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Full Length Research Paper

Discrimination of Xihulongjing tea grade using an electronic tongue

Hong Xiao[1,2] and Jun Wang[1]*

[1]College of Biosystems Engineering and Food Science, Zhejiang University, 268 Kaixuan Road, Hangzhou 310029, China.
[2]Department of agricultural science, College of Yingdong Biological Engineeringy, Shaoguan University, Daxue Road, Shaoguan 512005, China.

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Five grades of Xihulongjing tea (grade: AAA, AA, A, B and C, from the same region and processed with the same processing method) were discriminated using α-Astree II electronic tongue (e-tongue) coupled with pattern recognition methods including principal component analysis (PCA), canonical discriminant analysis (CDA) and back-propagation neural networks (BPNN). Results of PCA and CDA showed that the grades of the samples were discriminated with the exception of a few overlap samples between grade AA and grade A. The discrimination of accuracy of the training sample set and the predicted sample set was 95.7 and 97.5%, respectively, by the analysis of BPNN. 92.9% of all the cross-validated training sample set and 100% of the predicted sample set were exactly grouped by CDA. The sensory evaluation of the samples was consistent with the evaluation based on the e-tongue. The results show that the e-tongue is a potential tool to identify the tea quality.

Key words: Electronic tongue, tea, grade, pattern recognition.

INTRODUCTION

Tea is one of the most widely consumed drinks in the world because of its healthy, dietetic and therapeutic benefits. Three types of teas are classified based on their fermentation processing: green (unfermented tea), oolong (semifermented tea) and black (fermented tea). Green tea is one of the most popular beverages in the East and Southeast Asia countries, not only because of its prominent pharmaceutical activity (Robinson, 2001; Chen et al., 2005; Bettuzzi et al., 2006; Mbata et al., 2008), but also because of its excellent taste. Xihulongjing tea, as a brand of green tea and headed the list of the famous tea in China, is well known for its graceful appearance, brilliant green color, fragrant aroma and brisk taste in the world (Zhang, 2007).

In recent years, with the increase of yields and demands, the production and consumption of famous green teas have played a great role in Chinese tea industry and thus the quality evaluation of green tea has also become more and more important in tea production (Gong, 2000). The grade of tea is measured mainly on the basis of leaf appearance and internal attributes (taste, aroma, and liquor color) (Lu and Shi, 2001). The taste of tea is one of the most important factors in its quality grading and generally has been assessed by the professional tea tasters. However, the results of sensory test are usually affected by some subjective factors such as emotion, exhaustion and physiological conditions. Moreover, it takes years of experience to acquire the skills of quality evaluation of tea.

At present, analytical instruments have enabled the discovery and quantification of many of the key taste and aroma compounds known today in food (Linforth, 2000). Thus, researchers have endeavoured to develop instrumental methods for assessing the quality of tea (Liang et al., 2005; Seetohul et al., 2006; Yu and Wang, 2007; Sultana et al., 2008). An electronic tongue (e-tongue) is a biomimetic sensing device that detects liquid information with a set of sensors array and has advantages such as objectivity, low cost, easy-to-handle measurement set-up

*Corresponding author. E-mail: jwang@zju.edu.cn. Tel: +86-571-86971881. Fax: +86-571-86971139.

and rapidness. The e-tongue, combined with powerful pattern recognition techniques, has been applied to evaluations of some foodstuff, for example, beer (Legin et al., 1997; Toko, 2000), mineral water (Winquist et al., 1997), milk (Winquist et al., 1997; Toko, 2000), juice (Legin et al., 1997; Winquist et al., 1997), coffee (Toko, 1998; Schreyer and Mikkelsen, 2000; Miyanaga et al., 2003), tomato (Vermeir et al., 2007; Beullens et al., 2008), etc.

