Food Science and Technology Research, **21** (2), 207–212, 2015

doi: 10.3136/fstr.21.207

http://www.jsfst.or.jp

Technical paper

Identification of Fake Green Tea by Sensory Assessment and Electronic Tongue

Yanjie Li[1*], Jincan Lei[2] and Dawei Liang[3]

[1] *College of life science and engineering, Chongqing Three Gorges University, Wanzhou, 404100, P.R. China.*
[2] *Postdoctoral Station of Science and Technology of Instrumentation, College of Optoelectronic Engineering, Chongqing University, Chongqing, 400044, PR China*
[3] *College of automation, Chongqing University, Chongqing, 400044, P.R. China.*

Received October 14, 2014 ; Accepted December 24, 2014

Identification of fake green tea is performed by sensory assessment which has significant drawbacks in terms of objectivity. In this works, sensory assessment and electronic tongue were utilized for identifying fake green tea. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were used to assess the feasibility and effectiveness of discrimination of electronic tongue. The PCA and HCA revealed a distinct separation between samples, which corresponded with the results of sensory assessment. Artificial Neural Network (ANN), including back propagation neural network with the Levenberg-Marquardt training algorithm (LMBP) and radial basis function neural network (RBF), were used as an automatic classifier and showed good performance in the training set and the prediction set. The results suggest that electronic tongue can be used for distinguishing fake Dongting Biluochun Tea from certified products characterized by protection of geographical indications product certification with pattern recognition methods instead of sensory assessment.

Keywords: fake green tea, identification, electronic tongue, PCA, HCA, ANN

Introduction

Green tea is a non-fermented tea and made by suitable fresh tea leaves after high temperature fixing, rolling and drying. It is well-known that high quality green tea presents three remarkable features: light yellow-green transparent tea soup, light emerald leaves, light aroma and slightly sweet. Green tea has been the highest yield and the most popular tea drinks in many Asian countries, especially in China, because it has some important physiological effects, such as anti-aging (Mahmood and Akhtar, 2013), regulation of lipornetabolism (Wong *et al*., 2014), reducing the risk of cancer (Fujiki *et al*., 2012) and reducing radiation damage (Rhodes *et al*., 2013). With the increasing of consumer demand and production cost, more and more fake green tea, especially some fake famous Chinese green teas, such as fake Dongting Biluochun Tea, has entered the wholesale and retail markets in recent years. Traditionally, identification of Dongting Biluochun Tea has been performed by artificial sensory assessment. However, the method has disadvantages in terms of objectivity and repeatability because the trained tea tasters is probably affected by the psychology and physiology. In addition, the trained tea tasters cannot tolerate large numbers of samples because they fatigue rapidly with increasing number of samples (Hidayat *et al*., 2010).

Recently, there have been some methods about identification of geographical origin of tea and quality evaluation of tea, including near-infrared spectroscopy (He *et al*., 2012), chemical fingerprint spectrum (Wang *et al*., 2014; Deng and Yang, 2013), machine vision system (Gill *et al*., 2011; Laddi *et al*., 2013), electronic nose (Cheng *et al*., 2013) and electronic tongue (He *et al*., 2009). As a new analytical measures, electronic tongue based on electrochemical, photochemical and enzymatic sensor array is widely used in foodstuff studies of identification and quality control, such as analysis of characterization and age of wine (Peris and Escuder-

*To whom correspondence should be addressed. E-mail: bio2018@gmail.com

Gilabert, 2013; Rudnitskaya *et al*., 2010), analysis of milk adulteration (Escuder-Gilabert and Peris, 2010) and mineral water (Sipos *et al*., 2012), analysis of taste of beer (Cetó *et al*., 2013), identification of geographical origins of cocoa beans (Teye *et al*., 2014), identification of tea grade (Banerjee *et al*., 2012). So far, the feasibility and effectiveness of the identification of fake green tea have been rarely reported by electronic tongue. In this paper, sensory assessment and an electronic tongue were intended to respectively distinguish fake Dongting Biluochun Tea from certified products characterized by protection of geographical indications product certification with pattern recognition methods, including Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA) and Artificial Neural Network (ANN) which contained back propagation neural network with the Levenberg-Marquardt training algorithm (LMBP) and radial basis function neural network (RBF).

