

Determination of Total Ash and Acid-insoluble Ash of Chinese Herbal Medicine *Prunellae Spica* by Near Infrared Spectroscopy

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Rapid and accurate determination of total ash and acid-insoluble ash of Chinese herbal medicine would be valuable for the quality illustration because current approaches are laborious and time-consuming. This study investigated the feasibility of near infrared spectroscopy in predicting simultaneously the total ash and acid-insoluble ash contents of *Prunellae Spica* (one of the most widely used Chinese herbal medicine). Samples were collected from ten different geographic regions and scanned under the near-infrared spectroscopy region ($4000\text{--}12000\text{ cm}^{-1}$). Models between the spectral data and the results of reference analysis were developed, and subsequently validated. Calibration models based on partial least squares were accurate for the prediction of total ash ($R^2=0.914$; standard errors of prediction=0.373), and acid-insoluble ash ($R^2=0.905$; standard errors of prediction=0.452).

Key words—near infrared spectroscopy; total ash and acid-insoluble ash; *Prunellae Spica*; Chinese herbal medicine

INTRODUCTION

Total ash and acid-insoluble ash contents are important indices to illustrate the quality as well as purity of herbal medicine. Total ash includes “physiological ash”, which is derived from the plant tissue itself, and “non-physiological ash”, which is often from environmental contaminations such as sand and soil. Total ash content alone is not sufficient to reflect the quality of herbal medicines, since the plant materials often contain considerable levels of physiological ash, calcium oxalate in particular. Thus, the acid-insoluble ash content is another index to illustrate the quality of Chinese herbal medicine.

Accurate prediction of the total ash and acid-insoluble ash of Chinese herbal medicine is quite important, particularly in terms of quality assessment. According to the empirical methodology “Determination of ash” outlined in the Pharmacopoeia of the People’s Republic of China (Volume I, Edition 2005),¹⁾ the total ash and the acid-insoluble ash contents of Chinese herbal medicine are determined by an ignition method, which requires carbonizing the samples until constant weight is achieved. The procedure is very complicated, laborious, and it is too time-consuming (at least 8–10 h) to be used for rapid measurement. Therefore, there is a strong demand for a

simple and rapid analytical technique to improve the analysis efficiency.

Near-infrared spectroscopy (NIRS), in combination with chemometric analysis, is of interest to determine a wide variety of products and parameters, because it is fast, non-destructive, and it can analyze solid samples without sample pre-treatment. NIRS has successfully been used to predict the ash content of Mediterranean shrub,²⁾ green crop cereals,³⁾ flour,⁴⁾ roasted coffee,⁵⁾ alfalfa hay,⁶⁾ natural alpine swards,⁷⁾ diets of grazing young ostriches,⁸⁾ and olive tree leaves.⁹⁾ However, relatively few works have reported the use of NIRS to determine the ash content of traditional Chinese medicine. Cho *et al.*¹⁰⁾ found that prediction result of the ash content of deer antler using NIRS showed good correlation with the reference ignition method. Woo *et al.*¹¹⁾ confirmed the feasibility of NIRS in determination of ash content in *Cornu Cervi Parvum*. While most of traditional Chinese medicine are plant materials, the possibility of NIRS to determinate the total ash and acid-insoluble ash of Chinese herbal medicines has not been evaluated.

The objective of this study was to study the feasibility of NIRS in the analysis of total ash and acid-insoluble ash of *Prunellae Spica*, one of the most widely used Chinese herbal medicine. This herb is the dried fruit spike of *Prunella vulgaris* L. (Fam. Labiaceae), grown in the regions throughout China. It is collected

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in summer when the spike becomes brownish-red, removed from foreign matter, and dried in the sun. It has been routinely prescribed in China for quenching fire of the liver, counteracting inflammation of the eye, reducing nodulation, and inducing subsidence of swelling. The ash and acid-insoluble ash contents of *Prunellae Spica* must be controlled according to legal regulations (the Pharmacopoeia of the People's Republic of China, Volume I, Edition 2005).¹⁾ And this is the first study to our knowledge, to use NIRS to predict total ash and acid-insoluble ash of Chinese herbal medicine.