Some researchers also attempted to use various e-tongue to study different kinds of tea. An e-tongue based on voltammetry combined with a set of voltage pulses has been employed for the discrimination of nine different teas (black tea and green tea) and the best result was achieved when the combination large amplitude pulse voltammetry (LAPV) and staircase were merged together (Ivarsson et al., 2001; Tian et al., 2007). The discrimination of black tea and green tea and the quantitative analysis of main components responsible for the taste of Korea green tea were conducted using a disposable all-solid-state potentiometric e-tongue microsystem based on screen-printing technology (Lvova et al., 2003). It has been found that the e-tongue microsystem was capable of discriminating different kinds of teas (Lvova et al., 2003). Japanese researchers have established methods for evaluating the astringency and umami taste intensity of green tea by a taste sensor system (Hayashi et al., 2006; Hayashi et al., 2008; Chen et al., 2008).

The studies mentioned above have established some bases for the application of quality evaluation of tea by e-tongue technology, but they mostly focused on the different types of tea (green tea and black tea) or different producing areas. As it is known, the taste of green tea is primarily depended on the tea cultivars, time of plucking, ecological environment, cultivation and the processing conditions, etc (Le Gall et al., 2004). However, few studies have been reported on the application of e-tongue for identifying different quality grades of famous quality green tea with the same tea cultivar which was grown in the same region and processed by the same procedure.

The objective of this research is to explore the method to classify five grades of Xihulongjing tea by means of the e-tongue coupled with pattern recognitions (PCA, CDA and BPNN). Sensory test was also carried out in order to confirm the validity of the e-tongue for this application.

MATERIALS AND METHODS

Materials

Five grades of Xihulongjing tea (spring tea), supplied by Hangzhou Xihulongjing tea Ltd., were picked from the same area (120:10E, 30:15N) and processed by the same procedure. Sample grade priority was AAA, AA, A, B and C (Picked day: March 25, March 30, April 2, April 12 and April 19, respectively). Samples of grade AAA, AA and A were picked before Qingming Festival (usually on April 4th or 6th) and the others were picked after Qingming Festival. Measurements were repeated 22 times for each grade and a total of 110 samples were prepared for the experiment.

Electronic tongue and data acquisition

Experiments were performed using an α-Astree II electronic tongue (Alpha M.O.S company, France) which included an array of seven different liquid cross-selective sensors (ZZ, BA, BB, CA, GA, HA, JB), a 16-position autosampler and associated interface electronic module. The sensors were based on an innovative electrochemical potentiometric sensor technology: the Chemical Modified Field-Effect-Transistor (CHEMFET) technology. Each sensor was composed of an organic coating sensitive to the species in the samples and a transducer, which allows converting the response of the membrane into signals that will be analyzed. The sensor response was the voltage difference between the sensor and the Ag/AgCl reference electrode. Therefore, an integral signal for each sample was comprised of a vector with 7 individual sensor determinations.

For each sample, 3 g of tea were infused with 150 ml deionized boiled water for 5 min and then the leaves were removed. The infusion was quickly cooled down to 25 ± 2℃ and 80 ml solution of each sample was used in the measurement. The measurement procedure was controlled by a computer program. The measurement phase lasted for 120 s, which was enough for the sensors to give stable values. The interval for data collection was 1 s. A computer recorded the response of the e-tongue every second. When the measurement was completed, the acquired data was properly saved for later use. In this research, the signal of each sensor at 120th second was used in analysis. The sensors were cleaned with distilled water after a sample testing and were calibrated before testing a different grade sample with 0.01 mol/ L HCl.

Sensory quality assessment of tea samples

The tea quality was assessed by a professional tea taster from the Department of Tea Science at Zhejiang University. 150 g tea (each grade tea) were prepared for the examination of appearance, then 3 g tea were taken from the uniformly mixed 150 g tea and infused with 150 ml water at 100℃ for 5 min. The taster examined the appearance, then smelled the infused leaves, tested the aroma and smell of the liquor and finally tasted it (no swallowing the liquid, at about 50℃) (Lu and Shi, 2001). The grading standard was based on a total score of 100, of which 30% was awarded for the appearance of dry tea, 25% for the aroma, 10% for the liquor color, 25% for taste, 10% for the infused leaves (Lu and Shi, 2001) (Table 1).

