JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Application of Near-Infrared Reflectance Spectroscopy to the Evaluation of Rutin and D-*chiro*-Inositol Contents in Tartary Buckwheat

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Tartary buckwheat [*Fagopyrum tataricum* (L.) Gaench] is rich in rutin and D-*chiro*-inositol (DCI), which have beneficial effects in the treatment of hemorrhagic diseases and insulin-resistant diseases, respectively. The current methods of extraction and detection of rutin and DCI are complex and time-consuming; a simple way of analyzing these compounds in the native matrix would be desirable. In this work, near-infrared reflectance spectroscopy (NIRS) was applied to determine the contents of rutin and DCI in tartary buckwheat. The spectral data were compared with those determined from high-performance liquid chromatography (HPLC) methods. Models for predicting rutin and DCI contents in buckwheat were developed using a partial least-squares algorithm. Cross-validation procedures indicated good correlations between HPLC data and NIRS predictions ($R^2 = 0.76$ for rutin and $R^2 = 0.86$ for DCI). The rutin content ranged from 0.998 to 1.75%, while the DCI content covered 0.179–0.200%. The results showed that NIRS, a well-established and widely applied technique, could be applied to determine rutin and DCI in tartary buckwheat rapidly and nondestructively.

KEYWORDS: Rutin; D-*chiro*-inositol; NIRS; tartary buckwheat

INTRODUCTION

Tartary buckwheat [*Fagopyrum tataricum* (L.) Gaench] has been receiving increasing attention as a potential functional food. The various compounds contained in buckwheat such as flavonoids, fagopyrins, and buckwheat sterols have been claimed nutritionally and medically beneficial (*1*).

Rutin, a flavonol glycoside, is an active compound contained in buckwheat, which has been reported as the sole cereal source of rutin (*2*). It has been reported that tartary buckwheat [*Fagopyrum tataricum* (L.) Gaench] contained more rutin in seeds than common buckwheat (*Fagopyrum esculentum* Moench) (*3*). Rutin has shown the capability to antagonize the increase of capillary fragility associated with hemorrhagic disease (*4*), reduce high blood pressure (*5*), and show antioxidant and lipid peroxidation activities (*6*). It also has a lipid-lowering activity by decreasing the absorption of dietary cholesterol as well as lowering plasma and hepatic cholesterol (*7*).

D-*chiro*-Inositol (DCI) is another active compound in buckwheat seed with insulin-like bioactivity (*8–10*). As an epimer of *myo*-inositol, DCI is probably the main mediator of insulin metabolism by enhancing the action of insulin and decreasing blood pressure, plasma triglycerides, and glucose concentrations (*11, 12*). Therefore, DCI has great potential to work as an adjunctive drug in the treatment of insulin resistance diseases such as type 2 diabetes and polycystic ovary syndrome (*13, 14*). Cogram et al. have also reported that DCI was more effective than *myo*-inositol in preventing folate-resistant mouse neural tube defects (*15*).

In general, the contents of rutin and DCI in buckwheat seed vary depending on the species, varieties, and the environmental conditions under which they grow. The methods of analyzing rutin in buckwheat are complex, particularly in the step of extraction. The procedure for DCI detection is more complex, in which a silylation process is needed before being detected by gas chromatography. Although high-performance liquid chromatography (HPLC)-based methods are relatively simple, they are still too complicated to apply in practice. A nondestructive near-infrared reflectance spectroscopy (NIRS) may provide a rapid and convenient method to detect DCI in an efficient and cost-effective way.

The NIRS method has been used for the analysis of various agricultural and food products. NIRS is a fast, nondestructive analytical method, and there is no special need for sample preparation. In principle, the method is based on chemometrics, which generates correlations between the experimental data and the chemical composition or physical properties of the tested samples through mathematical and statistical procedures. The analysis software is designed for the quantitative analysis of spectra consisting of bands showing considerable overlap. That means that it could determine the content of more than one component in each sample simultaneously. It has shown good prediction with regard to moisture, fat, and protein in common buckwheat flour (*16, 17*).