Material and Methods

Sample preparation and preprocessing Biluochun Tea is made from leaves of Biluochun tea plant which is grown in several origins in China. Dongting Biluochun Tea is picked and made from leaves of Biluochun tea plant which is grown in Dongting Mountain in Jiangsu Province, China. Compared with other origins of Biluochun Tea, Dongting Biluochun Tea is sold at a higher price in the markets because of unique aroma and good taste of tea soup. Dongting Biluochun Tea can be divided into the new tea and aging tea (old tea or chen tea) according to the length of storage time. New Dongting Biluochun Tea preserved for less than one year is well accepted in the markets because of limited production. Because of poor taste, aging Dongting Biluochun Tea, namely more than one year storage of new Dongting Biluochun Tea, has not been recognized in the markets based on consumption habits.

In April to August 2013, 400 samples which were sold in the name of new Dongting (Mountain) Biluochun Tea were obtained from wholesale market in Chongqing, China. Each sample was immediately placed into sealed aluminum foil pouch (12 cm × 8 cm) and stored at 4℃. Sensory assessment of all samples was performed according to Chinese product of geographical indication-Dongting (Mountain) Biluochun Tea GB 18957/2008. A total of 1.0 g of each sample was weighed accurately, brewed with 50 mL of boiled water for 10 min, filtered through gauze, and then tea soup was collected for analysis of electronic tongue.

Data acquisition An electronic tongue system consists of four parts: auto-sampler, sensor array (Alpha M.O.S.Co., Toulouse, France), data acquisition and data analysis system. A sketch of electronic tongue system is shown as Fig.1. The sensor array has one Ag/AgCl reference electrode and seven chemical sensors: BA, BB, CA, GA, HA, JB and ZZ. Each sensor can achieve selective adsorption of free chemicals in liquid by a sensitive thin film on the surface, and the potentiometric differences between the sensors and the Ag/AgCl reference electrode are caused by molecular interactions between the molecules in solution and the molecules of the sensor membrane material.

