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Prediction of Total Phenolics and Flavonoids Contents in Chinese Wild Rice (*Zizania latifolia*) Using FT-NIR Spectroscopy

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ABSTRACT

Total phenolics and flavonoids contents in Chinese wild rice were predicted using Near Infrared (NIR) spectroscopy as a rapid method. A Partial Least Square (PLS) algorithm was applied to perform the calibration. The models were calibrated by cross-validation and the chosen number of PLS factor was achieved according to the lowest Root Mean Square Error Cross-Validation (RMSECV) in calibration set. The correlation coefficient (R) and Root Mean Square of Error Prediction (RMSEP) in the test set were used as the evaluation parameters for the optimal model as follows: R = 0.985; RMSEP = 2.41 and the Residual Predictive Deviation (RPD) = 6.06 for total phenolics contents prediction by Multiplication Scatter Correction (MSC) model. For flavonoids contents prediction, R = 0.978, RMSEP = 1.23 and RPD = 4.81 by non preprocessing model. It could be concluded that NIR spectroscopy has significant potential in the nondestructive determination of total phenolics and flavonoids.

Key words: Chinese wild rice, phenolic, flavonoids, FT-NIR spectroscopy

INTRODUCTION

Wild rice was an ancient grain that has been used to treat a variety of ailments in Chinese medical practice (Zhai *et al.*, 1996). Consuming Chinese wild rice can drastically improve blood lipid profiles and low-grade inflammation and suppress oxidative stress in rats fed with a high saturated fat and cholesterol diet (Zhang *et al.*, 2009). Composition analysis of wild rice species has revealed their functional chemical components including phenolic. Phenolics compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Benavente-Garcia *et al.*, 1997; Samman *et al.*, 1998; Middleton *et al.*, 2000; Puupponen-Pimia *et al.*, 2001; Manach *et al.*, 2005).

Colorimetric and chromatographic techniques are the traditional methods used to estimate the nutritional value and food quality. Previous studies had reported the disadvantages of these methods, including requirement of chemical, samples pretreatment, costly and more time required (Ibern-Gomez *et al.*, 2002; McGlone *et al.*, 2003).

Recently, both multivariate analysis and near infrared spectroscopy combined with chemometric methods were used as a rapid method to predict the phytochemical components concentration and biological qualitative analysis (Cen and He, 2007). Near infrared spectroscopy is based on the strong absorption of overtones and combinations of fundamental vibrations in near

infrared region (750-2500 nm) by several functional groups, such as C-H (aliphatic), C-H (aromatic), C-O (carboxyl), O-H (hydroxyl) and N-H (amine and amide), in the organic compounds (Williams and Norris, 1987).

Rice nutrient characteristics, such as protein content of milled whole-grain sample (Delwiche *et al.*, 1996; Barton *et al.*, 2002), amino acid content of milled rice flour (Wu *et al.*, 2002; Zhang *et al.*, 2011) and fatty acids (Li and Shaw, 1997) were estimated by NIR spectroscopy. Furthermore, rheological and cooking properties have been evaluated by NIR spectroscopy and multivariate calibration techniques models (Delwiche *et al.*, 1996; Windham *et al.*, 1997; Bao *et al.*, 2001; Meullenet *et al.*, 2002).

The objective of this study was to explore the potential application of NIR Spectroscopy and different regression tools to determine total phenolics and flavonoids contents in Chinese wild rice.

MATERIALS AND METHODS

Rice samples: Three hundred and forty samples of hulled grains Chinese wild rice (*Zizania latifolia*) were milled by cyclone miller. The samples were obtained from the Nanjing Research Institute of Plants of the Chinese Academy of Sciences.

Extraction: One gram of whole-meal rice samples was extracted with 50 mL of MeOH/HCl (99:1 v/v) for 24 h at 25°C. The extracts were centrifuged at 5000×g for 15 min and the supernatants were stored at 4°C (Shen *et al.*, 2009).

