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Volatile profile analysis and quality prediction of Longjing tea (*Camellia sinensis*) by HS-SPME/GC-MS

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Abstract: This study aimed to analyze the volatile chemical profile of Longjing tea, and further develop a prediction model for aroma quality of Longjing tea based on potent odorants. A total of 21 Longjing samples were analyzed by headspace solid phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS). Pearson's linear correlation analysis and partial least square (PLS) regression were applied to investigate the relationship between sensory aroma scores and the volatile compounds. Results showed that 60 volatile compounds could be commonly detected in this famous green tea. Terpenes and esters were two major groups characterized, representing 33.89% and 15.53% of the total peak area respectively. Ten compounds were determined to contribute significantly to the perceived aroma quality of Longjing tea, especially linalool (0.701), nonanal (0.738), (*Z*)-3-hexenyl hexanoate (−0.785), and β-ionone (−0.763). On the basis of these 10 compounds, a model (correlation coefficient of 89.4% and cross-validated correlation coefficient of 80.4%) was constructed to predict the aroma quality of Longjing tea. Summarily, this study has provided a novel option for quality prediction of green tea based on HS-SPME/GC-MS technique.

Key words: Partial least square (PLS) regression, Green tea, Headspace solid phase microextraction (HS-SPME), Volatile profile, Quality prediction
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1 Introduction

Green tea is a non-fermented tea that is consumed around the world and is especially popular in East Asian countries. Normally, the quality of green tea is determined by human sensory evaluation based on "shape, color, aroma, and taste". Among these characteristics, aroma is an essential criterion in the evaluation of sensory scores and the commercial description of tea. Besides the conventional sensory evaluation of aroma quality, gas chromatography-olfactometry (GC-O) and aroma extract dilution analysis (AEDA) are also commonly applied to odor description and the determination of potent odorants in green tea (Kumazawa and Masuda, 1999; 2002). Unfortunately, these methods all rely upon highly trained personel, and are likely affected by individual and subjective factors, such as age, emotion, and preference.

Recently several attempts have been made towards the objective evaluation of green tea quality by GC-mass spectrometry (GC-MS) (Pongsuwan *et al.*, 2007; 2008; Jumtee *et al.*, 2011). The experimental procedures presented in those studies were time-consuming or involved complex sample pretreatment. Togari *et al.* (1995) and Wang *et al.* (2009) have also investigated a correlation between sensory properties and the volatile chemical profiles of green teas. Having considered the previous work in this area, we now present a study in which the final aroma quality of green tea can be predicted by potent

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odorants extracted by headspace solid phase microextraction (HS-SPME). HS-SPME has been proven to be a fast, simple, and convenient method for the analysis of volatile compounds in tea (Augusto and Zini, 2002; Lv *et al.*, 2012). This technique has also been successfully applied to the quality assessments of apples (Lavilla *et al.*, 1999), strawberries (Azodanlou *et al.*, 2003a), vegetable oils (Jeleń *et al.*, 2000), mandarin juices (Alvarez *et al.*, 2011), and tomatoes and apricots (Azodanlou *et al.*, 2003b). Therefore, it would be interesting to investigate the feasibility of this method as a tool to predict the aromatic quality of green tea.

Longjing tea is the most famous Chinese green tea, and is popular for its light green color, fragrant scent, special tight, and plat shape (Yu and Zhang, 2002). There have been several reports on the volatile chemical analysis of Longjing tea (Kawakami and Yamanishi, 1983; Kumazawa and Masuda, 2002; Wang and Ruan, 2009); however, their researches were limited to the analysis of either a single sample or Longjing produced in just one region. Therefore, we have compiled a comprehensive analysis of the volatile chemical profiles of Longjing tea of a large sample number and from different producing regions.

In this study, we aimed to analyze the volatile profile of Longjing tea by HS-SPME/GC-MS combined with sensory evaluation, and utilize partial least square (PLS) regression to build an accurate model to predict aroma quality of Longjing tea based on the presence of potent odorants.