EXPERIMENTAL

Materials and Solvents Ashless filter paper was obtained from Hangzhou Xinhua Paper Industry Co., Ltd. (Hangzhou, China). Hydrochloric acid (analytical grade) was purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China).

Sample Collection Chinese herbal medicine varies in composition with different geographic regions. 72 *Prunellae Spica* samples were obtained from ten production regions in China (Beijing, and the provinces of Jiangsu, Anhui, Zhejiang, Hubei, Hunan, Henan, Jiangxi, Chengdu, Guangdong), to cover a wide range of concentrations both for the total ash and acid-insoluble ash, as required for NIRS calibration.

Reference Analyses Chemical reference data for the total ash and acid-insoluble ash of *Prunellae Spica* were obtained by the reference ignition method outlined in the Pharmacopoeia of the People's Republic of China (Volume I, Edition 2005).¹⁾ The ash contents were measured on a duplicate of each sample.

The procedure of the determination of total ash was performed as follows: each sample was pulverized to control the particle size below 24 meshes and then mixed well. 4 g powdered sample was placed in a tared crucible, weighed accurately, and slowly carbonized using a muffle-oven at 575°C until the sample turned into white ash to constant weight. The ash was weighed, and the percentage of the total ash was calculated.

The procedure of the determination of acid-insoluble ash was performed as follows: 10 ml of dilute hydrochloric acid was added with great care to the ash obtained from the determination of the total ash, and the crucible was covered with a watch glass. The

crucible was heated on a water bath for 10 minutes. Then the watch glass was rinsed with 5 ml of hot water. The rinsing was added to the crucible and filtered with an ashless filter paper. The residue was transferred to the filter paper with water, and the filter paper was washed with water for several times till the filtrate yielded no reactions of chlorides. The filter paper together with the residue was transferred to the original crucible, which was then dried and ignited to constant weight. The acid-insoluble ash was weighed, and the percentage of acid-insoluble ash was calculated.

Recording of the Near Infrared Spectra from *Prunellae Spica* Samples NIR spectra were recorded on a multi purpose analyzer (MPA) FT-NIR spectrometer from Bruker (Bruker Optics Inc., Ettlingen, Germany) equipped with a PbS detector. Spectra were recorded in the reflectance mode. Each individual spectrum was the average of 64 scans done with a resolution of 8 cm⁻¹ over the wavelength range of 4000–12000 cm⁻¹.

Data Process and Chemometric Analysis OPUS (Optics User Software) version 5.0 from Bruker was employed for the data process, including spectra-pretreatment, optimum wavelength region selection and quantification of the total ash and acid-insoluble ash contents.

The pretreatment for the spectral data included constant offset elimination, straight line subtraction, vector normalization, min-max normalization, multiplicative scatter correction, internal standard, two derivatives (first and second order), first derivative + straight line subtraction, first derivative + vector normalization, and first derivative + multiplicative scatter correction.

The data determined by the reference ignition method for all samples were added to the corresponding NIR spectral files. For each constituent, the spectra were sorted from lowest to highest constituent value and split into two independent subsets with a ratio of 2 : 1, a calibration set with 48 samples and a validation set with 24 randomly selected samples. In order to select a suitable validation set, main caution was taken to verify that it covered appropriately and uniformly the whole range of constituent concentrations.

The relationship between the spectra and reference values was studied using a partial least squares (PLS) method, and the PLS models were optimized by

cross-validation. With cross-validation, each sample was removed one at a time from the sample set, a new calibration was performed and a predicted score was calculated for the sample removed. This procedure was repeated until every sample had been left out once using the leave-one-out method. Performance of the calibration was expressed as coefficient of determination in calibration (R^2) and standard error of cross-validation (SECV).

The established PLS models was subsequently used to predict the total ash and acid-insoluble ash of the remaining 24 samples (validation set). The accuracy of the NIRS prediction equations was then assessed using linear regression analysis between NIRS-predicted values and those obtained by the reference ignition method. The most important statistics were squared coefficient of determination in validation (r^2) and standard error of prediction (SEP).

$$\text{SECV} = \sqrt{\frac{\sum (\hat{C}_i - C_i)^2}{n-1}}$$

$$\text{SEP} = \sqrt{\frac{\sum (\hat{C}_i - C_i)^2}{m}}$$

where C_i is the reference measured values; \hat{C}_i , the NIRS predicted values; n , the number of samples in the calibration set; m is the number of samples in the validation set.