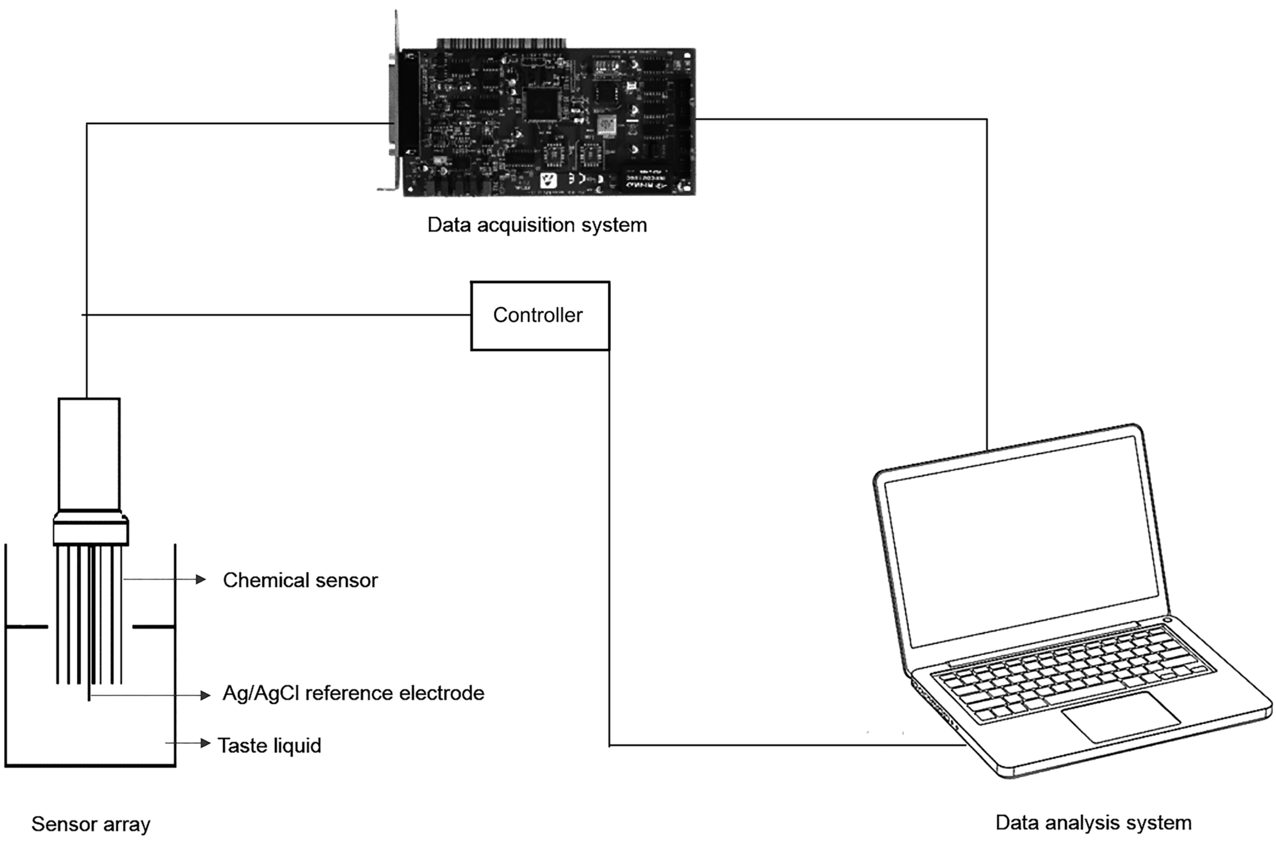

Fig. 1. A sketch of analysis system based on electronic tongue

Twenty five milliliter of tea soup of each sample was measured and loaded. Each sample was repeatedly measured three times by sensor array with 100 seconds response time according to the results of preliminary experiments. The room temperature was maintained at 25℃ during trial.

Data processing method and software Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA) and Artificial Neural Network (ANN) were used for identification of samples. All data processing were performed in MATLAB 2013a (Mathworks, Natick, USA) for windows 7.

Results and Discussion

Sensory Assessment The sensory assessment of these green tea samples was strictly performed in accordance with Chinese product of geographical indication-Dongting (Mountain) Biluochun Tea GB 18957/2008. According to the national standard, Dongting Biluochun Tea has seven grades in decreasing order of quality (Supreme I, Supreme II, Grade I, Grade II and Grade III). and identification criteria of tea are as follows: the dry tea has exquisite appearance of slender and coiled, much showy white pekoe and dark emerald-green; the tea soup shows light fresh green and limpid bright in color and slight sweet or floral aroma in taste; the securinega (tea leaves after brewing with boiled water) is yellow-green, bright and uniform.

A definitive result was provided by three trained and experienced tea tasters after identification of all samples was identified. On the basis of the judgment ofthe appearance of dry tea, the characteristics of tea soup (including color, aroma and taste) and the appearance of securinega, three trained tea tasters agreed that all tea samples were divided into four groups. The four groups were new Dongting Biluochun Tea (Grade I and Grade II) which was made from leaves of Biluochun tea plant grown in Dongting Mountain (Jiangsu Province, China) and was preserved for less than one year, counterfeit Dongting Biluochun Tea produced by non-Dongting Mountain regional Biluochun fresh tea leaves (Biluochun tea plant is also grown in Zhejiang and Sichuan Provinces, China) in accordance with the processing technology of Dongting Biluochun Tea, adulterated Dongting Biluochun Tea which was a mixture of genuine Dongting Biluochun Tea and other green teas in different proportion, and aging Dongting Biluochun Tea (old tea or chen tea), namely more than one year storage of new Dongting Biluochun Tea, was sold in the market in the name of new Dongting Biluochun tea because of attractive price differential between new tea and old tea. The four groups were respectively labelled as S1, S2, S3 and S4. Customarily, the latter three groups (S2, S3 and S4) appeared on the market in the name of new Dongting Biluochun Tea (S1) are false.