2 Materials and methods

2.1 Tea samples

A total of 21 Longjing tea samples (spring-harvested, fine tea) were collected from the three traditional tea-producing regions (six samples from Qiantang, six samples from Xihu, and nine samples from Yuezhou). All tea samples were kept in aluminum foil bags and stored in the dark at 4 °C before analysis.

2.2 Sensory evaluation

Three grams of tea sample were infused with 150 ml boiled and filtered water, and the tea slurry was poured out after 5 min of brewing. The aroma of the tea leaf remains was assessed by three national sensory evaluators of tea from Institute of Tea Science of Zhejiang University. The evaluators were all well-trained experts with more than eight years of experience in the sensory evaluation of tea. Group-evaluation was performed according to GB/T 23776-2009 (AQSIQ and SAC, 2009) (Chinese National Standard-Methodology of sensory evaluation of tea). First, the prime evaluator provided an aroma score (70–100 points) and notes description, which was then confirmed or revised by the other two evaluators until a consistent evaluation was reached.

According to Chinese national standard GB/T 23776-2009, seven samples with floral, tender, long-lasting notes, and aroma scores of 90–99 points were classified as Grade One, while 12 samples with green, faint notes, and aroma scores of 80–89 points were classified as Grade Two. Since all of the Longjing samples were spring-harvested and fine tea, their final aroma scores were all above 80 points.

2.3 HS-SPME analysis

Ten grams of Longjing tea samples were infused with distilled water (30 ml, 100 °C) in a 150-ml glass septum vial, and kept in a 60 °C water bath for 6 min. After the equilibration, commercially available SPME fibre (Supelco, Bellefonte PA, USA) coated with 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) (Supelco) was rapidly inserted into the headspace of the vial. The extraction was kept at room temperature for 60 min. The PDMS/DVB fibre was preconditioned for 5 min in the injection port of the GC at 220 °C before each extraction. The extraction was carried out in triplicate for each tea sample.

2.4 GC-MS analysis

An Agilent 6890 gas chromatograph coupled with an Agilent 5973N mass spectrometer was used to perform the aroma analysis. An HP-5MS capillary column (30 m×0.25 mm×0.25 μm; Agilent Technologies, USA) was equipped, with purified helium as the carrier gas, at a constant flow rate of 1 ml/min. After extraction, the fibre was desorbed in the injector port of the GC at 220 °C for 3.5 min. The oven temperature was held at 50 °C for 5 min and then increased to 220 °C at a rate of 3 °C/min. Ion source temperature was at 200 °C and spectra was produced in the electron impact (EI) mode at 70 eV. The mass spectrometer was operated in the full scan, and the peak area was determined by ChemStation software (Agilent Technologies).

2.5 Compound identification

Peak identifications were made by searching NIST98 MS data library (a match quality of 95% minimum was used as a criterion) and comparison of their retention indices (RIs) with the published data. In order to get the Kovats' RI for each peak, 1 µl *n*-alkane mixture (C_8–C_{20}, Sigma-Aldrich, USA) was injected under the same GC conditions.

2.6 Data analysis

The relative percentages of the detected peaks were obtained by peak-area normalization, all relative response factors being taken as one. The chromatographic data were unit variance transformed before partial least square (PLS) regression analysis. PLS regression was performed by SIMCA-P (Version 11.5, Umetrics, Umeå, Sweden). The efficiency and reliability of the PLS regression model were explained by the parameters as follows: the fraction of sum of squares of all the *Y*'s explained by the PLS component (R2*Y*), the fraction of the total variation of the *Y*'s that can be predicted by the PLS component (Q2*Y*), and root mean square error of the fit for observations in the work set (RMSEE). The cross validation threshold for the PLS component was 0.05. The PLS component was significant when $Q2Y>0.05$. Pearson's correlation analysis and tukey's test of the significance of differences were processed by SPSS statistical package (Version 16.0 for Windows, SPSS Inc., Chicage, IL, USA).

