Research Article

Analysis of Antioxidant Activity of Chinese Brown Rice by Fourier-Transformed Near Infrared Spectroscopy and Chemometrics

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This paper develops a rapid method using near infrared (NIR) spectroscopy for analyzing the antioxidant activity of brown rice as total phenol content (TPC) and radical scavenging activity by DPPH (2,2-diphenyl-2-picrylhydrazyl) expressed as gallic acid equivalent (GAE). Brown rice ($n = 121$) collected from five producing areas was analyzed for TPC and DPPH by reference methods. The NIR reflectance spectra were measured with compact powders of samples and no treatment was used. Full-spectrum partial least squares (FS-PLS) and interval PLS (iPLS) were used as the regression methods to relate the antioxidant activity values to the NIR data. The spectral range of $4800\text{--}5600\text{ cm}^{-1}$ plus $6000\text{--}6400\text{ cm}^{-1}$ has the best correlation with TPC, while the range of $4400\text{--}5200\text{ cm}^{-1}$ plus $6000\text{--}6400\text{ cm}^{-1}$ is the most suitable for predicting DPPH. With standard normal variate (SNV) transformation and the selected wavelength ranges, the root mean squared error of prediction (RMSEP) is $0.062\text{ mg GAE g}^{-1}$ for TPC and $0.141\text{ mg GAE g}^{-1}$ for DPPH radical, respectively. The multiple correlation coefficients of predictions for TPC and DPPH are 0.962 and 0.974, respectively. The developed NIR method might have a potential application to quality control of brown rice in the domestic market.

1. Introduction

As one of the oldest domesticated grains, rice serves as the staple food for half of the global population. China is the first leading producer of rice in the entire world [1] and there are lots of varieties of rice cultivated in China. Brown rice has attracted much attention in recent years for its special taste and healthy effects [2, 3]. Compared with nonbrown rice, brown rice varieties not only provide high-quality protein, high fiber and vitamin contents, but also have a much more higher content of antioxidative compounds, including anthocyanidins (aglycones), cyanidin and malvidin [4]; polymeric procyanidins [5]; the phenolic compounds: anisole, protocatechuic acid methyl ester, syringaldehyde, and vanillin [6–9]; the phenolic compounds ferulic and sinapinic acids. A more detailed review of the antioxidative compounds is referred to in [3]. Brown rice has the potential to improve human health because the antioxidative compounds have the ability

to inhibit the formation or to reduce the concentrations of reactive cell-damaging free radicals.

The contents of antioxidative compounds in brown rice mainly depend on the varieties, cultivation conditions, and processing. It is well known that long-term storage can degrade the quality of brown rice because of oxidation. In China, for economic reasons, it is profitable to sell degraded or fraud brown rice by extracting pigments and other active components before putting them on the supermarket shelf. Therefore, it is necessary to develop a rapid and reliable method for determining the quality of brown rice, especially its antioxidant activity.

Traditional methods for evaluating antioxidant activity generally can fall into two classes: direct determination of antioxidant capacity and determination of the levels of the main antioxidant components [10]. The methods for direct determination include ferric reducing/antioxidant power (FRAP), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)

(ABTS), Trolox equivalent antioxidant capacity (TEAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and oxygen radical absorbance capacity (ORAC) [11]. For determining the contents of antioxidant components, high performance liquid chromatography (HPLC), high performance capillary electrophoresis (HPCE), and colorimetric determination have been used and the antioxidant components analyzed include flavone C-glucosides, total flavonoids, and total phenolic content (TPC) [12, 13]. However, the above methods are time consuming, laborious, and inconvenient to use considering the large number of samples to be analyzed.

Compared with traditional methods, NIR spectroscopy has many advantages including less sample preparation, reduced analysis time and cost. Therefore, NIR has been widely used for rapid analysis of antioxidant activity in various food products [14]. In this work, we investigated the feasibility of using NIR spectroscopy for rapid analysis of the antioxidant activity of brown rice in Chinese market. The objectives of this paper include (1) developing a quantitative model between the NIR spectra and two antioxidant activity indexes, namely, TPC and DPPH; (2) selecting useful wavelength intervals by interval partial least squares [15]; (3) comparing the predictive performances of calibration models based on different data preprocessing methods.