Statistic analysis

One-Way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test were carried out on the data of 110 samples by SPSS 11.0. Data obtained in the experiment were also processed by multivariable analysis methods including Principal Component Analysis (PCA), Canonical Discriminant Aanalysis (CDA) and Back Propagation Neural Network (BPNN). Analysis of data were carried out with DPS version 3.11 (the Data Processing System Statistical Software package) (Tang and Feng, 1997) and SPSS11.0.

PCA is a useful and common statistical method for finding patterns in data of high dimension. It helps to reduce the number of dimensions without much loss of information. The PCA method was used for sensors array output treatment as a mean to visualize different groups.

CDA is also termed Linear Discriminant Analysis based on Fisher rule. It is a useful complement to PCA. Its application in this study

Table 1. Sensory score (total score of 100).

Attribute	Appearance of dry tea	Taste	Aroma	Liquor color	Infused leave
Significant	30%	25%	25%	10%	10%

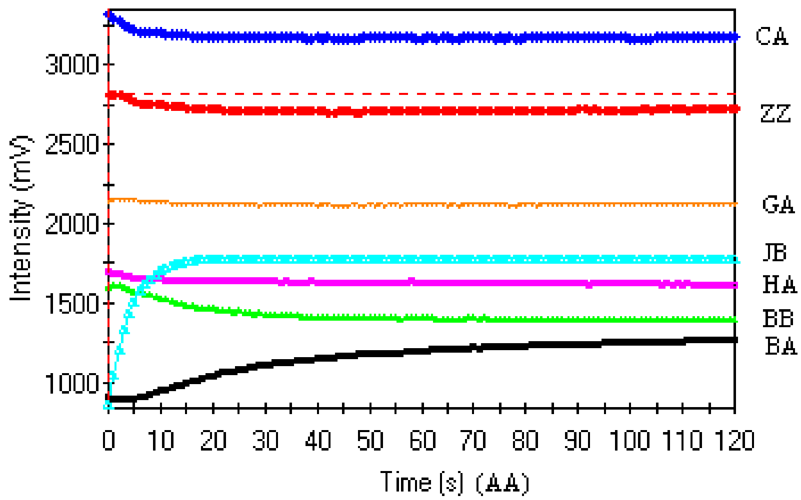

Figure 1. Response of electronic tongue to Xihulongjing tea grade AA.

was to assess the adequacy of Xihulongjing tea classification, focusing on the response signals obtained from the e-tongue.

BPNN is one of the most widely used artificial neural networks. Generally, the BPNN architecture comprises one input layer, hidden layers and an output layer. In the research, BPNN was employed to predict the classification of different grades of Xihulongjing tea.

RESULTS AND DISCUSSION

Response of e-tongue for Xihulongjing tea and ANOVA

The typical response signal of seven sensors for one grade Xihulongjing tea (grade AA) was shown in Figure 1. Y-axis represented the signal intensity, which was the voltage difference between the sensor and the reference electrode while the X-axis represented measurement time. The sensors according to the response values were arranged in the sequence of CA>ZZ>GA>JB>HA>BB>BA and all sensors became stable after 100 seconds.

There are some differences in the average response values (mean of 22 samples response value at 120th second) of each sensor to five grade tea samples (Figure 2), which may be mainly attributed to the variations of teas' taste. As revealed by the analysis of variance (ANOVA), the values of sensor CA and JB were significantly different between the teas picked before the Qingming Festival (the samples of grade AAA, AA and A) and after the Qingming Festival (the samples of grade B and grade C) ($p < 0.05$, n = 22). For the sensor of HA, there were significant differences among the samples of grade AAA, grade B and grade C ($p < 0.05$, n = 22). All samples showed no significant difference in the sensor of BB. However, it was difficult to deduce an overall conclusion on the difference of the sensor's response values to five kinds of tea samples due to the limitation of ANOVA. Thus, it is necessary to employ pattern recognition procedures achieving a more reliable tea grade differentiation.