3 Results and discussion

3.1 Volatile compounds

HS-SPME/GC-MS was used to characterize the volatile compounds present in each of the 21 Longjing samples. Sixty volatile compounds were commonly detected in these samples, and they jointly represented an average of 73.00% of the headspace extracts. Forty-eight peaks were identified by their mass spectra and RI, including three monoterpenes, eleven sesquiterpenes, three terpene alcohols, two alcohols, six esters, three aldehydes, four ketones, and seven alkanes, as listed in Table 1. Twelve volatile compounds detected in Longjing samples remained unidentified or only partially characterized. A typical GC-MS total ion chromatogram (TIC) of the volatile chemical profile of Longjing tea is shown in Fig. 1. The relative contributions (peak area percentages) from the corresponding volatile compounds are listed in Table 1.

It is worth noting that 16 compounds were detected in Longjing tea for the first time: myrcene, limonene, ocimene, decanal, α-cubebene, α-ionene, copaene, hexyl hexanoate, β-caryophyllene, γ-muurolene, ar-curcumene, γ-cadinene, δ-cadinene, cadine-1,4-diene, 1-hexadecene, hexanoic acid, and anhydride. Twelve of these compounds are terpenes, indicating that the PDMS/DVB fibre coating might have good selectivity for terpene compounds. A more in-depth study is necessary to investigate the sensory contribution of these "new" odorants to Longjing aroma.

Linalool (9.77%), (*Z*)-3-hexenyl hexanoate (8.19%), and geraniol (5.73%) were three principal

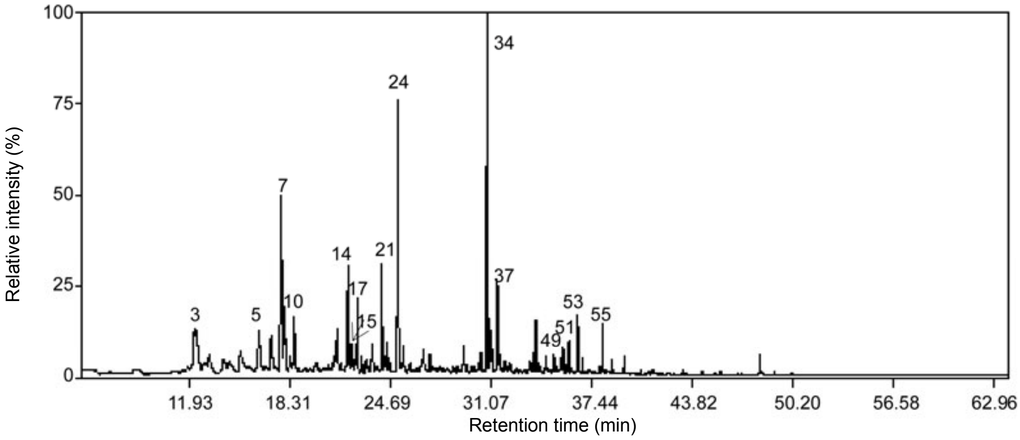

Fig. 1 Typical total ion chromatogram of the volatile compounds in a Longjing tea sample
The peak numbers refer to the compounds listed in Table 1

