0.107
EC	0.107	−0.288	0	0.107	0.107	0.107
EGCG	0.214	−0.162	0.143	0.214	0.214	0.214
GCG	−0.071	−0.505	−0.214	−0.071	−0.071	−0.071
ECG	0.324	−0.109	0.108	0.324	0.324	0.324
CG	−0.143	−0.505	−0.250	−0.143	−0.143	−0.143
Total	−0.429	−0.739	−0.643	−0.429	−0.429	−0.429

(4.74 mg g^{-1}) and EC (1.02 mg g^{-1}) in the pu-erh tea (Table 5). EGCG, GCG and CG were not detected in sample 6. Our previous studies showed that the major constituents of the eight tea catechins were EGCG and GC in green tea,[16] and GC and ECG in black tea.[7] This suggests that the composition of tea catechins in the pu-erh tea is different from that of green and black teas. Furthermore, the concentration of the tea catechins in the pu-erh tea was much lower than that in green and black teas. Table 5 shows that the mean concentration of the total catechins in the seven pu-erh tea samples was 8.41 mg g^{-1}, while it was 112 mg g^{-1} in green tea[16] and 33.3 mg g^{-1} in black tea.[7] This means that the concentration of total catechins in the Pu-erh tea is only about 7.5% of that of green tea and 25% of that of black tea. Tea fermentation is an oxidation process of tea polyphenols, especially tea catechins. The results suggest that the pu-erh tea is a more fully fermented tea than black tea.

Spearman's linear correlation analysis showed that concentration of catechins had no statistically significant correlation to individual quality attributes and TQS of pu-erh tea (Table 6). This may suggest that pu-erh tea quality could not be predicted by concentration of individual tea catechins or total tea catechins because they were deeply oxidized during fermentation, resulting in a low concentration.

Infusion colour difference and its correlation to sensory quality

The ΔL of Pu-erh tea infusions ranged from −54.6 to −43.5, meaning that the pu-erh tea infusions are

Table 7. Infusion colour indicators of various tea samples

Sample no	ΔL	Δa	Δb	ΔE
1	−43.5 ± 0.71	25.5 ± 0.71	43.8 ± 1.13	66.8 ± 0.42
2	−50.6 ± 0.85	29.0 ± 0.71	43.6 ± 0.71	72.8 ± 0.57
3	−46.3 ± 1.84	26.4 ± 0.57	45.2 ± 0.28	69.9 ± 1.27
4	−51.1 ± 1.56	25.2 ± 0.28	13.9 ± 0.28	72.0 ± 0.99
5	−54.6 ± 1.70	34.5 ± 1.42	45.8 ± 1.13	79.2 ± 1.13
6	−51.9 ± 1.27	28.1 ± 0.14	44.9 ± 0.14	74.2 ± 1.27
7	−49.2 ± 0.28	31.2 ± 0.57	46.3 ± 1.41	74.5 ± 0.71
Mean	−49.6 ± 3.69	28.6 ± 3.37	40.5 ± 11.8	72.8 ± 3.89

Mean ± SD, $n = 2$.

Table 8. Spearman's correlations coefficients between colour parameters and quality attributes

	Appearance	Aroma	Liquor	Taste	Infused	TQS
L	0.536	0.306	0.464	0.536	0.536	0.536
A	−0.500	−0.595	−0.679	−0.500	−0.500	−0.500
B	−0.607	−0.811*	−0.821*	−0.607	−0.607	−0.607
E	−0.786*	−0.685	−0.821*	−0.786*	−0.786*	−0.786*

* Correlation is significant at the 0.05 level (two-tailed).