RESULTS AND DISCUSSION

Compositional Data and Near Infrared Spectra of Prunellae Spica Samples The mean, range and standard deviation values of total ash and acid-insoluble ash contents of Prunellae Spica that resulted from the reference analyses and subsequently used for calibration set and validation set are summarized in Table 1. Prunellae Spica, like other herbal materials, show a variation in the variety and contents of compounds according to differences in growing conditions, such as soil type, climate, *etc.* In the present study, Prunellae Spica samples were collected from different geographic regions (10 production regions in China), and as shown in Table 1, they cover a wide

range of concentrations both for the total ash and acid-insoluble ash, calibration and validation sets are statistically similar both for the total ash and acid-insoluble ash contents.

Figure 1 shows the spectra of the ground Prunellae Spica samples over the spectral range of 4000–12000 cm^{-1} . It is noted that those NIR spectra of Prunellae Spica with different contents of total ash and acid-insoluble ash are very similar to each other, and the overlapping bands due to overtones and combination modes makes the quantitative or qualitative analysis not straight forward. Therefore, chemometrics methods were applied in the current study to extract information from the spectra for the total ash and acid-insoluble ash study of the Prunellae Spica samples.

NIRS Analysis Quantification of the total ash and acid-insoluble ash of the Prunellae Spica samples was performed using PLS algorithm. PLS is a commonly used statistical approach to quantitative analysis based on the partial least squares algorithm. PLS is capable of quantifying sample components when the correlation between concentration and absorbance is very complex.¹²⁾ The statistical evaluation of calibration and validation of Prunellae Spica for the total ash and acid-insoluble ash are summarized in Table 2. The accuracy of prediction, expressed in terms of r^2 and SEP values, appeared satisfactory. r^2 for both total ash and acid-insoluble ash were greater than 0.90, suggesting a good correlation between the references and the PLS model. And the linear regression relationships between the NIRS-predicted values and the reference measured values for validation sets are also charted (Figs. 2 and 3). The results indicated that NIRS offered the possibility of rapid determination of the total ash and acid-insoluble ash of Prunellae Spica.

NIRS is often used for the analysis of organic constituents, through the detection of several functional groups associated with H bonding, such as C-H, N-H and O-H. Meanwhile, inorganic minerals theoretical-

Table 1. Total Ash and Acid-insoluble Ash Contents of the Prunellae Spica Samples Used for NIRS Calibration and Validation

Constituent	Calibration set		Validation set	
	Mean \pm SD	Range	Mean \pm SD	Range
Total ash (%)	10.41 \pm 1.26	8.73~13.60	10.23 \pm 1.22	8.75~12.98
Acid-insoluble ash (%)	2.128 \pm 1.311	0.68~5.38	2.130 \pm 1.308	0.69~5.10

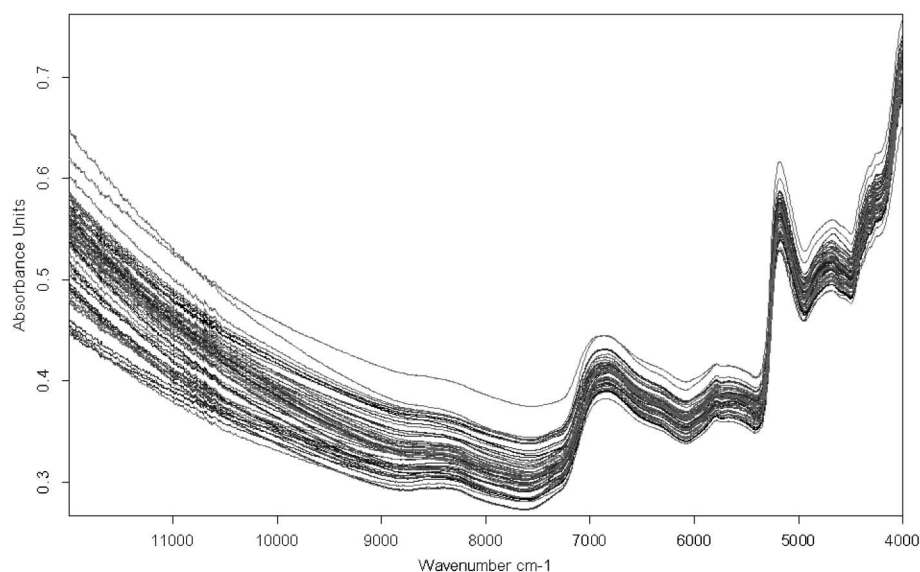


Fig. 1. NIR Spectra of Prunellae Spica Samples over the Range of 4000–12000 cm^{-1}