Table 1 Volatile compounds commonly detected in Longjing tea by HS-SPME/GC-MS

No.	Compound[a]	RI[b]	ID method[c]	Relative content (%)		No.	Compound	RI	ID method	Relative content (%)	
				Mean	Range					Mean	Range
1	1-Octen-3-ol	984	MS, RI	0.32	0.10–0.68	31	α-Cubebene	1349	MS, RI	0.56	0.15–1.05
2	Unknown	989	–	0.51	0.12–1.56	32	α-Ionene	1354	MS, RI	0.14	0.10–0.19
3	Myrcene	992	MS, RI	2.21	1.18–5.75	33	Copaene	1373	MS, RI	0.38	0.28–0.54
4	Limonene	1031	MS, RI	0.49	0.15–1.34	34	(*Z*)-3-hexenyl hexanoate	1383	MS, RI	8.19	3.50–14.63
5	Ocimene	1052	MS, RI	1.26	0.35–2.50	35	Hexyl hexanoate	1387	MS, RI	1.27	0.64–1.93
6	Unknown	1074	–	2.26	1.21–3.59	36	(*E*)-2-Hexenyl hexanoate	1391	MS, RI	1.41	0.49–2.54
7	Linalool	1101	MS, RI	9.77	4.65–22.45	37	Jasmone	1398	MS, RI	1.51	0.83–2.72
8	Nonanal	1104	MS, RI	3.02	1.04–4.20	38	Tetradecane	1400	MS, RI	1.53	0.77–2.56
9	Phenylethyl alcohol	1111	MS, RI	0.86	0.45–1.33	39	Longifolene	1402	MS, RI	0.41	0.14–1.30
10	Unknown	1116	–	2.21	0.60–4.58	40	Cedrene	1410	MS, RI	0.92	0.29–3.11
11	Linalool oxide (pyranoid)	1168	MS, RI	0.13	0.04–0.35	41	β-Caryophyllene	1418	MS, RI	0.67	0.20–1.55
12	Unknown	1172	–	0.98	0.49–1.53	42	α-Ionone	1428	MS, RI	0.32	0.18–0.56
13	Naphthalene	1178	MS, RI	0.51	0.11–0.91	43	Geranyl acetone	1454	MS, RI	0.58	0.38–0.81
14	(*Z*)-3-Hexenyl butyrate	1188	MS, RI	3.19	1.12–5.52	44	β-Farnesene	1458	MS, RI	0.73	0.20–3.13
15	Methyl salicylate	1192	MS, RI	1.51	0.80–2.89	45	Alkane	1463	MS	0.29	0.19–0.52
16	Safranal	1197	MS, RI	0.53	0.12–1.05	46	Unknown	1472	–	0.31	0.04–0.68
17	Dodecane	1200	MS, RI	1.84	0.47–5.54	47	γ-Muurolene	1476	MS, RI	0.12	0.05–0.18
18	Decanal	1206	MS, RI	0.50	0.33–0.69	48	α-Curcumene	1483	MS, RI	0.10	0.03–0.35
19	Unknown	1213	–	0.47	0.21–0.96	49	β-Ionone	1486	MS, RI	1.08	0.50–2.06
20	Unknown	1221	–	0.81	0.32–2.41	50	Pentadecane	1500	MS, RI	0.62	0.46–0.93
21	Unknown	1234	–	2.71	0.98–5.72	51	α-Farnesene	1509	MS, RI	1.09	0.44–2.91
22	Unknown	1238	–	0.44	0.20–1.06	52	γ-Cadinene	1513	MS, RI	0.34	0.07–0.73
23	Unknown	1244	–	0.30	0.11–0.74	53	δ-Cadinene	1523	MS, RI	1.51	0.23–2.73
24	Geraniol	1256	MS, RI	5.73	3.77–10.91	54	Cadine-1,4-diene	1532	MS, RI	0.23	0.06–0.47
25	Hexanoic acid, anhydride	1263	MS, RI	0.80	0.48–1.49	55	Nerolidol	1565	MS, RI	1.50	0.88–3.38
26	Unknown	1273	–	0.37	0.15–0.52	56	1-Hexadecene	1593	MS, RI	0.07	0.03–0.15
27	Indole	1291	MS, RI	0.86	0.35–1.61	57	Hexadecane	1600	MS, RI	0.78	0.45–1.18
28	Tridecane	1300	MS, RI	0.42	0.15–0.89	58	Heptadecane	1700	MS, RI	0.12	0.05–0.15
29	2-Methyl naphthalene	1306	MS, RI	0.19	0.09–0.36	59	Octadecane	1800	MS, RI	0.04	0.02–0.07
30	Methyl anthranilate	1340	MS	0.16	0.07–0.32	60	Caffeine	–	MS	0.85	0.32–2.90
	Monoterpenes (3)			4.07	2.03–9.59		Esters (6)			15.53	8.83–23.25
	Sesquiterpenes (11)			6.83	3.61–9.94		Aldehydes (3)			4.23	1.57–5.81
	Terpene alcohols (3)			17.47	12.11–28.55		Ketones (4)			3.52	2.62–4.76
	Alcohols (2)			1.17	0.60–1.54		Alkanes (8)			5.60	2.52–9.45
	Total			73.00	60.36–81.40						