Table 9. Concentration of volatile components of various tea samples

Sample no	1	2	3	4	5	6	7	Mean
n-Valeraldehyde	0	0	0	0	0	0	0	0
n-Caproaldehyde	0	0	0	0	0.45 ± 0.03	0	10.1 ± 0.21	1.51 ± 3.79
1-Penten-3-ol	0.14 ± 0.01	0	0.54 ± 0.03	0.81 ± 0.02	0.39 ± 0.01	0.50 ± 0.03	2.07 ± 0.03	0.64 ± 0.69
Ethyl caproate	0.14 ± 0.03	0	0	0.54 ± 0.06	0.29 ± 0.01	0.30 ± 0.07	0.49 ± 0.01	0.25 ± 0.22
2-Methyl-2-hepten-6-one	0	0	5.75 ± 0.21	0	0.23 ± 0.03	0.08 ± 0.01	0.27 ± 0	0.90 ± 2.14
Linalool oxide I	3.23 ± 0.21	32.0 ± 0.71	12.2 ± 0.28	3.99 ± 0.01	1.63 ± 0.04	3.37 ± 0.10	3.17 ± 0.03	8.51 ± 10.9
Linalool oxide II	5.37 ± 0.03	56.4 ± 1.98	5.08 ± 0.04	3.81 ± 0.01	2.54 ± 0.06	2.53 ± 0.04	2.44 ± 0.06	11.2 ± 20.0
Linalool	3.00 ± 0.07	0	3.25 ± 0.01	2.07 ± 0.07	1.80 ± 0.14	1.67 ± 0.10	1.44 ± 0.03	1.89 ± 1.08
Phenyl aldehyde	0.64 ± 0.06	0	0.21 ± 0.01	0.59 ± 0.04	0.57 ± 0.03	0.46 ± 0.01	0.47 ± 0	0.42 ± 0.23
Terpineol	0	0	3.16 ± 0.14	1.48 ± 0.01	0	2.23 ± 0.04	1.11 ± 0.01	1.14 ± 1.24
Benzyl acetate	2.04 ± 0.06	19.4 ± 0.57	3.23 ± 0.01	2.57 ± 0.10	4.46 ± 0.08	3.42 ± 0.03	1.70 ± 0.14	5.26 ± 6.30
Citral	0.66 ± 0.03	27.4 ± 0.57	10.3 ± 0.28	0.60 ± 0.03	1.08 ± 0.04	0.66 ± 0.08	0.42 ± 0.03	5.87 ± 10.1
Citronellol	0	0	0.47 ± 0.03	0	0	0	0	0.07 ± 0.18
Nerol	0	2.99 ± 0.14	0.40 ± 0.03	0	0.73 ± 0.01	0.09 ± 0.01	0	0.60 ± 1.09
Geraniol	0.33 ± 0.04	2.44 ± 0.28	2.47 ± 0.10	0.32 ± 0.03	1.13 ± 0.04	0.33 ± 0.01	0.44 ± 0.06	1.07 ± 0.99
Trans-geraniol	2.89 ± 0.13	60.1 ± 1.56	1.53 ± 0.04	0	0.26 ± 0.01	1.05 ± 0.03	0.22 ± 0.03	9.44 ± 22.4
β-Ionone	0.77 ± 0.03	80.2 ± 3.11	0.69 ± 0.06	0	1.12 ± 0.03	0.10 ± 0.01	1.63 ± 0.01	12.1 ± 30.0
Benzoic acid	0.88 ± 0.04	12.9 ± 1.56	0.80 ± 0.07	1.08 ± 0.01	1.22 ± 0.03	0.38 ± 0.03	1.33 ± 0.04	2.66 ± 4.53
Total volatiles	20.1	294	50.1	17.9	17.9	17.2	27.3	63.5

Mean ± SD, $\mu g\, g^{-1}$, $n = 2$.

darker than distilled water. The average values of Δa and Δb of the pu-erh tea infusions were 28.6 and 40.5, respectively, suggesting that the pu-erh tea infusions are red and yellow in colour. Because distilled water was used as control in the present experiment, ΔE represents a total colour difference between the tea infusion and distilled water. The average ΔE of the pu-erh tea infusions ranged from 66.8 to 79.2, showing that they are darker and have a deeper hue than water (Table 7).

Spearman's linear correlation analysis showed that there were significantly negative correlations of ΔE to appearance, liquor, taste, infused leaf and TQS. Δb was significantly correlated with aroma and liquor, whereas the other infusion colour difference parameters were not significantly correlated to individual quality score and TQS (Table 8). These results suggested that higher-grade pu-erh tea infusion had a lighter colour and hue.