There were some differences in the appearance of dry tea and securinega, and in color and taste of tea soup among the four groups of tea samples. With a slender shape, dark emerald-green and pekoe-covered surface, the dry tea of S1 could be clearly distinguished from the dry tea of S2, S3 and S4 which were blackish dark yellow. In addition, S2 and S3 were some pekoe, and while S4 was little pekoe. The tea soup of S1 (new Dongting Biluochun Tea) showed light fresh green and limpid bright in color and slight sweet in taste. The tea soup of S2 (counterfeit Dongting Biluochun Tea), S3 (adulterated Dongting Biluochun Tea) and S4 (aging Dongting Biluochun Tea) appeared slightly turbid and brown. The taste of S2 and S3 were somewhat bitter and astringent tastes, while S4 was obvious bitter and astringent tastes, even off-flavor. The securinega of S1 and S2 was yellow-green, bright and uniform, while the securinega of S4 was dark yellow. A small portion of securinega of S3 was the same as S1. The numbers of S1, S2, S3 and S4 are 96, 100, 91 and 113, respectively. There are 400 samples in all.

Principal Component Analysis (PCA) Principal component analysis (PCA) is a multivariate statistical methods, which transform a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables by orthogonal linear transformation (Pearson, 1901). PCA can reduces the dimension of the data set and identifies new meaningful underlying variables based on information of original data (Kemsley, 1996). In addition, the scatter plot of PCA can show visual clustering results.

In this work, 10 samples of each groups were randomly selected and mixed again for analysis with electronic tongue system. By using MATLAB software, the three principal components (PC1, PC2 and PC3) were obtained (Fig.2). From the figure, 40 samples appear in cluster trend along three principal component axes, just associated with four groups of tea samples divided by sensory assessment. The PC1, PC2 and PC3 can explain 50.12%, 33.55% and 1.67% of total variance respectively, and the total cumulative variance reaches 85.34%. The S1 was clearly separated from S2, S3 and S4, furthermore, S2, S3 and S4 could also be separated each other. The figure demonstrated the capability of PCA to identify samples, and proved the feasibility and validity of electronic tongue for identification of fake Dongting Biluochun

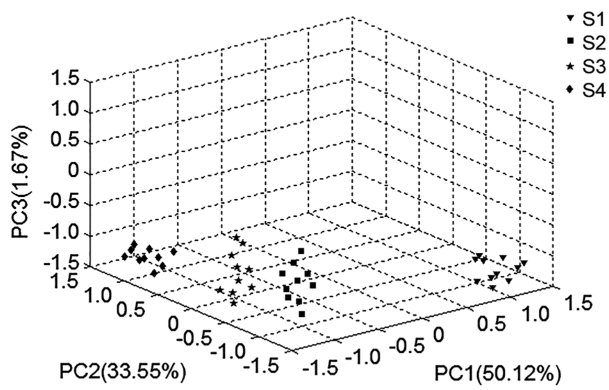

Fig. 2. Principal Components score plot of 10 samples of each tea groups based on electronic tongue. S1, new Dongting Biluochun Tea (Grade I and Grade II); S2, counterfeit Dongting Biluochun Tea; S3, adulterated Dongting Biluochun Tea; S4, aging Dongting Biluochun Tea (old tea or chen tea)