[a] Compounds are listed in order of retention time. [b] RI, retention indices as determined on HP-5MS column using the homologous series of *n*-alkanes. [c] Method of identification: MS, identification by comparison with mass spectra stored in NIST98 library; RI, identification by retention index and comparison with those reported in the literatures

compounds extracted by HS-SPME in Longjing samples. Though with quantitative variations in different samples, myrcene (2.21%), nonanal (3.02%), (*Z*)-3-hexenyl butyrate (3.19%), and three unknown compounds (Nos. 6, 10, and 21) were also detected in abundance. It has previously been reported that linalool and geraniol were the major volatile compounds in Longjing tea (Kawakami and Yamanishi, 1983; Wang and Ruan, 2009), which is similar to our results. Additionally, Kawakami *et al*. (1983) had identified (*Z*)-3-hexenyl hexanoate in Longjing essential oil, and our study further discovered that this compound was present in Longjing tea of high amount.

Concerning the classes of substances, terpene alcohols (17.47%) and esters (15.53%) were two major chemical groups identified as contributors to Longjing tea aroma (Table 1). In addition, many terpenes (33.89%) were identified in headspace chemical analysis of Longjing tea, suggesting that these compounds might contribute the most to Longjing aroma.

3.2 Volatile compounds and perceived aroma quality

Pearson's linear correlation analysis was performed between the relative content data and the sensory aroma scores of 19 Longjing samples of Grade One and Grade Two. Results showed that 10 volatile compounds significantly correlated with perceived aroma quality ($P<0.05$), as listed in Table 2. Among them, linalool (0.701), nonanal (0.738), safranal (0.633), and β-farnesene (0.672) showed strong positive correlation with aroma scores, whereas (*Z*)-3-hexenyl hexanoate (−0.785), hexyl hexanoate (−0.527), β-ionone (−0.763), and an unknown compound (No. 21) (−0.653) showed strong negative correlation with aroma scores. When tukey's significance test was carried out on the chromatographic data, it was discovered that the relative contents of linalool, nonanal, safranal, and β-farnesene were significant higher in Longjing samples of Grade One compared with that of Grade Two. On the other hand, significantly lower relative contents of (*Z*)-3-hexenyl hexanoate and β-ionone were detected in Grade One Longjing samples when compared with Grade Two. Therefore these compounds appear to be the most potent odorants in Longjing tea, and can be further used to build an odorant-based model to predict aroma quality.

High-quality Longjing teas possess fragrant, tender, and long-lasting aroma, whereas those of low quality have green and faint aroma (AQSIQ and SAC, 2008) (Chinese national standard GB/T 18650-2008). Linalool and nonanal have been characterized as aroma-active compounds contributing to floral or fruity odors in Longjing tea infusions (Table 2), and linalool has a high flavor dilution (FD) factor ≥100 (Kumazawa and Masuda, 1999; Cheng *et al*., 2008). Furthermore, they were also reported to have significant positive correlation with the perceived aroma of Longjing tea or oolong tea (Wang and Ruan, 2009;

Table 2 Key volatile compounds correlated with aroma quality and their odor descriptions