Volatile constituents and their correlation to sensory quality

Concentration of the detected volatile constituents varied greatly between compounds and between the pu-erh tea samples. *N*-valeraldehyde was not detected in all pu-erh tea samples and citronellol was detected only in sample 3. The mean concentration of the detected individual volatile compound ranged from 0.07 (citronellol) to 12.1 $\mu g\,g^{-1}$ (β-ionone). The concentration of β-ionone was the highest, and linalool oxide II the next, while citronellol was the lowest (Table 9). Total concentration of the detected volatile constituents was the highest in sample 2, being 4.6 times the average of the seven samples, and 17.1 times that in the lowest sample, no 6.

Spearman's correlation analysis showed that the correlation of concentration of linalool to individual quality attributes and TQS was significant statistically. Concentration of *n*-caproaldehyde was significantly correlated to liquor and aroma scores. Correlation of the other detected volatile compounds to quality was not statistically significant (Table 10).

Regressive relationship of chemical composition to total quality

Table 10 shows that there are some chemical parameters which are significantly correlated to individual quality attributes and TQS for pu-erh tea. It will be interesting to extract some representative indicators to construct mathematic models to estimate the TQS of pu-erh tea. Principal component analysis (PCA) begins by finding a linear combination of variables (component) that accounts for as much variation in the original variables as possible. It then finds another component that accounts for as much of the remaining variation as possible and is uncorrelated with the previous component, continuing in this way until there are as many components as original variables. Usually, a few components will account for most of the variation, and these components can be used to replace the original variables. This method is most often used to reduce the number of variables in the data file. Principal component analysis of the present data set showed that geraniol had the highest communality value (0.979), and citral (0.973) the next highest. According to the size of communalities, polyphenols (0.949) in component 2, linalool oxide I (0.632) in component 4 and caffeine (0.813) in component 5 were extracted. Δa (0.655), amino acids (0.652) and *n*-caproaldehyde (0.651) had close communalities in component 3 and they were extracted for further analysis (Table 11). Table 12 showed that the cumulative variance from principal

Table 10. Spearman's correlations coefficient between volatiles and quality attributes

Volatiles[a]	Appearance	Aroma	Liquor	Taste	Infused	TQS
n-Caproaldehyde	−0.668	−0.809*	−0.802*	−0.668	−0.668	−0.668
1-Penten-3-ol	−0.679	−0.685	−0.607	−0.679	−0.679	−0.679
Ethyl caproate	−0.667	−0.345	−0.432	−0.667	−0.667	−0.667
Linalool oxide 1	−0.519	−0.823*	−0.741	−0.519	−0.519	−0.519
Linalool oxide 2	0.500	0.487	0.571	0.500	0.500	0.500
Linalool	0.964**	0.757*	0.893**	0.964**	0.964**	0.964**
Phenyl aldehyde	0.393	0.108	0.286	0.393	0.393	0.393
Terpineol	0.071	0.126	0.143	0.071	0.071	0.071
Phenyl aldehyde	−0.334	−0.280	−0.259	−0.334	−0.334	−0.334
Citral	0.179	0.180	0.107	0.179	0.179	0.179
Citronellol	0.559	0.300	0.360	0.559	0.559	0.559
Nerol	0.204	−0.206	0	0.204	0.204	0.204
Geraniol	0.185	0	0	0.185	0.185	0.185
Trans-geraniol	0.162	−0.282	−0.144	0.162	0.162	0.162
β-Ionone	0.714	0.631	0.607	0.714	0.714	0.714
Benzoic acid	0.071	−0.126	−0.107	0.071	0.071	0.071
Total volatiles	0	−0.162	−0.071	0	0	0

[a] The linear correlation of *n*-valeraldehyde and 2-methyl-2-hepten-6-one to quality attributes were 0 and so they are not listed in the table 1.

* Correlation is significant at the 0.05 level (two-tailed).