Classification of Xihulongjing tea by e-tongue

Principal component analysis

PCA reduces the data dimension to some principal components and enables the extraction of the differences between samples and the main variables. The data of 110 samples were analyzed by PCA. The loading plot showed that the relationship between the variables and facilitated an observation of the contributions of the variables to each PC from the positive and negative values of the weights (Figure 3). PC1 was mainly contributed by sensor ZZ, BB, BA, GA, HA and JB because of their high positive values in PC1. The greatest effect in PC2 was exerted by sensor CA.

Figure 4 showed the classification pattern based on 7 variables. The first component contributed 70.71% of the total variance, the second component contributed 22.22% and the third component was 3.10%, with a 96.03% in total. There was a clear discrimination between tea samples picked before the Qingming Festival (AAA, AA, A)

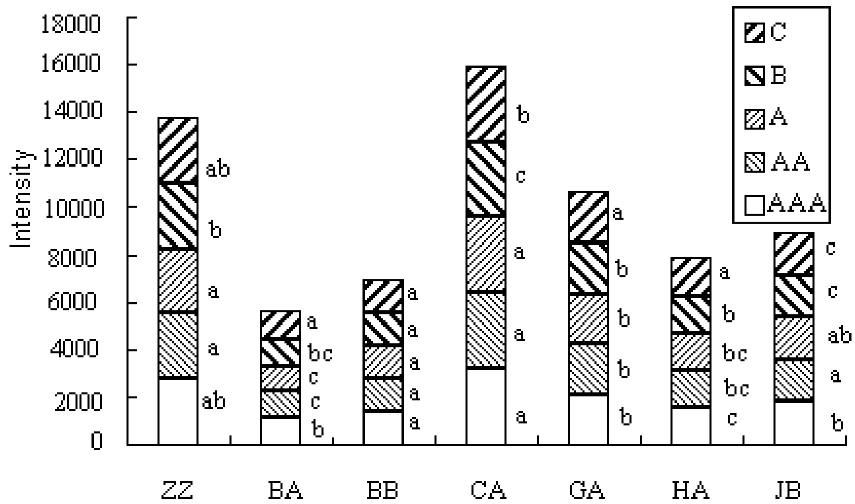

Figure 2. Average responses of electronic tongue to different grades of Xihulongjing tea. Different lower case letters were significantly different at $p < 0.05$ (n = 22).

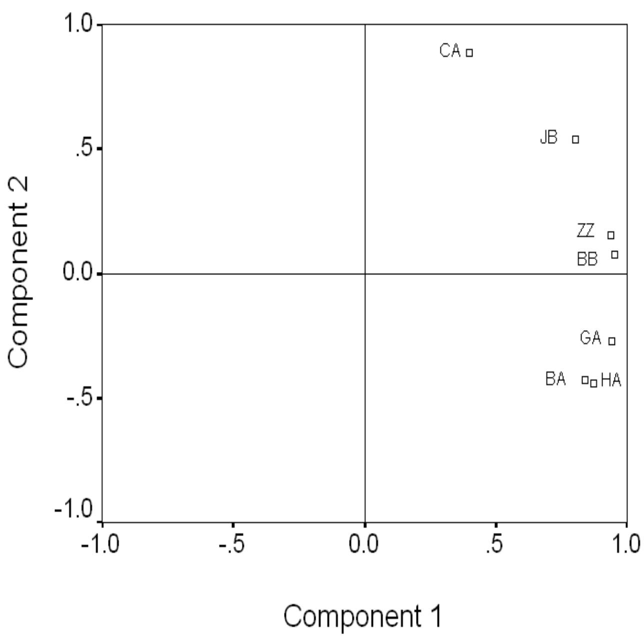

Figure 3. The loading plot for PCA in teas.