2. Materials and Methods

2.1. Brown Rice. Brown rice samples were collected from domestic markets and the producing areas are Yunnan (25), Hunan (21), Sichuan (27), Guizhou (25), and Shanxi (23). All the 121 samples are harvested and analyzed in 2013. All the samples were cleaned and stored at 25°C before analysis.

2.2. Analysis of Antioxidant Activities

2.2.1. Sample Preparation. The method in [16] was used. About 10 g of rice sample was ground by a crusher and extracted with 100 mL of mixture solvent (acetone: water, 75:25 v/v) for 1.5 h at 25°C. The extracts were then centrifuged at 950 g for 15 min. The supernatant was then kept at -20°C for further analysis.

2.2.2. Reference Analysis of Total Phenolic Content (TPC). A modified version of the Folin-Ciocalteu assay [17] was used to determine the TPC values for the prepared extracts. For the analysis, 20 μ L of extract, gallic acid standard, and blank were analyzed in parallel. Firstly, 1.58 mL of distilled water was added, followed by 100 μ L of Folin-Ciocalteu reagent. The mixtures were fully mixed and within 8 min, 300 μ L of sodium carbonate was added. The mixtures were magnetically stirred and allowed to incubate in dark for 30 min at 40°C. The absorbance at 765 nm was measured. The TPC values of samples were computed using the standard curve of aqueous gallic acid solutions and the unit was mg GAE/g.

2.2.3. Reference Analysis of DPPH. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extracts was measured using the method in [18]. Firstly, the blank was prepared by adding 100 μ L methanol to 1.4 mL of DPPH

radical methanolic solution (10^{-4} M). Separately, 100 μ L of the prepared extract was added to 1.4 mL of DPPH radical methanolic solution. The absorbance at 517 nm was measured.

2.2.4. NIR Analysis. The NIR diffuse reflectance spectra of all the samples were collected in 4000–12000 cm^{-1} on a Bruker-TENSOR37 FTIR system (Bruker Optics, Ettlingen, Germany) using OPUS software. All the spectra were measured with a PbS detector and an internal gold background as the reference. The resolution was 4 cm^{-1} and the scanning interval was 1.929 cm^{-1} . Therefore, each spectrum had 4148 wavelengths. The scanning number was 64, because more scans did not reduce the signal-to-noise ratio significantly.

2.3. Chemometrics. To obtain a set of representative objects for training/validating the calibration models, the DUPLEX algorithm [19] was used to split the measured objects into two data sets, one for training and the other for validation. DUPLEX alternatively picks up the two furthest objects in the objects pool for the training set and test set.

The quantitative modeling was performed using PLS and iPLS [15]. The iPLS algorithm was used to select informative spectral intervals for predicting TPC and DHHP. The principle of iPLS is to split the full spectra into smaller equidistant regions and, afterwards, build separate PLS regression models for each subinterval, using the same number of latent variables. Thereafter, a modeling error is calculated for each subinterval and for the full-spectrum model. The regions with the lowest error are selected and combined to build a final PLS model. An advantage of iPLS is that it can represent the predictive ability of each interval in a graphical display and enable a fast and reasonable selection of spectral intervals.

3. Results and Discussions

The content ranges reference values of antioxidant activity in calibration samples were 1.0933–6.0464 for TPC and 0.7833–5.8125 (mg GAE/g) for DPPH, respectively. The content ranges of TPC and DPPH in validation samples were 1.2135–5.9406 and 0.8121–5.4903 (mg GAE/g), respectively. This indicates that the DUPLEX method can split the data properly for calibration and validation. To reduce the unwanted spectral variations, three preprocessing methods, including smoothing, taking second-order derivative (D2) [20], and standard normal variate (SNV) transformation [21], were applied to the raw data. The raw NIR spectra and smoothed, D2, SNV spectra are shown in Figure 1. As seen from Figure 1, both D2 and SNV can reduce shifts caused by backgrounds. The DUPLEX algorithm was performed on the raw data to divide the 121 objects into a training set of 80 samples and a test set of 41 samples. For both full-spectrum PLS (FS-PLS) and iPLS models, leave-one-out cross-validation (LOOCV) was used to estimate model complexity and the errors of both FS-PLS and iPLS models were estimated by root mean squared error of cross-validation (RMSECV). The calibration and prediction results of FP-PLS models are listed in Table 1.