Determination of total phenolics content: Total phenolics content was measured by Folin-Ciocalteu reagent according to (Singleton *et al.*, 1999). Six hundred microliter of freshly diluted 10-fold Folin-Ciocalteu reagent and 960 mL of sodium carbonate solution (75 g L^{-1}) were added to (120 μL) extract. The absorbance was measured at 760 nm after 5 min of reaction at 50°C, using a UV spectrophotometer. Gallic acid was used as standard and TPC was expressed as mg Gallic acid (GAL) equivalent per 100 g dry weight of sample.

Determination of total flavonoids: Total flavonoid content was determined by a colorimetric method (Bao *et al.*, 2005) with minor modi cation. The 0.5 mL of sample extract diluted with 2 mL double distilled H_2O and mixed with 0.15 mL 5% $NaNO_2$. After 5 min, 0.15 mL of 10% $AlCl_3.6H_2O$ solution was added, standing mixture for 5 min and then 1 mL of 1 M NaOH was added. After 15 min for mixture reaction the absorbance was determined at 415 nm. Total flavonoids contents were calculated using the standard Rutin curve and expressed as mg Rutin Equivalent (mg RE) per 100 g of dry weight of sample.

FT-NIR analysis: Near infrared spectra of hulled grain samples set (n = 340) were recorded at room temperature (20±0.5°C), in transmission mode in the spectral range 12,000-4000 cm^{-1} employing vials with an 8 mm path length and an FT-NIR spectrometer (MPA, Bruker Optics, Ettlingen, Germany). The resolution of the measurement was fixed at 8 cm^{-1}, the background and sample scan time was 64 sec and the scanner velocity was 10 kHz. Instrument control, processing and data analysis were performed using OPUS software (v.5.5 Bruker Optics, Ettlingen, Germany).

Spectra preprocessing: All samples spectra were preprocessed by seven mathematic methods: Constant Offset Elimination (COE) shifts the spectrum in order to set the y minimum to zero,

Table 1: Statistical analysis of calibration and test sets: Mean, range, Standard Deviations (SD) and Standard Error of Laboratory (SEL) for total phenolics and flavonoids contents of Chinese wild rice grain

Compounds and items	Calibration set n = 204	Test set n = 136
Polyphenols		
Mean	104.03	103.12
Range	77.17-136.82	78.06-132.61
SD	16.31	14.61
SEL	1.53	2.28
Flavonoids		
Mean	185.03	186.10
Range	175.71-201.03	174.41-198.15
SD	5.31	5.92
SEL	0.55	0.94

Total phenolics content expressed as mg GAL/100 g, flavonoids contents expressed as mg RE/100 g

Straight Line Subtraction (SLS) a straight line is fitted into spectrum and then subtracted from it; this accounts for tilt in the spectrum, Vector Normalization (VN) the spectrum will be normalized by first calculating the average intensity value and subsequent subtraction of this value from the spectrum. Then, the sum of the squared intensities was calculated and the spectrum was divided by the square root of this sum, Minimum-Maximum Normalization (MMN) was used to transform the data into a desired range by subtracting the minimum value from each individual spectrum and then dividing the range of this spectrum, Multiplication Scatter Correction (MSC) was used for the correction of scattered light on the basis of different particle sizes, First Deviation (FD) and Second Deviation (SD) eliminated baseline drifts and small spectral differences were enhanced. To avoid enhancing the noise, which is a consequence of derivative, spectra were first smoothed. This smoothing was done by using the Savitzky-Golay algorithm, which is a moving window averaging method: a window is selected, where the data are fitted by a polynomial of a certain degree. The central point in the window is replaced by the value of the polynomial (Naes *et al.*, 2002; Chen *et al.*, 2006a; Tripathi and Mishra, 2009; Sinija and Mishra, 2011). The calibration models were developed on the original and the preprocessed spectra using Partial Least Squares (PLS) regression, respectively.