No.	Compound	Correlation coefficient[a]	Relative content (%)[b]		Odor description
			Grade One (n=7)	Grade Two (n=12)	
3	Myrcene	0.462*	2.88	1.91	Sweet (Jøgensen *et al*., 2000; Pino *et al*., 2002)
7	Linalool	0.701**	**13.11**	**8.34**	Floral (Kumazawa and Masuda, 1999; Cheng *et al*., 2008)
8	Nonanal	0.738**	**3.70**	**2.86**	Floral, green, orange-like (Kumazawa and Masuda, 1999; Cheng *et al*., 2008)
16	Safranal	0.633**	**0.72**	**0.46**	Herbal (Zhu *et al*., 2008; Lv *et al*., 2012)
21	Unknown	−0.653**	2.03	2.80	
34	(*Z*)-3-hexenyl hexanoate	−0.785**	**5.98**	**9.18**	Fruity, green (Kumazawa and Masuda, 1999; Zhu *et al*., 2008)
35	Hexyl hexanoate	−0.527**	1.09	1.37	Fruity, green (Pino *et al*., 2002)
43	Geranyl acetone	−0.472*	0.54	0.61	Tea leaves and floral (Kumazawa and Masuda, 1999; Cheng *et al*., 2008)
44	β-Farnesene	0.672**	**1.35**	**0.45**	Fruity (Choi, 2003; 2005)
49	β-Ionone	−0.763**	**0.83**	**1.21**	Tea leaves and woody (Cheng *et al*., 2008)

[a] Pearson's linear correlation analysis: $^{*}P<0.05$; $^{**}P<0.01$. [b] Bold relative content data representing significant difference of corresponding compound between Grade One and Grade Two (Tukey's test, $P<0.05$)

Wang *et al*., 2010). Safranal was also characterized as an herbal odorant in green tea (Zhu *et al*., 2008) and Pu-erh tea (Lv *et al*., 2012). β-Farnesene was determined to have fruity odor (Choi, 2003; 2005). These four odorants all have positive correlation coefficients >0.6 with the aroma quality, and thus might contribute to the top notes of Longjing tea.

(*Z*)-3-Hexenyl hexanoate, hexyl hexanoate, and β-ionone negatively correlated with aroma quality, which was in agreement with the overall odor impression of the individual compounds. β-Ionone has been determined to have tea leaves and woody odor in Longjing tea (Cheng *et al*., 2008). (*Z*)-3-Hexenyl hexanoate was determined to have a fruity and green odor in green tea (Kumazawa and Masuda, 1999; Zhu, *et al*., 2008). Finally, hexyl hexanoate was also reported to have a fruity and green odor (Pino *et al*., 2002). Similar to our results, it has been reported that the amount of (*Z*)-3-hexenyl hexanoate in high grade Sen-cha was five times that observed in low grade Sen-cha (Shimoda *et al*., 1995).

The chemical odorants observed in our study were quite different from the most active odorants detected in Longjing tea infusions in the previous studies (Kumazawa and Masuda, 1999; Cheng *et al*., 2008). The difference may be attributed to the different forms of tea aroma used for extraction. Extracts of tea infusion (without leaves) were analyzed in the previous study, while the headspace of tea infusion (with leaves) was analyzed in our work. Influenced by water solubility and gas/liquid partition coefficients of the odorants, the aroma constituents of infusion headspace are likely very different from that of infusion extracts. The aroma of headspace extracts might be closer to the initial form of tea aroma smelled. A further study would be required to analyze the odor activity values (OAVs; ratio of concentration to odor threshold in water) of the odorants in Longjing tea.

It should be noted here that the characterization of specific odor-active compounds and the odor threshold of odorants were not considered in the present study because the final aroma quality was perceived as a complex mixture of odorants rather than a single odor-active compound. Due to this added complexity, we applied Pearson's linear correlation analysis to identify the odorants with the biggest contributions to the perceived aroma scores, and used these compounds to assess the aroma quality of Longjing tea.