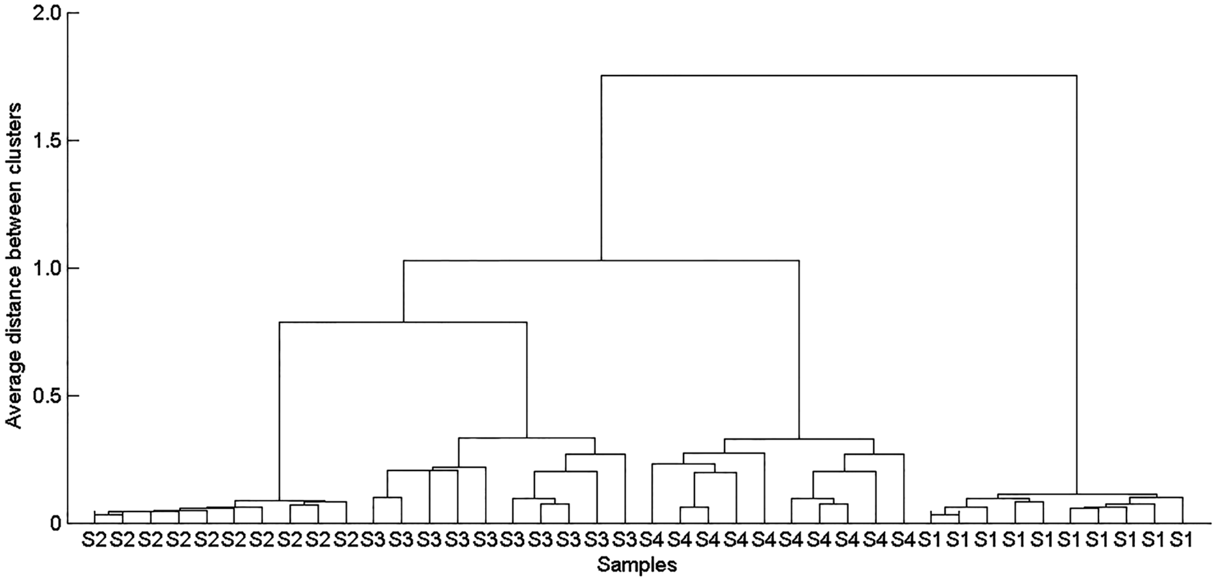

Fig. 3. A dendrogram of 10 samples of each tea groups based on electronic tongue. S1, new Dongting Biluochun Tea (Grade I and Grade II); S2, counterfeit Dongting Biluochun Tea; S3, adulterated Dongting Biluochun Tea; S4, aging Dongting Biluochun Tea (old tea or chen tea)

Tea. Similar results were obtained when the above random sampling was repeated many times.

Hierarchical Cluster Analysis (HCA) Cluster analysis is a form of exploratory data analysis aimed at grouping observations in a way that minimizes the difference within groups while maximizing the difference between groups. HCA organizes observations in a tree structure based on similarity or dissimilarity between clusters. The algorithm of HCA starts with each observation as its own cluster, and successively combines the clusters based on most similar until all observations are in a category (Fraley and Raftery, 1998; Patidar *et al*., 2012).

Ten samples of each group were randomly selected and mixed again, and HCA based on unweighted pair group method with arithmetic mean for computing distance between clusters was performed by MATLAB. The results of HCA were accordant with the results of sensory assessment, which all samples are clearly divided into four groups (Fig.3). Seen from the figure, the results showed that there were more similarities among S2, S3 and S4, and S1 and subclass containing S2, S3 and S4 revealed definite similarities. The results of HCA were also the same as the results of PCA plot. Similar result were obtained when the above random sampling was repeated many times.

Because of different chemical compounds, there are different taste and color of tea soup between S1, S2, S3 and S4. Many chemical compounds of new tea leaves, such as acids, esters, alcohols, amino acids and vitamins, form some water-insoluble compounds or volatile compounds based on oxidation reaction and condensation reaction caused by illumination, heat, water content and oxygen for long-term storage. The results of both PCA and HCA show an electronic tongue based on potential difference of sensor array can successfully achieve the identification of Dongting Biluochun Tea, consistent with sensory assessment. It suggests that an electronic tongue with pattern recognition methods is valid and practicable for identification of fake Dongting Biluochun Tea.

Artificial Neural Network (ANN) The previous PCA and HCA successfully showed their capabilities to distinguish between veritable and fake green tea. As a kind of unsupervised pattern recognition method, they are not suitable for classification and an automated prediction but more suitable for clustering and assessing the linear separability of samples. Compared to non-targeted clustering, some supervised pattern recognition methods which processes discrimination on the premise of the relationship between the characteristics of samples and the target of classification are commonly used for classification and prediction, such as support sector machine (SVM) and ANN. The ultimate goal of this work is to automatically recognize and distinguish fake green tea, and the two kinds of ANN, back propagation neural network with the Levenberg-Marquardt training algorithm (LMBP) and radial basis function neural network (RBF), are used as an automatic classifier in this works. With the advantages of Gauss-Newton method and gradient descent algorithm, LMBP has a better performance than traditional back propagation neural network (BPNN) based on gradient descent algorithm in practical application. According to Cover's Theorem (Cover, 1965), RBF is also a suitable tool for classification of complex systems by transforming training set into a higher-dimensional space via some non-linear transformation for linear separability. Because of self-learning, self-organizing and self- adaptive function, RBF is better approximation and global optimal ability, simpler network structure and faster training time than LMBP (Meng *et al*., 2010 ; Nie and Linkens, 1993). However, RBF requires more hidden layer neurons than LMBP. In this works, the structure of both LMBP and RBF consists of three parts:

Table 1. Identification results of LMBP and RBF models

ANN	Training time (s)	MSE	Identification rate (%)	
			Training set	Prediction set
LMBP	46.1258	8.7265×10^{-7}	100%	93.66%
RBF	22.7047	3.3205×10^{-7}	100%	99.85%

ANN, artificial neural network; MSE, mean square error; LMBP, back propagation neural network with the Levenberg-Marquardt training algorithm; RBF, radial basis function neural network; MSE, mean squared normalized error

the input layer, hidden layer, and output layer.