Table 2. Statistics of the Calibration and Validation Equations Developed by NIRS to Estimate the Total Ash and Acid-insoluble Ash of Prunellae Spica

Constituent	Mathematical pre-treatment	Wave length selected/ cm^{-1}	Calibration set		Validation set	
			^a R ²	^b SECV V	^c r ²	^d SEP
Total ash (%)	First derivative + straight line subtraction (17 smoothing point)	7502–6098.1 ; 5025.8–4597.6	0.932	0.225	0.914	0.373
Acid-insoluble ash (%)	Vector normalization	7502–6800 ; 5450.1–4597.6	0.929	0.210	0.905	0.452

^a Coefficient of determination in calibration;

^b Standard error of cross-validation;

^c Coefficient of determination in validation;

^d Standard error of prediction.

ly do not show absorbance in the NIRS region, so they usually cannot be predicted by NIRS directly. However, previous studies have shown the feasibility of the prediction of inorganic components using NIRS. NIRS technology has been widely used to determine metal contents in freshwater sediment,¹³⁾ soils,^{14–16)} red paprika¹⁷⁾ and cheese.¹⁸⁾ The first study on mineral elements in plants using NIRS was published in 1981, and it determined Ca, P and K in forage and grain.¹⁹⁾ Hallett *et al.* precisely predicted the Al, Ca, K, Mg, and Mn concentrations in white pine and red oak foliage.²⁰⁾ Huang *et al.* reported that NIRS could be utilized for the prediction of K, Ca, Mg in dried milled straw and Mg in cut straw.²¹⁾ Good results were also obtained in prediction of minerals in legumes, alfalfa and woody plant.^{22–24)} It is reported that NIRS can measure these mineral constituents owing to the correlation between the minerals and the or-

ganic components, either through associations with organic molecules, chelates or forming salts which affects hydrogen bonding in samples.^{9,23,25)} The majority constituents of ash are mineral elements. No publications reported the composition and concentrations of the minerals in Prunellae Spica. However, it was reported that Ca constitutes 40–60% of the ash forming atom species in wood and bark.²⁶⁾ And the ashes of some agricultural fuels (such as sunflower husk, coconut shell, and wood) consist of SiO₂, Fe₂O₃, TiO₂, P₂O₅, Al₂O₃, CaO, MgO, SO₃, Na₂O, and K₂O.²⁷⁾ The feasibility of the prediction of mineral elements in plants using NIRS opens up the possibility for predictions of ash content. As mentioned before, NIRS has successfully been used to predict the ash content of Mediterranean shrub,²⁾ green crop cereals,³⁾ flour,⁴⁾ roasted coffee,⁵⁾ alfalfa hay,⁶⁾ natural alpine swards,⁷⁾ diets of grazing young ostriches,⁸⁾

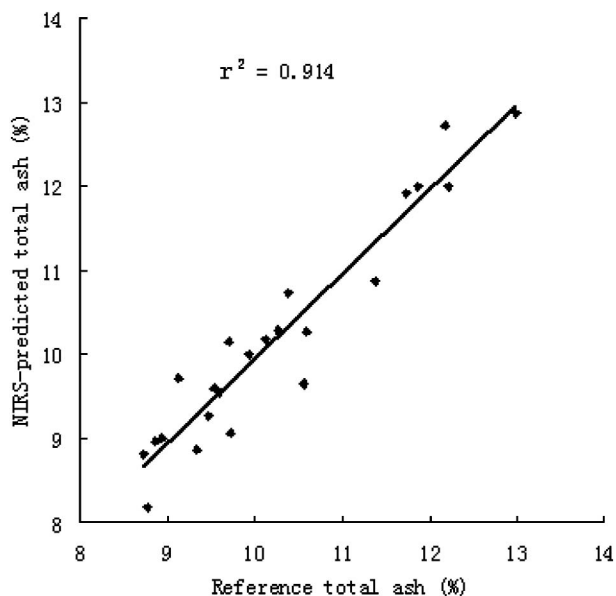


Fig. 2. Linear Regression Relationships between the NIRS-predicted and the Reference Values of the Total Ash in the Validation Set of Prunellae Spica Samples