** Correlation is significant at the 0.01 level (two-tailed).

Table 11. Component matrix extracted by principal component analysis[a]

Quality attributes	Component				
	1	2	3	4	5
Nitrogen	0.568	−0.327	−0.334	0.056	0.674
Amino acids	0.508	0.523	−0.608	0.030	0.313
Caffeine	0.384	0.224	0.381	0.063	0.806
Polyphenols	−0.040	0.975	−0.099	−0.006	0.065
C	−0.251	−0.616	0.630	0.175	0.334
CG	−0.499	0.605	0.500	−0.213	0.259
EC	−0.301	0.745	0.577	−0.141	0.011
ECG	0.640	0.039	0.536	0.389	0.097
EGC	0.575	0.614	0.288	−0.103	0.422
EGCG	−0.275	0.738	0.517	−0.299	0.134
GC	0.009	−0.779	0.390	0.465	0.158
GCG	0.031	0.708	0.670	−0.188	−0.032
Total catechins	−0.037	−0.646	0.595	0.416	0.228
ΔL	−0.031	0.773	−0.374	0.011	0.447
Δa	0.031	−0.677	0.645	0.094	0.064
Δb	0.198	−0.051	0.167	0.306	0.057
ΔE	−0.067	−0.758	0.584	0.041	−0.280
n-Caproaldehyde	−0.324	0.074	0.546	−0.626	0.135
1-Penten-3-ol	−0.563	0.145	0.541	−0.579	−0.005
Ethyl caproate	−0.689	−0.333	0.148	−0.549	−0.006
2-Methyl-2-hepten-6-one	−0.026	0.73	0.317	0.574	−0.189
Linalool oxide I	0.961	0.207	0.078	−0.087	−0.141
Linalool oxide II	0.977	−0.021	−0.044	−0.210	−0.037
Linalool	−0.624	0.485	−0.202	0.518	0.250
Phenyl aldehyde	−0.822	−0.329	0.262	−0.024	0.361
Terpineol	−0.374	0.590	0.082	0.212	−0.678
Benzyl acetate	0.975	−0.139	0.047	−0.119	−0.110
Citral	0.965	0.192	0.122	−0.009	−0.128
Citronellol	−0.004	0.746	0.278	0.574	−0.192
Nerol	0.976	−0.151	0.147	−0.028	−0.053
Geraniol	0.736	0.345	0.388	0.414	−0.127
Trans-geraniol	0.974	−0.046	−0.086	−0.216	−0.046
β-Ionone	0.968	−0.065	0.032	−0.233	−0.053
Benzoic acid	0.962	−0.073	0.067	−0.251	−0.020
Total volatiles	0.976	0.029	0.068	−0.189	−0.075

[a] Five components were extracted by principal component analysis.

Table 12. Total variance explained

Component	Initial eigenvalues		
	Total	Percentage variance	Cumulative (%)
1	13.12	37.48	37.48
2	8.94	25.53	63.01
3	5.25	14.99	78.01
4	3.33	9.50	87.51
5	2.78	7.95	95.45

component 1 to component 5 accounted for 95.13% of the total variance of the data set with 34 variables.

If the parameters of components 1–5 were used as independent variables and TQS as dependent variables to construct mathematical models, the results showed that statistical significance of the resulted Pearson's linear regressive equations was dependent on the parameter from principal component 3 (Table 13). If *n*-caproaldehyde was used as the independent variable, the equations were significant (models 3 and 6, $p < 0.01$). However, when *n*-caproaldehyde was replaced by Δa or amino acids, the equations were not significant (models 1, 2, 4 and 5, $p > 0.05$).

Table 14 shows that citral and geraniol of component 1 were significantly correlated to nine and eight chemical parameters, respectively. Chemical parameters from components 2–5 were significantly correlated to six chemical parameters and two infusion colour parameters. The correlation analysis found that the chemical parameters from the five components were also correlated to the other chemical parameters, which were not listed in Table 14 because they were not statistically significant. That was why the chemical parameters from the five components accounted for 95.13% of the variation in the 34 original variables listed in Table 11. If we further analyse the components in Table 14, it will be found that components 1 and 4 were a linear combination of variables of volatile compounds which were related to