3.3 PLS regression

The PLS regression method can analyze data with strongly collinear, noisy, and numerous *X*-variables, and also simultaneously model the response variables (Wold *et al*., 2001). The use of this method has been validated to predict tea quality using gas chromatographic profiles in some previous studies (Pongsuwan *et al*., 2007; 2008; Jumtee *et al*., 2011). In the present study, the relative contents of volatile compounds observed in Longjing tea samples were imported to the ***X***-matrix, while the aroma scores of 19 Longjing samples of Grade One and Grade Two were imported to the ***Y***-matrix. PLS regression was applied to verify the correlation between the two matrices.

PLS regression was first performed by importing all 60 volatile compounds to the ***X***-matrix and a good correlation between these compounds and sensory quality could be achieved as observed from the correlation coefficient (R2*Y*) of 0.861 (Fig. 2a). The cross-validation procedure was used to estimate the performance of the predictive model by a leave-one-out strategy, and the cross-validated correlation coefficient (Q2*Y*=0.524) was rather low, indicating a poor predictive model. This model explained only 15.2% of the *X* variances (R2*X*=15.2%), and hence 84.8% of the variations in *X* were not related to *Y* and were removed. These results further confirmed that the volatile compounds together were very valuable in the interpretation of sensory quality, but some *X* components not associated with aroma quality might have interfered with the prediction performance. Based on these considerations, it is essential to extract the main attributes associated with aroma quality from the bulk of volatile compounds, so as to reduce noise and increase the interpretation capacity for the prediction model. By Pearson's linear correlation analysis, 10 compounds strongly associated with aroma quality have already been determined (Table 2), and a secondary PLS regression was carried out using these relevant compounds. Interestingly, a much better predictive ability (Q2*Y*=0.804) was obtained. The explaining ability of modeling was improved as well, and 89.4% of the *Y* variances (R2*Y*=0.894) and 61.0% of the *X* variances (R2*X*=0.610) were explained (Fig. 2b). Using this regression model, most of the Longjing samples examined in this work received a high predicted aroma score, as listed in Table 3. The second PLS regression model utilized fewer variables and therefore considerably improved the accuracy of the predictive model.

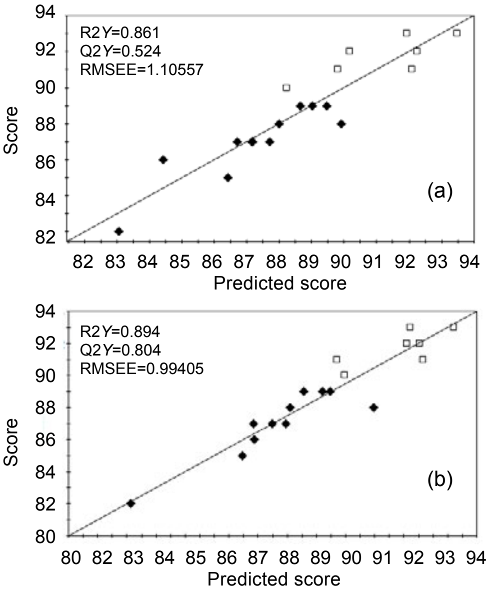

Fig. 2 Plot of the PLS regression between observed and predicted aroma scores of Longjing tea based on 60 volatile compounds (a) and 10 quality-relevant compounds (b)
Tea samples were classified as Grade One and Grade Two according to aroma scores determined by tea sensory experts. Open box, samples of Grade One; filled diamond, samples of Grade Two. R2*Y*, correlation coefficient; Q2*Y*, cross-validated correlation coefficient; RMSEE, root mean square error of estimation, the error rate of predictability of the regression model

Jumtee *et al.* (2011) have reported the prediction of Japanese green tea (Sen-cha) rankings by the analysis of aromatic components as compared to comprehensive rankings determined by leaf appearance, color, aroma, and taste of tea infusion. In the present study, we have verified the feasibility of using potent odorants to predict one of the most important ranking factors of green tea-aroma quality. Since the odorants directly contribute to tea aroma quality, a model based on some key aromatic components might have more potential to accurately predict the aroma quality of tea. Furthermore, HS-SPME is a very simple and convenient method of aromatic extraction and could considerably improve the efficiency and accuracy of the prediction of tea quality.