Quantities analysis of PLS models: The 340 spectra were divided into calibration set to establish the PLS model and test set to predict the robustness of the model. To avoid bias in subset division, the division was made as follows: All samples were sorted according to their respective y-value (viz., the reference measurement value of total phenolics contents and total flavonoids contents). In order to obtain a 3/2 division of calibration/test spectra, the two spectra of every five samples were selected into the test set (Chen *et al.*, 2006a). Finally, 204 spectra constituted calibration set and the remaining 136 spectra constituted the test set. It can be seen from Table 1, the range of y-value in the calibration set covers the range in the test set. The performance of the final PLS model was evaluated according to the Root Mean Square Error of Prediction (RMSEP) and the correlation coefficient (R) in the test set. A leave-one-sample-out cross-validation was performed. The RMSECV was calculated as follows:

$$\text{RMSECV} = \sqrt{\frac{\sum_{i}^{n} = 1(\hat{x} \setminus i - x_i)^2}{n}} \tag{1}$$

where, is the predicted value for sample, when the model was constructed with the sample i removed, is the measured value for sample and is the number of samples in the calibration set.

Optimal PLS factor was chosen according to the lowest RMSECV. This procedure was repeated for each of the preprocessed spectra. For the test set, the Root Mean Square Error of Prediction (RMSEP) was calculated as follows:

$$RMSEP = \sqrt{\frac{\sum_{i}^{n} = 1(x_i - \hat{x}_i)^2}{n}} \qquad (2)$$

where, is the measured value for test set sample i, $\hat{x}_i$ is the predicted value of the model for test sample and is the number of samples in the test set.

Correlation coefficients between the measured and predicted values were calculated for both the calibration and the test sets, using Eq. 3, where the mean of the measured values for all of samples in the calibration and the test sets:

$$R = \sqrt{1 - \frac{\sum_{i=1}^{n}(\hat{x}_i - x_i)^2}{\sum_{i=1}^{n}(x_i - \overline{x}_i)^2}} \qquad (3)$$

The Residual Predictive Deviation (RPD), defined as the ratio between the standard deviation and (RMSECV or RMSEP) was used to verify the accuracy of the calibration models. The acceptable model for the quantitative analysis is the highest RPD value model (Williams and Norris, 1987).

RESULTS AND DISCUSSION

Spectra investigation: Figure 1a shows non-processing spectra for the original data and the spectra after first derivative preprocessing was presented in Fig. 1b, showing the spectra overtone characteristic absorption of functional groups and the vibrations of the carbonyl group which are caused by the contents of phenolic, alkaloids, protein, volatile and nonvolatile acids and some aroma compounds (Chen *et al.*, 2007).

Quantification of total phenolics contents with PLS algorithm: Multiplication Scatter Correction (MSC) was the optimal model for predicting the total polyphenols content in Chinese wild rice using 4 PLS factors as shown in Table 2, according to the lowest value of RMSECV (Chen *et al.*, 2006a).

Table 2: Results of a PLS models for total phenolics contents in Chinese wild rice

Treatment methods	Optimum PLS factor	Calibration set				Test set				
		CE	R	RMSECV (%)	RPD	CE	R	RMSEP (%)	RPD	SEP
NP[a]	9	Y = 0.951x+8.913	0.972	1.23	4.31	Y = 1.000x-0.155	0.978	1.23	4.81	0.96
COE	9	Y = 0.916x+15.52	0.951	1.61	3.30	Y = 0.900x+17.76	0.961	1.78	3.33	0.87
SLS	7	Y = 0.913x+15.93	0.958	1.51	3.52	Y = 0.830x+30.97	0.954	1.88	3.15	0.81
VN	9	Y = 0.934x+12.04	0.962	1.44	3.69	Y = 0.879x+21.59	0.959	1.84	3.22	0.85
M.MN	9	Y = 0.922x+14.30	0.958	1.51	3.52	Y = 0.869x+23.48	0.961	1.81	3.27	0.84
MSC	7	Y = 0.914x+15.77	0.957	1.52	3.50	Y = 0.792x+38.00	0.956	1.93	3.07	0.78
FD	9	Y = 0.926x+13.58	0.958	1.52	3.50	Y = 0.794x+37.74	0.951	1.98	2.99	0.78
SD	6	Y = 0.862x+25.42	0.929	1.95	2.72	Y = 0.804x+35.74	0.935	2.15	2.75	0.81