Table 13. Mathematic models for estimation of pu-erh tea quality

Model no	Pearson's linear regressive equation	SS reg[a]	SS Res[b]	Significant level	R^2	SEE[c]
1	TQS = 134.22 + 0.78geraniol − 0.23polyphenols − 1.57Δ*a* + 0.30linalool oxide I + 0.24caffeine	128.103 (df = 5)[d]	22.105 (df = 1)	$p = 0.604$	0.853	4.70
2	TQS = 73.95 − 1.53geraniol − 0.32polyphenols + 1.76amino acids + 0.85linalool oxide I + 0.10caffeine	146.674 (df = 5)	3.535 (df = 1)	$p = 0.257$	0.976	1.88
3	TQS = 57.47 − 0.18geraniol + 0.33polyphenols − 1.14n-caproaldehyde − 1.38linalool oxide I + 0.21caffeine	150.207 (df = 5)	0.002 (df = 1)	$p = 0.006$	0.999	0.05
4	TQS = 133.94 − 0.12citral − 0.23polyphenols − 1.56Δ*a* + 0.34linalool oxide I + 0.25caffeine	127.738 (df = 5)	22.470 (df = 1)	$p = 0.609$	0.850	4.74
5	TQS = 73.56 − 0.25citral − 0.30polyphenols + 1.76amino acids + 0.74linalool oxide I + 0.09caffeine	146.077 (df = 5)	4.131 (df = 1)	$p = 0.278$	0.972	2.03
6	TQS = 57.42 − 0.03citral + 0.33polyphenols − 1.14n-caproaldehyde − 1.40linalool oxide I + 0.20caffeine	150.205 (df = 5)	0.004 (df = 1)	$p = 0.009$	0.999	0.00

[a] SS reg, sum of squares of regression; [b] SS res, sum of squares of residual; [c] SEE, standard error of the estimation; [d] df, degrees of freedom.

Table 14. Various linear combinations of variables

Component	Representative variable	Other variables in the linear combination[a]
1	Citral	Linalool oxide II (0.939**), linalool (0.958**), phenyl aldehyde (−0.773*), terpineol (0.802*), citronellol (0.936**), geraniol (0.989**), β-ionone (0.987**), benzoic acid (0.989**), total essential (0.985**)
	Geraniol	Linalool oxide II (0.928**), linalool (0.963**), terpineol (0.808*), citral (0.989**), citronellol (0.938**), β-ionone (0.965**), benzoic acid (0.969**), total essential (0.968**)
2	Polyphenols	GC (−0.791*), EGCG (0.758*), Δ*L* (0.866), Δ*E* (−0.809*)
3	*n*-Caproaldehyde	C (0.814*), 1-penten-3-ol (0.921**)
4	Linalool oxide I	Nerol (0.999**)
5	Caffeine	EGC (0.809*)

[a] Data in parenthesis are Spearman's correlation coefficients.
* Correlation is significant at the 0.05 level (two-tailed).
** Correlation is significant at the 0.01 level (two-tailed).

Table 15. Residual statistics

		Minimum	Maximum	Mean	Standard deviation	*N*
Model 3	Predicted value	77.40	91.62	84.01	5.00	7
	Residual	−0.02	0.04	0	0.02	7
	Standardized predicted value	−1.32	1.52	0	1.00	7
	Standardized residual	−0.41	0.84	0	0.41	7
Model 6	Predicted value	77.40	91.62	84.01	5.00	7
	Residual	−0.02	0.052	0	0.03	7
	Standardized predicted value	−1.32	1.52	0	1.00	7
	Standardized residual	−0.39	0.85	0	0.41	7

tea aroma. Component 5 was a linear combination of variables related to tea taste, ie caffeine and EGC. Component 2 was a linear combination of variables related to tea taste (polyphenols, GC and EGCG) and tea infusion colour (Δ*L* and Δ*E*), whereas component 3 was a linear combination of variables related to tea taste (C) and aroma (*n*-caproaldehyde and 1-penten-3-ol). That suggested that most variation in variables related to taste, aroma and infusion colour of the pu-erh tea samples was included in the five components.

Residual statistics showed that the predicted TQS value of models 3 and 6 ranged from 77.40 to 91.62, with a standard deviation of 5.00 (Table 15).

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