400 samples were divided into two subsets: 300 training sets for building the model and 100 prediction sets for testing the robustness of the model. The prediction performance, named R, was defined as :

$$R = (N_1/N_2) \times 100\% \qquad \cdots\cdots \text{Eq. 1}$$

Where R represents the identification accuracy (%); N_1 is number of correct classification; N_2 is number of total samples.

The performance of the ANN is be associated with some net parameters, such as number of hidden neurons in hidden layer and learning rate factor. The optimized number of hidden neurons in hidden layer of LMBP was 12 by trial-and-error method, and mean square error, maximum epoch and learning rate were set at 0.000001, 2000 and 0.01 respectively. The outputs of RBF are independent of the initial weights, and the numbers of RBF neuron in the hidden layer are attempted to continuously increase according to error goal until the error meets the requirements during the RBF training process. The goal of RBF was set at 0.000001and the value of DF (number of neurons to add between displays) was 4, and the remaining parameters took default values. The identification results from LMBP and RBF models are listed in Table 1. Seen from the table, the identification rates of LMBP and RBF models were 100% in training set, and the identification rates were 93.66% and 99.85% in the prediction set, respectively. By comparison, RBF is higher prediction accuracy and faster training time than LMBP. These results suggest that RBF model is more appropriate than LMBP for automatic identification and classification of fake green tea.

Conclusions

With the increased demand and rising costs, there are some fake famous green teas on the Chinese market, especially in rural and urban fringe. In order to overcome the subjectivity of sensory assessment, improve the efficiency of recognition and build an automatic classification and prediction models, an electronic tongue with pattern recognition methods was utilized for exploring the feasibility and availability of the identification of fake Dongting Biluochun Tea. The results showed that an electronic tongue system with pattern recognition could be used for identifying fake green tea instead of sensory evaluation. The two kinds of ANN achieved an automatic classifier for unknown samples, and the results of ANN suggested that RBF was more appropriate than LMBP whether in training time and prediction accuracy. Our results indicated that electronic tongue combined with pattern recognition methods has great potential for identification of fake green tea, even other fake or counterfeit foods.

Acknowledgements The authors gratefully acknowledge the financial support from Scientific and Technological Research Program of Chongqing Municipal Education Commission (Grant No. KJ1401001), China.

References

Banerjee, R., Tudu, B., Shaw, L., Jana, A., Bhattacharyya, N., and Bandyopadhyay, R. (2012). Instrumental testing of tea by combining the responses of electronic nose and tongue. *J. Food Eng.,* **110**, 356-363.

Cetó, X., Gutiérrez-Capitán, M., Calvo, D., and del Valle, M. (2013). Beer classification by means of a potentiometric electronic tongue. *Food Chem.,* **141**, 2533-2540.

Cheng, H., Qin, Z., Guo, X., Hu, X., and Wu, J. (2013). Geographical origin identification of propolis using GC–MS and electronic nose combined with principal component analysis. *Food Res. Int.,* **51**, 813-822.

Cover, T. M. (1965). Geometrical and statistical properties of systems of linear inequalities with applications in pattern recognition. *IEEE Trans. Comput.,* **14**, 326-334.

Deng, J. and Yang, Y. (2013). Chemical fingerprint analysis for quality assessment and control of Bansha herbal tea using paper spray mass spectrometry. *Anal. Chim. Acta,* **785**, 82-90.

Escuder-Gilabert, L. and Peris, M. (2010). Review: Highlights in recent applications of electronic tongues in food analysis. *Anal. Chim. Acta,* **665**, 15-25.

Fraley, C. and Raftery, A. E. (1998). How many clusters? Which clustering method? Answers via model-based cluster analysis. *Comput. J.,* **41**, 578-588.