PLS: Partial least square, CE: Calibration equation, R: Correlation coefficient, RMSECV: Root mean square of standard error in cross validation, RMSEP: Root mean square of standard error in prediction, RPD: Residual predictive deviation: SD/RMSECV or RMSEP; SEP, Standard error of prediction, NP: Non-processed, COE: Constant offset elimination, SLS: Straight line subtraction, VN: Vector normalization, M-MN: Minimum-max normalization, MSC: Multiplication scatter correction, FD: First deviation and SD: Second deviation
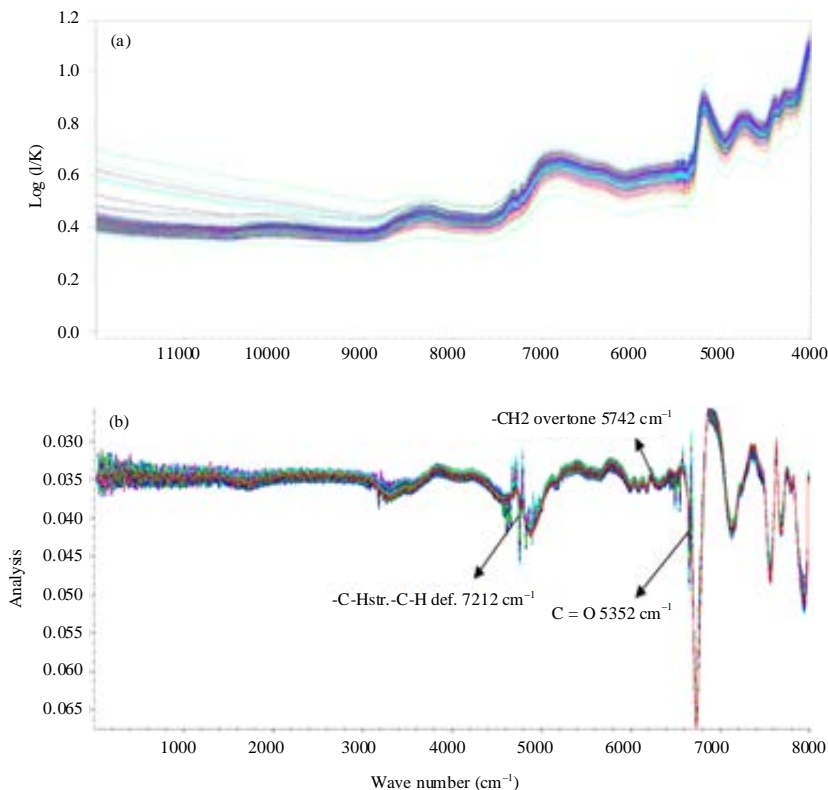
Fig. 1(a-b): Spectra of wild rice obtained from (a) Raw data non-processing and (b) First derivative

Figure 2 represents the regression correlation for calibration set (a) and test set (b) spectral samples by Multiplication Scatter Correction (MSC) preprocessing between the measured and NIR predicted values for total phenolic. The Root Mean Square of Standard Error in Cross Validation (RMSECV) 3.11, correlation coefficient (R) 0.981 and Residual Predictive Deviation (RPD) in cross validation 5.24 in calibration set. When the performance of PLS model was evaluated by samples in the test set, the Root Mean Square of Standard Error Prediction (RMSEP) 2.41, the correlation coefficient (R) 0.985, Residual Predictive Deviation (RPD) 6.06 and Standard Error of Prediction SEP 2.29. As shown in Table 2 the results of calibration models by different spectral preprocessing methods had a great power to predict the total phenolics contents according to RPD values higher than 3 and the RPD for optimum Multiplication Scatter Correction (MSC) model was 6.06. Generally a RPD values greater than three (range 3.1-4.9) could be recommended for screening purposes and more than five (range 5-6.4) for quality control (Williams and Norris, 1987). Prediction total phenolics content by NIRS models were reported in blueberries (Sinelli *et al*., 2008), green tea leaves (Chen *et al*., 2006a, b), forage legume (Goodchild *et al*., 1998) and green rooibos (Manley *et al*., 2006).