The aim of the study was to evaluate the capability of using the NIRS technique in the prediction of rutin and DCI contents in

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10.1021/jf072453u CCC: $40.75
Published on Web 01/01/2008

Table 1. HPLC Analysis of Rutin and D-*chiro*-Inositol in Tartary Buckwheat and Statistic Results for Validation Set Predicted by the Optimal Models

	calibration				validation				statistic		
	n^a	range (%)	mean (%)	SD[b]	n^a	range (%)	mean (%)	SD[b]	R^{2c}	t^d	SEP[e] (%)
rutin	96	0.998–1.750	1.556	0.086	24	1.167–1.678	1.439	0.005	0.8355	2.059	0.029
DCI	96	0.179–0.200	0.192	0.095	24	0.184–0.200	0.192	0.005	0.8856	2.018	0.004

[a] n = sample numbers. [b] SD = standard deviation. [c] Correlative coefficient between predicted values in the validation set. [d] $T_{0.05}$ (23) = 2.096. [e] SEP = standard error of prediction.

tartary buckwheat and to perform an exploratory study to construct the predicted database of functional components in tartary buckwheat.

MATERIALS AND METHODS

Chemicals and Reagents. DCI and rutin standards and trifluoroacetic acid (TFA, 99%) were purchased from Sigma-Aldrich (Shanghai, China). Acetonitrile (HPLC) was purchased from Fisher Chemicals (Shanghai, China).

Samples Preparation. One hundred twenty samples of buckwheat kernels from different tartary buckwheat varieties from different regions of China and other countries were analyzed. The samples were divided into two groups, with each group containing different hybrids and varieties to cover all the ranges of content. The larger group of samples (96 samples) was used for model calibration. The smaller group (24 samples) was used for testing the soundness of model predication. All samples were dried in an oven at 40° until the weight was constant, detected by the NIR instrument, and then smashed to powder passing through 80 meshes for chemical detection.

Extraction and Qualification of Rutin and DCI. *Rutin Extraction.* The extraction was performed by mixing 1 g of sample with 20 mL of ethanol:water (90%, v/v) solution in one conical flask, incubated in a water bath shaker under at 30 °C for 12 h. The extract was filtered through Whatman #4 filter paper, with 1 mL of supernatant being diluted to 25 mL in methanol. Then, 1 mL of the diluted solution was filtered through 0.45 μm Milex-HN syringe filters (13 mm) (Bedford, MA) before injection into the HPLC.

DCI Extraction. The extraction was performed by mixing 1 g of sample with 20 mL of ethanol:water (1:1, v/v) solution in one conical flask, incubated in a water bath shaker under room temperature for 30 min. The extract was filtered through Whatman #4 filter paper, with 1 mL of supernatant being transferred into a little vial equipped with a cover. The vial was then put into an oven to evaporate the solvent. Dried samples were then hydrolyzed with 2 N TFA for 4 h at 70 °C with the cover on. The reactant was evaporated to dryness again and then redissolved by 1 mL of methanol. This solution was filtered through 0.45 μm Milex-HN syringe filters (13 mm) (Bedford, MA) and transferred into HPLC autosampler vials for immediate HPLC-ELSD (evaporative light-scattering detector) analysis.

Rutin and DCI standards were respectively dissolved in methanol to prepare standard solutions at 0.1 mg/mL. The standard calibration curve of rutin was made with a gradient injection of 2, 4, 6, 8, and 10 μL of rutin standard solutions (200–1000 ng, $R^2 > 0.99$), while the standard calibration curve of DCI was made with a gradient injection of 1, 2, 4, 6, 8, and 10 μL of DCI standard solutions (100–1000 ng, $R^2 > 0.99$).

HPLC Analysis. The HPLC system consisted of two Shimadzu LC-20A pumps, a Shimadzu LC-20A autosampler, and a SPD-20A UV/vis detector (Tokyo, Japan).

Rutin HPLC Analysis. A Thermo BDS HYPERSIL C_{18} column (4.6 mm × 250 mm, Thermo Electron Inc., Waltham, MA) was used. The wavelength of the UV detector was set at 280 nm. The separation was performed using a mixture of acetonitrile and distilled water with a gradient elution (0−5 min, 30% acetonitrile; 5−15 min, 40% acetonitrile; 15−18 min, 50% acetonitrile; and 18−35 min, 30% acetonitrile concentration). A 10 μL amount of rutin extract was injected for each analysis.