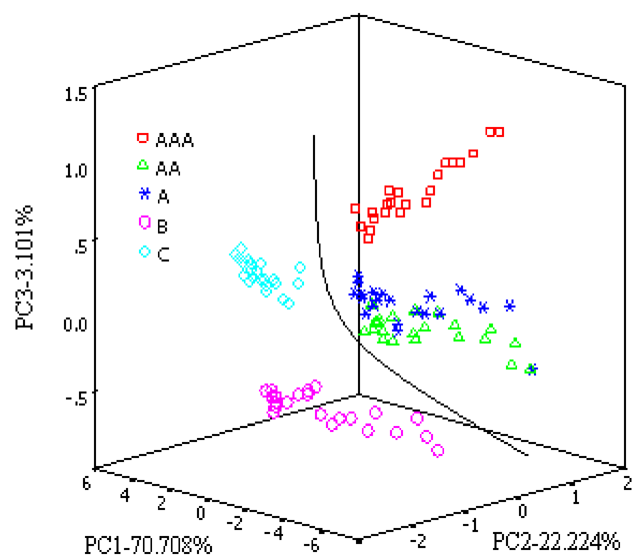

Figure 4. PCA plots of different grades of Xihulongjing tea.

and after the Qingming Festival (B, C). Samples of grade AAA, B and C were easily discriminated, while samples of grade AA and A were overlapped partially. PCA did not give a clear discrimination between different grades of teas; therefore, CDA was used for further discriminating the grade of Xihulongjing tea.

Canonical discriminant analysis

Discriminant analysis using a canonical discriminant function with leave-one-out cross validation was performed to assess the adequacy of the tea's grade classification (110 samples). Compared with the PCA, CDA can give a quantitative result of category. It was found that the samples were obviously separated into five clusters except for a few samples of grades AA and A (Figure 5). The first two discriminant functions accounted for 98.7% of the total discriminating power. The differentiation between the samples picked before the Qingming Festival (AAA, AA, A) and after the Qingming Festival (B, C) was shown to be related to the direction of the first discriminant function. The second discriminant function contributed to the discrimination between samples of grade B

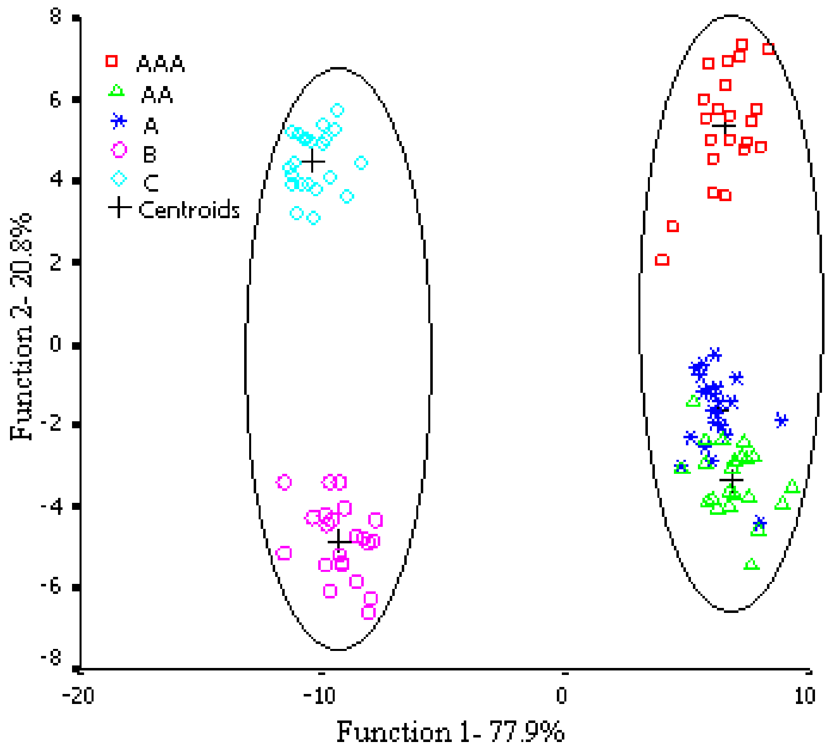

Figure 5. CDA of different grades of Xihulongjing tea.