Quantification of flavonoids contents with PLS algorithm: Total flavonoids contents were predicted by the PLS algorithm using different spectral preprocessing methods. According to the calibration set and test set of calibration models, non-preprocessing spectral model was the lowest
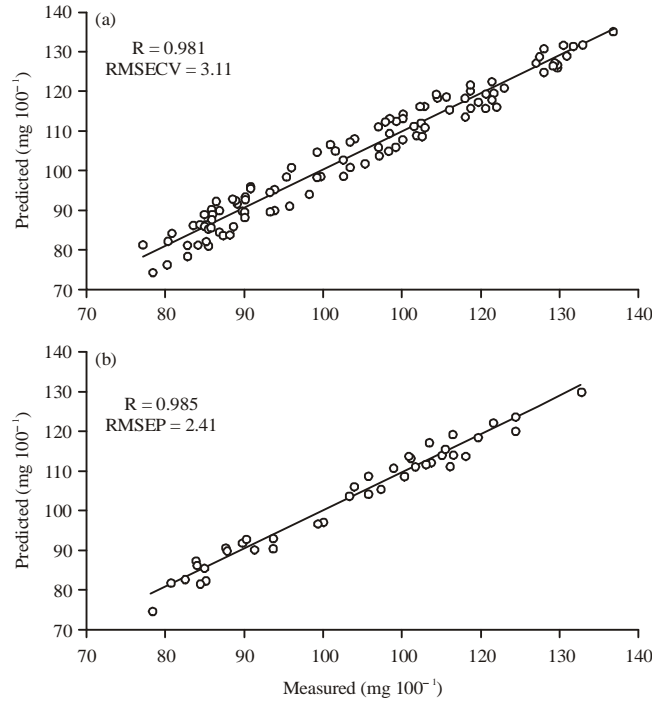
Fig. 2(a-b): (a) Regression lines calibration set and (b) Test set of a PLS model by Multiplication Scatter Correction (MSC) spectral preprocessing for total phenolics using FT-NIR spectroscopy

Table 3: Results of a PLS models for flavonoids contents in Chinese wild rice grain

Treatment methods	Optimum PLS factor	Calibration set				Test set				
		CE	R	RMSECV (%)	RPD	CE	R	RMSEP (%)	RPD	SEP
NP[a]	6	Y = 0.946x+5.540	0.971	3.82	4.27	Y = 0.938x+6.359	0.973	3.28	4.45	2.20
COE	5	Y = 0.956x+4.460	0.972	3.77	4.33	Y = 0.936x+6.373	0.959	4.08	3.58	2.22
SLS	9	Y = 0.951x+5.004	0.971	3.86	4.23	Y = 0.902x+10.32	0.948	4.56	3.20	2.17
VN	7	Y = 0.959x+4.116	0.978	3.30	4.94	Y = 0.910x+9.024	0.957	4.14	3.53	2.16
M.MN	5	Y = 0.946x+5.499	0.972	3.78	4.31	Y = 0.978x+2.189	0.958	4.20	3.48	2.33
MSC	4	Y = 0.963x+3.806	0.981	3.11	5.24	Y = 0.961x+4.229	0.985	2.41	6.06	2.29
FD	7	Y = 0.949x+5.202	0.974	3.65	4.47	Y = 0.921x+8.276	0.948	4.59	3.18	2.21
SD	5	Y = 0.909x+9.398	0.953	4.87	3.35	Y = 0.863x+13.99	0.953	4.37	3.34	2.12

PLS: Partial least square, CE: Calibration equation, R: Correlation coefficient, RMSECV: Root mean square of standard error in cross validation, RMSEP: Root mean square of standard error in prediction, RPD: Residual predictive deviation: SD/RMSECV or RMSEP, SEP: Standard error of prediction, NP: Non-processed, COE: Constant offset elimination, SLS: Straight line subtraction, VN: Vector normalization, M-MN: Minimum-maximum normalization, MSC: Multiplication scatter correction, FD: First deviation and SD: Second deviation

RMSECV with 9 PLS factors. However more than 10 factors correlated with a high risk of over-fitting and the model with 10 factors was taken in account (Zou *et al.*, 2007). In the present study the highest PLS factor was 9 as shown in Table 3.

