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Analytical Methods

Non-destructive determination of total polyphenols content and classification of storage periods of Iron Buddha tea using multispectral imaging system

Chuanwu Xiong [a], Changhong Liu [a], Wenjuan Pan [a], Fei Ma [a], Can Xiong [a], Li Qi [b], Feng Chen [c], Xuzhong Lu [d], Jianbo Yang [d], Lei Zheng [a,e,*]

[a] School of Biotechnology and Food Engineering, Hefei University of Technology, Hefei 230009, China
[b] R&D Center, Hefei Meiya Optoelectronic Technology Inc., Hefei 230088, China
[c] Department of Food, Nutrition and Packaging Sciences, Clemson University, Clemson, SC 29634, United States
[d] Rice Research Institute, Anhui Academy of Agricultural Sciences, Hefei 230031, China
[e] School of Medical Engineering, Hefei University of Technology, Hefei 230009, China

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ABSTRACT

Total polyphenols is a primary quality indicator in tea which is consumed worldwide. The feasibility of using near infrared reflectance (NIR) spectroscopy (800–2500 nm) and multispectral imaging (MSI) system (405–970 nm) for prediction of total polyphenols contents (TPC) of Iron Buddha tea was investigated in this study. The results revealed that the predictive model by MSI using partial least squares (PLS) analysis for tea leaves was considered to be the best in non-destructive and rapid determination of TPC. Besides, the ability of MSI to classify tea leaves based on storage period (year of 2004, 2007, 2011, 2012 and 2013) was tested and the classification accuracies of 95.0% and 97.5% were achieved using LS-SVM and BPNN models, respectively. These overall results suggested that MSI together with suitable analysis model is a promising technology for rapid and non-destructive determination of TPC and classification of storage periods in tea leaves.

1. Introduction

The tea plant (*Camellia sinensis* (L.)) is native to Southeast Asia but is currently cultivated in more than 30 countries around the world. Tea is the most popular flavored and functional drink worldwide (Katiyar & Mukhtar, 1996; Li, Lo, Pan, Lai, & Ho, 2013). The nutritional value of tea is mostly from the tea polyphenols that are reported to possess a broad spectrum of biological activities, including antioxidant properties (Dufresne & Farnworth, 2001; Shizuo, 2011), reduction of various cancers, inhibition of inflammation, and protective effects against diabetes, hyperlipidemia and obesity (Claudia, Graciela, & Rosana, 2008; Khan & Mukhtar, 2007; Mukhtar & Ahmad, 2000; Weisburger, Rivenson, Garr, & Aliaga, 1997; Yang & Koo, 1997).

The three major types of tea, green tea, oolong tea, and black tea, differ in terms of the manufacture and chemical composition (Chen, Zhao, Fang, & Wang, 2007; Li et al., 2013). Iron Buddha tea (*C. sinensis* (L.) O. Kuntze), as the premium variety of semi-fermented oolong tea which was originally developed in Anxi county of Fujian Province, China around 300 years old (Chen, Guo, Zhao, & Ouyang, 2012). In comparison with other oolong tea varieties, Iron Buddha tea is famous for complex volatiles, partly resulted from its fermentation during preparation (Schuh & Schieberle, 2006). Empirically, the longer oolong tea is stored and further oxidized gradually, the better it is in terms of taste (Chen, Kuo, Yang, Li, & Tzen, 2013). Tea polyphenols in Iron Buddha tea chiefly consist of green tea catechins and a small proportion of black tea theaflavins and thearubigins due to its partial fermentation process (Li et al., 2013). During storage epimerization and oxidation were reported as the main cause of changes in tea catechins, tea catechins stability is dependent on temperature, oxygen availability, and relative humidity (Ananingsih, Sharma, & Zhou, 2013; Sang, Lambert, Ho, & Yang, 2011).

With the increasing consumption of the tea, the quality of tea is getting more and more attention. The amount of tea polyphenols has been regarded as a primary quality indicator of tea (Obanda, Owuor, & Taylor, 1997). To date, many analysis methods have been

* Corresponding author at: School of Medical Engineering, Hefei University of Technology, Hefei 230009, China. Tel./fax: +86 551 62919398.

E-mail addresses: yjianbo@263.net (J. Yang), lzheng@hfut.edu.cn, lei.zheng@aliyun.com (L. Zheng).

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developed to determine the total polyphenols contents (TPC) after tea preparation, such as colorimetric measurements (Claudia et al., 2008; ISO, 2005), titration method with potassium permanganate (Turkmen, Sari, & Velioglu, 2006) and HPLC (Chen et al., 2013; Lee, Hwang, Lee, & Choung, 2014; Xu, Song, Li, & Wan, 2012). However, these analytical methods are not only time consuming, no environmental-friendly and labor-intensive but also destructive (Kim, Park, & Choung, 2007; Lee et al., 2014). In order to overcome these disadvantages, non-destructive methods, especially those based on optical properties, are urgently required (Chen, Zhao, Liu, Cai, & Liu, 2008).

Recently, the spectroscopic techniques which have been developed with improved computer capacity and powerful chemometric tools have facilitated to extend in an increasing number of fields, thereby allowing efficient management of spectra and chemical data obtained from the samples. To date, near infrared reflectance (NIR) spectroscopy has been widely used in many sectors such as agricultural sector (Cen & He, 2007), petrochemical sector (Balabin & Safieva, 2008) and pharmaceutical sector (Dou, Sun, Ren, Ju, & Ren, 2005). It was known today that many studies on applying NIR with multivariate calibration methods analyzed the content of major catechins and total polyphenols in green tea (Chen, Zhao, Huang, Zhang, & Liu, 2006; Chen et al., 2008). These studies show that NIR spectroscopy has a high potential in measurement of active compositions in green tea. However, these studies are only used in milled tea powder.