Table 2. Classification results of different grades Xihulongjing tea by CDA.

Grade		Predicted membership				
		AAA	**AA**	**A**	**B**	**C**
AAA	Count	21	0	1	0	0
AA		0	19	3	0	0
A		0	1	21	0	0
B		0	0	0	22	0
C		0	0	0	0	22
AAA	Rate %	95.5	0	4.5	0	0
AA		0	86.4	13.6	0	0
A		0	4.5	95.5	0	0
B		0	0	0	100	0
C		0	0	0	0	100

and those of grade C and also contributed to classifying three grades of sample picked before Qingming festival (April 5).

95.5% of cross-validated grouped samples were correctly classified (Table 2). The accuracy rate of samples from grade of AAA to grade of C was 95.5% (one sample misclassified into grade A), 86.4% (three samples misclassified into grade A), 95.5% (one sample misclassified into grade AA), 100 and 100%, respectively.

Both the PCA and the CDA analysis of sensor signals for the all samples showed the similar results of classification, which was due to the characteristics in quality among different grades of tea. In contrast with the samples picked after Qingming festival (grade B and grade C), the samples of grade AAA, AA and A were picked in early spring, which had a greater content of amino acids and lower content of flavanols. It is known that the quality of tea is highly related with the age of the tea leave used and the younger leaves can achieve better tea quality. Different times of plucking led to the differences in taste between the two group samples, which enabled the e-tongue to distinguish them from each other. The partial overlapping between the samples of grade AA and grade A might be their similar taste

Table 3. Results of BP-ANN.

Grade	Training set				Testing set		
	Number	Expected output	Misclassified number	Recognition (%)	Number	Misclassified number	Prediction (%)
AAA	14	[1 0 0 0 0]	0	100	8	0	100
AA	14	[0 1 0 0 0]	2	85.7	8	0	100
A	14	[0 0 1 0 0]	1	92.9	8	1	87.5
B	14	[0 0 0 1 0]	0	100	8	0	100
C	14	[0 0 0 0 1]	0	100	8	0	100
Total	70		3	95.7	40	1	97.5

Table 4. Results of Canonical Disciminant Analysis.

Grade	Training set			Testing set		
	Number	Misclassified number	Recognition (%)	Number	Misclassified number	Prediction (%)
AAA	14	1	92.9	8	0	100
AA	14	3	78.6	8	0	100
A	14	1	92.9	8	0	100
B	14	0	100	8	0	100
C	14	0	100	8	0	100
Total	70	5	92.9	40	0	100

because of short intervals of plucking. These results indicate that the e-tongue can make different responses to five grades of samples and have a good discrimination capability.

Grade prediction of Xihulongjing tea by e-tongue

Back propagation neural network

The total 110 samples (22 samples for each grade) were randomly divided into a training set (70 samples, 14 samples for each grade) and a testing set (40 samples, 8 samples for each grade). The seven sensors measurements obtained from the E-tongue at 120th second were used for inputs. The number of hidden layer neurons was determined by trial and error and set at 5. The output layer had five neurons to represent the sample grade. According to the number of inputs and outputs, the network topology was designed 7×5×1. The value for learning rate and momentum were 0.1 and 0.6. The training epoch was 1500. The classification accuracy was 95.7% for the training set and 97.5% for the testing set (Table 3). Two samples of grade AA and one sample of grade A were misclassified in the training set. The prediction of all samples was 100% except for the samples of grade A (87.5%).