DCI HPLC Analysis. An Alltech Prevail Carbohydrates ES 5 μm column (4.6 mm × 250 mm, Alltech, Deerfield, IL) was used. The separation was performed using a mixture of acetonitrile and distilled water with a gradient elution (0−20 min, 80% acetonitrile; and 20−50 min, 65% acetonitrile concentration), the flow rate was set at 1 mL/min, and the elution after the column was sent to a ELSD (Alltech). ELSD conditions were optimized to achieve maximum sensitivity, the temperature of the drift tube was set at 95 °C, the nebulizing gas flow rate was set at 2.2L/min, and the gain was set at 1. A 10 μL amount of DCI extract was injected for each analysis.

NIRS Analysis. A Multi-Purpose Analyzer FT-NIR spectrometer (Bruker Optics Inc., Billerica, MA) was used to obtain the spectra. Approximately 15 g of sample was packed into a sample cup (diameter, 50 mm × height, 75 mm). The sample was irradiated with near-infrared monochromatic light, and diffuse reflectance was collected with lead sulfide detectors in the frequency range of 4000−12000 cm^{-1} to give a total of 64 data points per sample. Each sample was scanned twice, and the two spectra were averaged.

To correlate the spectral data to the HPLC data, multivariate analysis was performed with a commercial spectral analysis program "OPUS-QUANT" (version 5.5, Bruker Inc., Germany). Partial least-squares (PLS) regression was used for model regression. The performance of the final

Table 2. PLS Regression Statistics of Cross-Validation for Rutin and D-*chiro*-Inositol in Tartary Buckwheat

	preprocessing	n^a	R^{2b}	PLS vectors	RMSECV	content range	mean	frequency range (cm^{-1})
				rutin				
1	second derivative	85	0.35	4	0.0292	0.180–0.202	1.56	7502.1–6800.1
2	second derivative	87	0.43	7	0.0325	0.180–0.202	1.564	7502.1–6098.1 5450.1–4246.7
3	constant offset elimination	84	0.73	6	0.0220	0.179–0.202	1.565	6102–5446.3
4	constant offset elimination	85	0.76	8	0.0208	0.179–0.202	1.565	6102–5446.3 4601.6–4246.7
				DCI				
1	vector normalization	95	0.87	9	0.0016	0.180–0.202	0.193	6102–5446.3 4601–4246.7
2	vector normalization	93	0.85	8	0.0016	0.180–0.202	0.193	5774.1–5446.3 4601.6–4246.7
3	min−max normalization	94	0.86	9	0.0016	0.179–0.202	0.193	6102–5446.3 4601.6–4246.7
4	first derivative+ vector normalization	93	0.86	6	0.0016	0.179–0.202	0.193	6102–5446.3 4601.6–4246.7

[a] n = sample numbers used for calibration (samples with large residuals were omitted in a cross-validation procedure). [b] R^2 = coefficient of determination, and RMSECV = root-mean-square error of cross-validation.

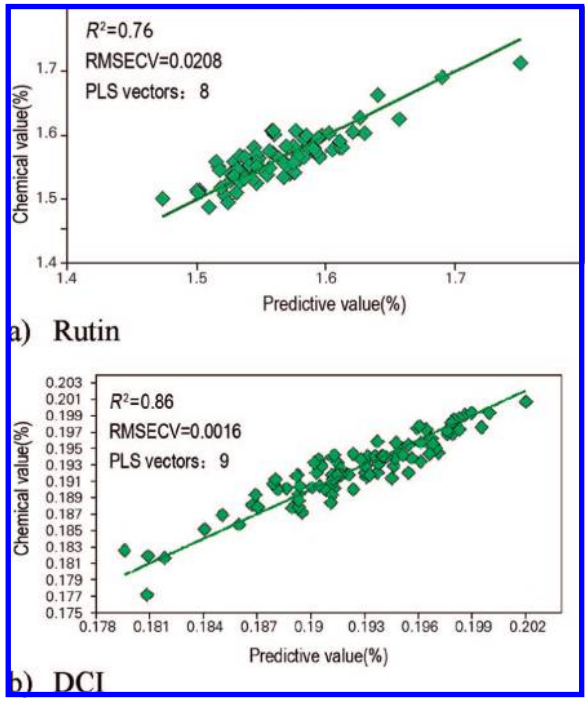

Figure 1. Scatter plot NIRS prediction values vs HPLC measurement for the determination of actual content of rutin (**a**) and D-*chiro*-inositol (**b**) in tartary buckwheat samples.