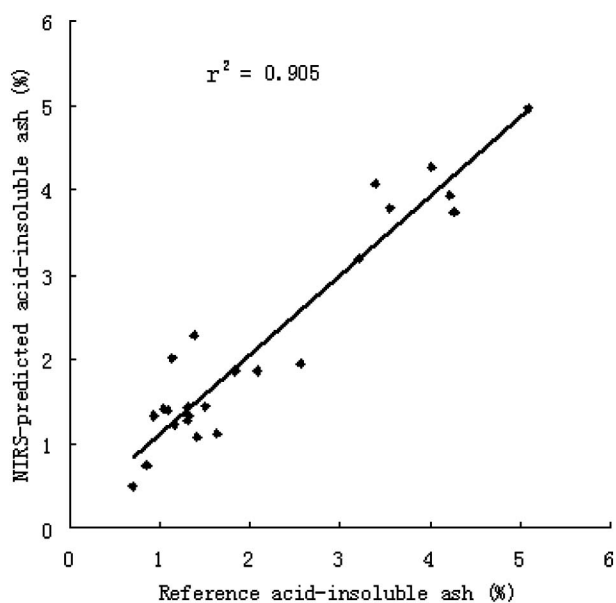


Fig. 3. Linear Regression Relationships between the NIRS-predicted and the Reference Values of the Acid-insoluble Ash Contents in the Validation Set of Prunellae Spica Samples

and olive tree leaves.⁹⁾ And in this study, the NIRS method proved to be rapid, simple alternative for the analysis of total ash and acid-insoluble ash of Chinese herbal medicine Prunellae Spica.

As mentioned before, the total ash of the plant materials includes “physiological ash”, which is derived from the plant tissue itself, and “non-physiologi-

cal ash”, which is often from environmental contaminations such as sand and soil. Upon treatment with dilute acid and further ignition, the residues of the total ash left behind is the acid-insoluble ash, including the silica materials such as sand and siliceous earth. Since total ash consists of “physiological ash”, which is derived from variable amounts of soil minerals incorporated into the plant, it can be concluded that total ash is more correlated with the organic matrix than the acid-insoluble ash. It was speculated that, the more correlated with organic matrix, the more spectral active the inorganic minerals are. Total ash was more spectrally active, and it got better NIRS prediction result than the acid-insoluble ash.

The main advantage of NIRS is its ability to quickly analyze several characteristics for a large number of samples that conventionally require elaborate preparative work and a lot of time. In this study, when using the ignition method to get the reference values of total ash and acid-insoluble ash, we found that this method was rather laborious and time-consuming. At least 5 times of weighing, 4 times of ignition, and 4 times of cooling needed for the determination of total ash, and at least another 2 times of weighing, 2 times of ignition, 2 times of cooling needed for the determination of acid-insoluble ash. So it required a minimum of 21 h to finish the measurement of total ash and acid-insoluble ash of one Prunellae Spica sample. People need to be very patient and careful during these boring procedures. And because of the limited space of muffle-oven, at most only 8 samples could be analyzed at one time. Unlike the ignition method, NIRS is non-destructive, and it involves no chemicals and no pretreatment of the sample powder. With the NIRS technique, the analysis of one sample could be performed in one minute, and thus it is possible to finish the determination of hundreds of samples in one day and the analysis efficiency can be improved greatly.

CONCLUSIONS

The results of this study confirmed that NIRS could be used to estimate the total ash and acid-insoluble ash contents of Prunellae Spica. This method was much more simple and rapid than the reference ignition method, and had the ability to predict the two constituents simultaneously. Further studies with other kinds of herbal medicines, such as herb, flower, folium, rhizome, bark, and fruit, are next steps in

evaluating the potential of NIRS for the rapid ash analysis for a wider range of Chinese herbal medicine.

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