The regression correlation for the calibration set (a) and the test set (b) spectral samples by non-preprocessing between the measured and prediction values for flavonoids were illustrated in Fig. 3. The performance of this model was shown in Table 3 with RMSECV 1.23, correlation coefficient (R) 0.972 and RPD 4.31 for cross validation in calibration set. While in test set, RMSEP
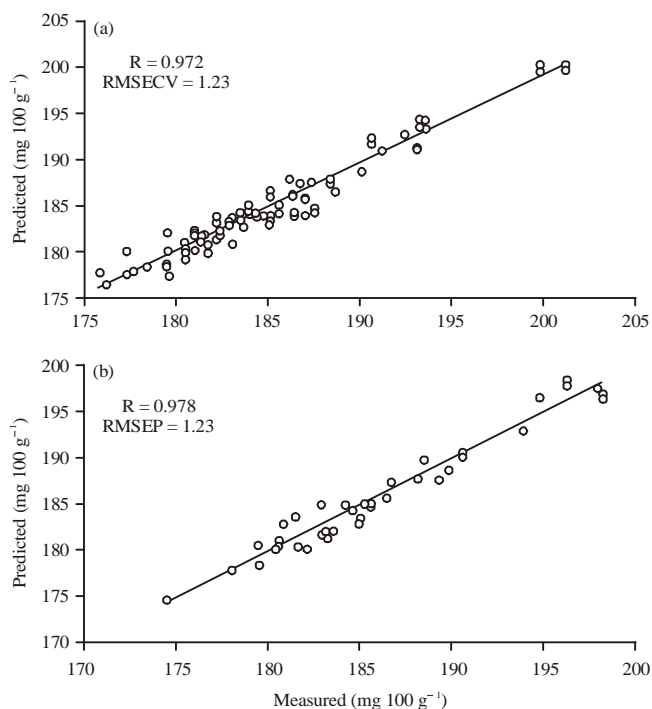
Fig. 3(a-b): (a) Regression lines calibration set and (b) Test set of a PLS model by spectral non-preprocessing for flavonoids using FT-NIR spectroscopy

1.23, correlation coefficient (R) 0.978, SEP 0.96 and RPD 4.81 indicating that method can be considered as a very good method for prediction purposes (Williams and Norris, 1987; Fearn, 2002). Prediction of flavonoids by NIRS models were reported in blueberries (Chen *et al.*, 2007) and fresh *Ginkgo biloba* leaf (Shi *et al.*, 2012).

Generally, the correlation coefficient (R) values for quantitative predictions models could be 0.83-0.90 and the R values for quality control models 0.92-0.96 and the accurate models R values greater than 0.98 for all applications (Lebot *et al.*, 2009). In this study, total polyphenol models R values were 0.95-0.98 and flavonoids models R values were 0.92-0.97 implying that those models were suitable for quantitative predictions.

CONCLUSION

The overall results sufficiently demonstrated that total phenolics and flavonoids contents in Chinese wild rice can be determined by NIR spectroscopy. The MSC and non preprocessing were confirmed to be optimal PLS models to determine total phenolics and flavonoids contents, respectively. The MSC model was achieved with R = 0.985, RMSEP = 2.41 and the Residual Predictive Deviation (RPD) = 6.06 for phenolics content prediction. For flavonoids content prediction the non preprocessing model was achieved with, R = 0.978, RMSEP = 1.23 and RPD = 4.81. Therefore, the NIRS technique was proven to be a quick, non-destructive, less expensive method to determine chemicals in plant samples. In our study, total phenolics and flavonoid contents by NIRS technique was in good agreement with chemical analysis, indicating that the NIRS technique could be potentially used for quick prediction for total phenolics and flavonoids contained in Chinese wild rice.

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