PLS regression model was evaluated in terms of root-mean-square error of cross-validation (RMSECV), standard error of prediction (SEP), and the correlation coefficient (R^2). For PLS, a leave-one-sample-out cross-validation was involved; the spectrum of one sample of the calibration set was removed from this set, and a PLS regression model was built up with the remaining spectra of the calibration set. The left-out sample was predicted with this model, and the procedure was repeated with leaving out each of the samples of the calibration set. For the test set, SEP estimated the precision of the developed NIRS model. Finally, correlation coefficients between the predicted and the chemical data were calculated for both the calibration and the test set.

RESULTS AND DISCUSSION

To build up a robust PLS model, all 120 average spectra data were divided into a calibration group and a validation group by a ratio of 4:1. The contents of rutin and DCI in all 120 samples were detected by the HPLC method. The content ranges, means, and standard deviations of chemical values of components for samples in the calibration set and validation set are shown (**Table 1**), and both contents of rutin and DCI ranged widely. The results suggest that the samples used in this study are representative of the diversity in buckwheat.

The regression models of the cross-validations for rutin and DCI with different preprocessing methods, PLS vectors, and wavelength ranges were developed (**Table 2**). For rutin, different preprocessing methods, PLS vectors, and wavelength ranges selected led large differences in the value of R^2 and RMSECV (**Table 2**). Four models developed for rutin all had similar content ranges, and among them, #4 had the highest R^2 of 0.76 and the lowest RMSECV of 0.0208. The optimized equation was developed by choosing a reprocessing method of constant offset elimination, a PLS vector of 8, and wavelength ranges of 6102–5446.3 and 4601.6–4246.7 cm^{-1}. The regression equation can be seen in **Figure 1a**.

For DCI, four models were developed as well (**Table 2**). Among them, #3 was the optimal model, which had an R^2 of 0.86 and a RMSECV of 0.0016. The optimized equation was developed by choosing the reprocessing method of min−max normalization, PLS vectors of 9, and wavelength ranges of 6102–5446.3 and 4601.6–4246.7 cm^{-1}. The regression equation can be seen in **Figure 1b**.