Fujiki, H., Imai, K., Nakachi, K., Shimizu, M., Moriwaki, H., and Suganuma, M. (2012). Challenging the effectiveness of green tea in primary and tertiary cancer prevention. *J. Cancer Res. Clin. Oncol.,* **138**, 1259-1270.

Gill, G. S., Kumar, A., and Agarwal, R. (2011). Monitoring and grading of tea by computer vision–A review. *J. Food Eng.,* **106**, 13-19.

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

He, W., Zhou, J., Cheng, H., Wang, L., Wei, K., Wang, W., and Li, X. (2012). Validation of origins of tea samples using partial least squares analysis and Euclidean distance method with near-infrared spectroscopy data. *Spectrochim. Acta. A Mol. Biomol. Spectrosc.,* **86**, 399-404.

Hidayat, W., Shakaff, A. Y. M., Ahmad, M. N., and Adom, A. H. (2010). Classification of agarwood oil using an electronic nose. *Sensors,* **10**, 4675-4685.

Kemsley, E. (1996). Discriminant analysis of high-dimensional data: a comparison of principal components analysis and partial least squares data reduction methods. *Chemometrics Intell. Lab. Syst.,* **33**, 47-61.

Laddi, A., Sharma, S., Kumar, A., and Kapur, P. (2013). Classification of tea grains based upon image texture feature analysis under different illumination conditions. *J. Food Eng.,* **115**, 226-231.

Mahmood, T. and Akhtar, N. (2013). Combined topical application of lotus and green tea improves facial skin surface parameters. *Rejuv. Res.,* **16**, 91-97.

Meng, K., Dong, Z. Y., Wang, D. H., and Wong, K. P. (2010). A self-adaptive RBF neural network classifier for transformer fault analysis. *IEEE Trans Power Syst.,* **25**, 1350-1360.

Nie, J. and Linkens, D. A. (1993). Learning control using fuzzified self-organizing radial basis function network. *IEEE Trans Fuzzy Syst.,* **1**, 280-287.

Patidar, A. K., Agrawal, J., and Mishra, N. (2012). Analysis of Different Similarity Measure Functions and their Impacts on Shared Nearest Neighbor Clustering Approach. *Int. J. Comput. Appl.,* **40**, 1-5.

Pearson, K. (1901). On lines and planes of closest fit to systems of points in space. *Philos. Mag.,* **2**, 559-572.

Peris, M. and Escuder-Gilabert, L. (2013). On-line monitoring of food fermentation processes using electronic noses and electronic tongues: A review. *Anal. Chim. Acta,* **804**, 29-36.

Rhodes, L. E., Darby, G., Massey, K. A., Clarke, K. A., Dew, T. P., Farrar, M. D., Bennett, S., Watson, R. E., Williamson, G., and Nicolaou, A. (2013). Oral green tea catechin metabolites are incorporated into human skin and protect against UV radiation-induced cutaneous inflammation in association with reduced production of pro-inflammatory eicosanoid 12-hydroxyeicosatetraenoic acid. *Br. J. Nutr.,* **110**, 891-900.

Rudnitskaya, A., Rocha, S., Legin, A., Pereira, V., and Marques, J. C. (2010). Evaluation of the feasibility of the electronic tongue as a rapid analytical tool for wine age prediction and quantification of the organic acids and phenolic compounds. The case-study of Madeira wine. *Anal. Chim. Acta,* **662**, 82-89.

Sipos, L., Kovács, Z., Sági-Kiss, V., Csiki, T., Kókai, Z., Fekete, A., and Héberger, K. (2012). Discrimination of mineral waters by electronic tongue, sensory evaluation and chemical analysis. *Food Chem.,* **135**, 2947-2953.

Teye, E., Huang, X., Han, F., and Botchway, F. (2014). Discrimination of Cocoa Beans According to Geographical Origin by Electronic Tongue and Multivariate Algorithms. *Food Anal. Methods,* **7**, 360-365.

Wang, L., Wei, K., Cheng, H., He, W., Li, X., and Gong, W. (2014). Geographical tracing of Xihu Longjing tea using high performance liquid chromatography. *Food Chem.,* **146**, 98-103.

Wong, C. P., Kaneda, T., and Morita, H. (2014). Plant natural products as an anti-lipid droplets accumulation agent. *J. Nat. Med.,* **68**, 253-266.