Canonical discriminant analysis

The aim to use CDA is to classify cases into three or more categories using continuous or dummy categorical variables as predictors. The training set and testing set were the same as BPNN. CDA showed 92.9% of cross-validated training samples were correctly classified and 100% of prediction samples were separated into each group (Table 4). The first two discriminant functions accoun-ted for 98% of the total discriminant power. In training samples, three samples of grade AA and one of grade AAA were classified into the group of grade A and one of grade A was separated into the group of grade AA. The prediction result of CDA was better than that of BPNN.

The models of BPNN and CDA displayed adequate capability to predict the classification of five grades of Xihulongjing tea, although there were some errors. This demonstrates that the e-tongue may be prospectively applied to evaluating quality of tea.

Sensory analysis of Xihulongjing tea

The sensory evaluation was conducted in the study in order to test the possibility of e-tongue applied to the quality evaluation of tea. It was observed that the results

Table 5. Sensory score of different grades of Xihulongjing tea.

Grade	Appearance	Liquor color	Aroma	Taste	Infused leaves	Total score
AAA	27.9	9	23.25	23.25	9.4	92.8
AA	28.5	9	22.75	22.75	9.4	92.4
A	27.6	8.9	22.75	23	9.2	91.5
B	26.1	8.6	21.75	22	8.7	87.2
C	24.9	8.2	21	21	8.3	83.4

of sensory panel and the e-tongue were very consistent. Total quality scores of the five grades of tea samples ranged from 83.4 to 92.8, in which the samples of first three grades (AAA, AA and A) had high scores (above 90) and the rest (grade B and grade C) had relatively low scores (Table 5). This indicated that five grades of samples had different attributes and the attributes of the samples picked before Qingming festival were distinct from those of the samples picked after Qingming festival. As it could be seen that the taste scores of the first three grades samples were very similar and the variations between the samples of grade AA and grade A were the least. To the aroma, the scores of both samples of grade AA and samples of grade A were completely the same, which implied that taste played a more important role in the grade classification of tea and simultaneously it also elicited their quality similarity. All these could further explain why overlaps between the two grades samples were observed in the analysis of e-tongue data.

On the other hand, compared with the previous studies, we find out that the misclassification occurs easily on tea with higher quality grade, which can be observed in the study on quality grade identification of green tea using electronic nose (Yu et al., 2008). It had also been pointed out in the interrelated research on Japanese green tea ranking by near-infrared reflectance spectroscopy that when the tea quality was higher, the prediction became more difficult (Ikeda et al., 2007). In this study, the e-tongue showed better classification between the famous high quality teas than the above previous ones, though there were errors. Moreover, the sensory analysis effectively proved the capability of the e-tongue to discriminate tastes of the teas. The e-tongue is a very promising tool, because the e-tongue can be thought of as analogous to both olfaction and taste and it can be used for the detection of all types of dissolved compounds, including volatile compounds which give odours after evaporation (Legin et al., 2002). Nevertheless, there may still be many problems to be further studied, since there are many complex factors affecting the quality of tea. Therefore, it is necessary to use more different quality teas to train the e-tongue, to explore other pattern recognition methods and hence make the models of discriminating quality of tea more robust step by step. It is expected that the e-tongue will be a powerful tool to evaluate the quality of tea in tea manufacture industry.

Conclusions

In this research work, an attempt was made to discriminate five grades of Xihulongjing tea (from the same region and processed by the same procedure) using the e-tongue. The classification result of the e-tongue was compared with the sensory analysis of professional tasters.

Five different tea grades were discriminated by PCA, CDA and BPNN based on the sensor responses signals of e-tongue. Accuracies of 95.7 and 92.9% were achieved in the classification using BPNN and CDA. 100% of predicted samples were correctly grouped using CDA, compared with the accuracy of 97.5% using BPNN. The sensory analysis explained and supported adequately the discrimination result of the e-tongue. The results demonstrate that the e-tongue combined with pattern recognition method can provide accurate taste information for the discrimination of different quality grade of tea.

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