For iPLS models, the total spectral range was sequentially (from 4000 cm^{-1} to 12000 cm^{-1}) split into 20 spectral

TABLE 1: Results of full-spectrum PLS (FS-PLS) models for predictions of antioxidant activity of brown rice.

	TPC (mg GAE/g)			DPPH (mg GAE/g)		
	RMSECV ^a	RMSEP ^b	LVs ^c	RMSECV	RMSEP	LVs
Raw data	0.242	0.303	7	0.269	0.283	8
Smoothing	0.196	0.232	6	0.243	0.244	9
D2	0.191	0.209	6	0.233	0.219	8
SNV	0.162	0.167	6	0.199	0.211	7

^aRMSECV: root mean squared error of cross-validation.^bRMSEP: root mean squared error of prediction.^cNumber of PLS components.

FIGURE 1: The raw NIR spectra and the smoothed, second-order derivative (D2), and standard normal variate (SNV) spectra of brown rice objects.

intervals with an equal width of 400 cm^{-1} . At each interval, a PLS model is built to predict TPC and DPPH values. The numbers of PLS components were determined to obtain the lowest RMSECV. With each data preprocessing method, three intervals with the lowest RMSECV values were selected and combined to build the final iPLS model. The spectral intervals selected for predictions of TPC and DPPH are listed in Table 2. The most accurate interval models were obtained by SNV preprocessing. With standard normal variate (SNV) transformation, the root mean squared error of prediction (RMSEP) is $0.062\text{ mg GAE g}^{-1}$ for TPC (selected spectral intervals, $4800\text{--}5600\text{ cm}^{-1}$ and $6000\text{--}6400\text{ cm}^{-1}$) and $0.141\text{ mg GAE g}^{-1}$ for DPPH radical (selected spectral intervals, $4400\text{--}5200\text{ cm}^{-1}$ and $6000\text{--}6400\text{ cm}^{-1}$), respectively.

The correction coefficients (r) of predictions for TPC and DPPH are 0.962 and 0.974, respectively. The RMSECV for each spectral interval with SNV for predictions of TPC and DPPH is shown in Figure 2. The results indicate that iPLS can effectively select useful spectral intervals for predicting TPC and DPPH. By comparing the results in Tables 1 and 2, wavelength selection can improve the model accuracy more significantly than data preprocessing, indicating that uninformative wavelengths can degrade the FS-PLS model.

4. Conclusions

A rapid method for determination of antioxidant activity was developed by near infrared (NIR) spectroscopy and

TABLE 2: Results of interval partial least squares (iPLS) models for predictions of antioxidant activity of brown rice.

	TPC (mg GAE/g)			DPPH (mg GAE/g)		
	Selected intervals	RMSEP ^a	LVs ^b	Selected intervals	RMSEP	LVs
Raw data	3, 6, 8	0.179	4	2, 5, 6	0.175	6
Smoothing	3, 4, 8	0.131	5	2, 4, 6	0.188	4
D2	2, 4, 5	0.117	4	1, 3, 4	0.181	4
SNV	3, 4, 6	0.062	4	2, 3, 6	0.141	5

^aRMSEP: root mean squared error of prediction by the final iPLS with selected spectral intervals.

^bNumber of components of the final iPLS models including all the selected spectral intervals.

FIGURE 2: Root mean squared errors of cross-validation (RMSECV) obtained for each spectral interval by interval partial least squares (iPLS) with standard normal variate (SNV). The number at each bar indicates the number of PLS components at each wavelength interval.

chemometrics. By comparison of the results by FS-PLS and iPLS, wavelength selection can significantly improve the calibration accuracy of TPC and DPPH. The most suitable data preprocessing method was SNV. With SNV transformation and the selected wavelength ranges, the RMSEP is 0.062 mg GAE g⁻¹ for TPC and 0.141 mg GAE g⁻¹ for DPPH radical, respectively. The proposed method will provide a useful alternative tool to the physical and chemical analysis methods for brown rice.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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