4 Conclusions

In summary, 60 volatile compounds were routinely detected in Longjing tea samples by HS-SPME/GC-MS and 16 new odorants were observed in this famous green tea. Linalool (9.77%), (*Z*)-3-hexenyl hexanoate (8.19%), and geraniol (5.73%) were identified as the three most abundant aromatic components in Longjing tea. Additionally, 10 compounds (linalool, nonanal, safranal, β-farnesene, myrcene,

Table 3 Aroma scores and notes descriptions of Longjing samples and aroma scores predicted by PLS regression

Sample No.	Aroma grade	Notes description[a]	Score[b]	Predicted score[c]	Sample No.	Aroma grade	Notes description	Score	Predicted score
1	1	Tender	92	91.6	11	2	Approach high	89	88.7
2	1	Tender, roasted	91	92.2	12	2	Woody, smoky	87	86.3
3	1	Tender	93	91.7	13	2	Woody, smoky	85	86.0
4	1	Chestnut, tender	92	92.0	14	2	Approach high	86	86.4
5	1	Tender	93	93.2	15	2	Pure	82	82.1
6	1	Tender, fresh, high	91	89.2	16	2	Approach high, roasted	89	89.0
7	1	Tender, fresh, high	90	89.5	17	2	Approach high	88	87.6
8	2	Approach high	89	88.1	18	2	Approach high	87	87.4
9	2	Clean, faint	87	87.5	19	2	Roasted	87	87.0
10	2	Clean, faint	88	90.5					

[a] High: strong and long-lasting aroma; Approach high: between high and faint. [b] Aroma scores assessed by group-evaluation according to GB/T 23776-2009. [c] Aroma scores predicted by PLS regression based on the 10 quality-relevant compounds listed in Table 2

(*Z*)-3-hexenyl hexanoate, hexyl hexanoate, geranyl acetone, β-ionone, and an unknown compound) were found to be the key aromatic components contributing to the sensory aroma quality of Longjing tea. The PLS regression method provided a good model for quality prediction of Longjing aroma resulting from contributions from the 10 compounds (correlation coefficient of 89.4% and cross-validated correlation coefficient of 80.4%). To the best of our knowledge, this is the first report using HS-SPME in quality prediction of green tea. Further investigation is needed in order to simplify aroma extraction procedures and include more key variables to improve the prediction model.

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Abstract: Dysfunction of inhibitory synaptic transmission can destroy the balance between excitatory and inhibitory synaptic inputs in neurons, thereby inducing epileptic activity. The aim of the paper is to investigate the effects of successive excitatory inputs on the epileptic activity induced in the absence of inhibitions. Paired-pulse orthodromic and antidromic stimulations were used to test the changes in the evoked responses in the hippocampus. Picrotoxin (PTX), γ-aminobutyric acid (GABA) type A ($GABA_A$) receptor antagonist, was added to block the inhibitory synaptic transmission and to establish the epileptic model. Extracellular evoked population spike (PS) was recorded in the CA1 region of the hippocampus. The results showed that the application of PTX induced a biphasic change in the paired-pulse ratio of PS amplitude. A short latency increase of the second PS (PS2) was later followed by a reappearance of PS2 depression. This type of depression was observed in both orthodromic and antidromic paired-pulse responses, whereas the GABAergic PS2 depression [called paired-pulse depression (PPD)] during baseline recordings only appeared in orthodromic-evoked responses. In addition, the depression duration at approximately 100 ms was consistent with a relative silent period observed within spontaneous burst discharges induced by prolonged application of PTX. In conclusion, the neurons may ignore the excitatory inputs and intrinsically generate bursts during epileptic activity. The depolarization block could be the mechanisms underlying the PPD in the absence of $GABA_A$ inhibitions. The distinct neuronal responses to stimulations during different epileptic stages may implicate the different antiepileptic effects of electrical stimulation.