Multispectral imaging (MSI) was developed by integrating the imaging and spectroscopy techniques together, which makes it possible to acquire both spatial and spectral information from a target object simultaneously. As a rapid and non-destructive analytical approach, MSI has recently been adopted to assess food safety and quality including contaminants detection (Kim et al., 2002), defect identification (Dissing et al., 2013; Xing, Saeys, & Baerdemaeker, 2006), constituent analysis (Lleó, Barreiro, Ruiz-Altisent, & Herrero, 2009; Lu, 2004; Peng & Lu, 2006, 2007), quality evaluation (Lunadei, Galleguillos, Diezma, Lleo, & Ruiz-Garcia, 2011; Lunadei et al., 2012; Løkke, Seefeldt, Skov, & Edelenbos, 2013; Panagoua, Papadopouloua, Carstensenb, & Nychasa, 2014; Sun et al., 2012), and also identification of transgenic rice (Liu et al., 2014b). According to our knowledge, there are no published studies about determination of TPC in tea using multispectral imaging.

Therefore, the development of rapid and non-destructive methods for determination of TPC in Iron Buddha tea is highly desired. The specific objectives of the study were to: (1) investigate the feasibility of using NIR and MSI to predict TPC in Iron Buddha tea powder; (2) compare the prediction abilities between NIR and MSI by partial least squares (PLS) method; (3) develop the TPC predication method in Iron Buddha tea leaves by using MSI; (4) test the ability of multispectral imaging technology to classify the tea based on storage periods by using least squares-support vector machine (LS-SVM) and back propagation neural network (BPNN).

2. Material and methods

2.1. Sample preparation

The test samples were grown in Huqiu Town of Anxi County, and were harvested and prepared by baking fresh teas at 120–140 °C for 72 h by Fuzhou Qingfang Tea Co., Ltd. which is a local tea manufacturer, after process the whole leaf was retained. Then the tea was kept in vacuumed plastic bag and stored at −18 °C. The test tea samples were provided with the year of 2004, 2007, 2011, 2012 and 2013 from same location and process, each year with 500 g.

Table 1
Descriptive statistics of total polyphenols content (g gallic acid/100 g) in tea leaves and tea powder.

Sample	Set	Year	Sample No.	Mean	SD	Range
Tea leaves	Calibration	2004	14	10.77	1.36	8.29–13.30
		2007	14	12.23	0.50	11.19–12.95
		2011	14	12.95	0.59	11.58–13.72
		2012	14	13.49	0.86	11.45–14.86
		2013	14	13.49	0.69	12.05–14.87
	Prediction	2004	7	10.70	1.38	8.68–12.74
		2007	7	12.19	0.54	11.28–12.86
		2011	7	12.95	0.55	12.00–13.63
		2012	7	13.48	0.69	12.46–14.41
		2013	7	13.48	0.68	12.52–14.65
	External validation	2004	7	10.82	1.48	8.79–13.24
		2007	7	12.28	0.48	11.46–12.88
		2011	7	13.01	0.53	12.13–13.66
		2012	7	13.61	0.75	12.51–14.70
		2013	7	13.57	0.71	12.66–14.80
Tea powder	Calibration	2004	10	10.95	0.266	10.42–11.24
		2007	10	12.22	0.271	11.93–12.82
		2011	10	12.27	0.243	11.89–12.63
		2012	10	12.88	0.302	12.41–13.34
		2013	10	13.54	0.254	13.20–13.89
	Validation	2004	5	11.08	0.316	10.79–11.49
		2007	5	12.12	0.219	11.89–12.40
		2011	5	12.23	0.192	12.01–12.41
		2012	5	12.74	0.145	12.52–12.87
		2013	5	13.41	0.441	12.99–14.13

SD: standard deviation.

The sample of each year were divided into two groups (250 g for each year), one group was directly measured by MSI, afterwards immediately using the same sample to test TPC by chemical analysis. The other group was milled into fine powder by small electric blender, after this process, the powder was sieved through 500 μm mesh. The sieved tea powder was made into pie samples by the special accessory of near infrared spectroscopy. After this procedure, the powder pie were intermediately performed the test by both NIR and MSI. Finally, powder pie was individually tested TPC by chemical analysis.

As a result, a total of 105 tea leaves and 75 tea powder samples were obtained in this study. Of these, 70 tea leaves and 50 tea powder samples were used for calibration set to build model, and remaining samples were used for prediction set to test the robustness of model. As shown in Table 1, the range of reference value of TPC in the calibration set can cover the range in the prediction set. Therefore, the sample distribution in the calibration and prediction sets is appropriate. Another 30 independent samples of tea leaves were collected as external validation set to verify the prediction models as shown in Table 1.

2.2. Chemical analysis

TPC was measured by colorimetric method using Folin–Ciocalteu assay (ISO, 2005). Polyphenols were extracted with 70% methanol in distill water (v/v) from a test portion of finely ground tea at 70 °C. The polyphenols in the extract were determined colorimetrically using Folin–Ciocalteu phenol reagent. Then measure the optical densities in 10 mm path length glass cells against distilled water on the spectrophotometer (UV–VIS 2802, Unico Shanghai) which was set at 765 nm. The glass cell was carefully cleaned between samples with distilled water and methanol. TPC of each sample was calculated from the average of independent determinations carried out in triplicate. The results were expressed as gallic acid equivalents (GAE), in g GAE /100 g dry material.