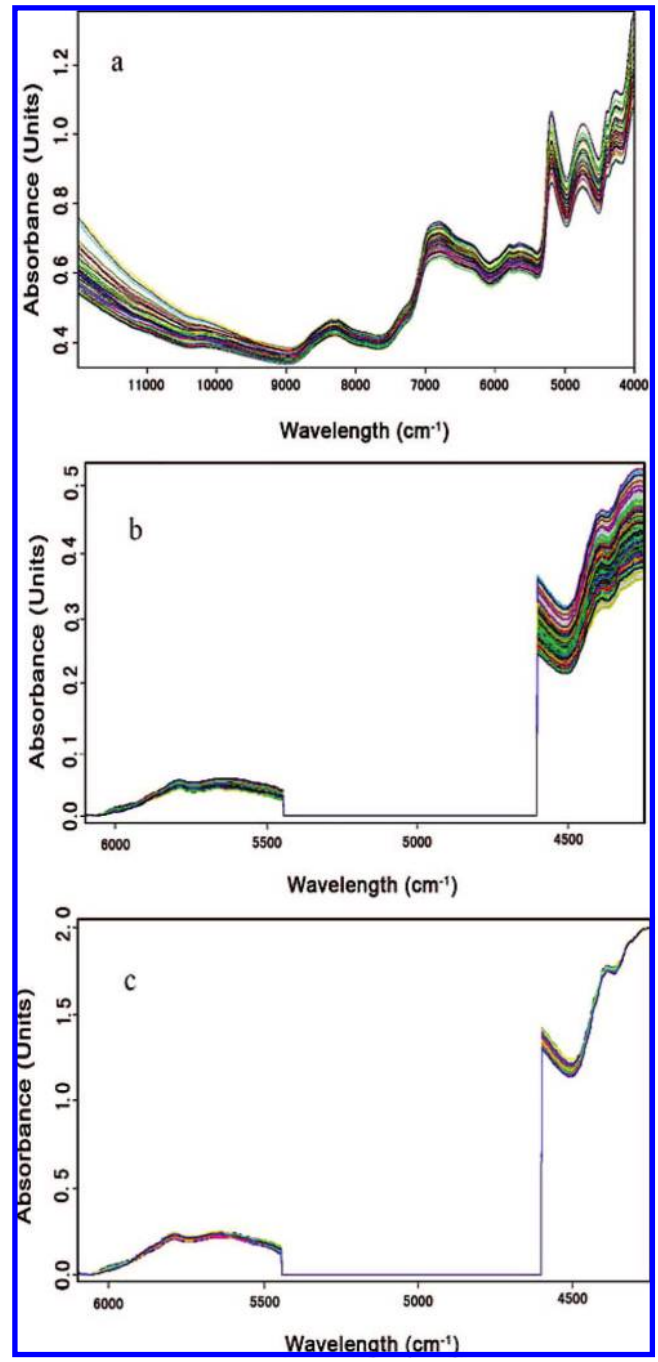

Figure 2. (**a**) Typical NIR spectra obtained from different tartary buckwheat samples. (**b**) The effect of constant offset elimination preprocessing to original spectra. (**c**) The effect of min−max normalization preprocessing to original spectra.

Data preprocessing is an important stage in performing a calibration. If the spectra of the same sample are not identical, a data preprocessing procedure must be chosen to eliminate offset or different linear baselines. The evidence was that the spectra of rutin were significantly improved with optimal preprocessing of constant offset elimination (**Figure 2b**). It cannot only minimize the baseline shift but also enhance the intrinsic matrix absorption of the material and enable the extraction of significant information from the original spectra matrix. For DCI, the min−max normal-

ization was the optimal preprocessing method, which is one of methods to eliminate different samples thickness (**Figure 2c**). In quantitative analysis, it is assumed that the layer thickness is the same in all measurements, but in fact, the operation will cause variations in sample thickness. By preprocessing the data, these variations can be eliminated to ensure a good correlation between the spectral data and the chemical values.

The number of PLS vectors is a crucial point for the quality of the calibration model. To handle the data conveniently, the spectral data and the chemical data are written in the form of matrices, in which each row represents a sample spectrum. The matrices were broken down into several factors, which were called principal components (PLS vector). Not all of the principal components are necessary to describe the relevant spectral features, so that only the relevant principal components were used to perform regression model. The model with too many PLS vectors will fit the training set better, but the prediction for other samples may become worse, which is called "overfitting" of a model. On the other hand, less PLS vectors would give a lower adaptability because of lacking enough information. In the research, reasonable PLS vector numbers were below 10.

The optimized models for both rutin and DCI needed to be validated using a validation group. The samples in the validation group were analyzed, predicted data for rutin and DCI were collected, respectively, and the statistical results are shown in **Table 1**. The SEP value means the SEP for the validated samples and reflects the deviated extent between predicted values and chemical values for the validated samples. The SEP decreased as the correlation coefficient increased. The significance of the model was tested using variance analysis (*T* test), and close agreement between experimental and predicted values was obtained. All *T* values of the predicted and measured chemicals for rutin and DCI were lower than the level of significance ($p > 0.05$). It indicated that chemical measurement and NIRS prediction had no significant deviation, and NIRS calibration models developed can be used for the prediction of the rutin and DCI content.

In conclusion, the first application of NIRS to simultaneously analyze the rutin and DCI in tartary buckwheat is reported. As compared with HPLC methods, the NIRS method has many advantages. First, it requires simple sample preparation. Second, it is fast; analyzing one sample can be finished within several minutes after the calibration equation has been developed. Because it is a spectral procedure, the NIRS procedure requires less chemical reagents, which not only reduces analytical cost but also provides a sage working environment.

ACKNOWLEDGMENT

We are grateful to L. Q. Wei, National Key Facility for Crop Gene Resources and Genetics Improvement, Institute of Crop Science, CAAS, for providing the NIRS equipment.

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Received for review August 15, 2007. Revised manuscript received November 28, 2007. Accepted November 29, 2007. The present study was supported by a Talent Fund (for G.R.) of the Chinese Academy of Agricultural Science, a Technology Transformation Fund (2005EFN-211400040), a Chest Fund (2005DIA4J01), and a Supporting Fund (2006BAD02B06-03) from The Ministry of Sciences and Technology, People's Republic of China.

JF072453U