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FAST ADULTERANT QUANTIFICATION IN CHINESE YAM POWDER USING VISIBLE, NEAR AND MID-INFRARED SPECTROSCOPY

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ABSTRACT Authentication of food products is of primary importance for both consumers and industries in both economic and public health terms. Because of their similar appearance and lower prices to Chinese yam powder (CYP), sweet potato powder (SPP) and cassava powder (CP) are two main adulterants for CYP in China CYP market. This paper compared the feasibility of using visible and near infrared (Vis-NIR) spectra and mid-infrared (MIR) spectra for the adulterant quantification of CYP. Adulterant samples were prepared by adding 5%, 10% 15% and 20% weights of SSP and CP, respectively. Least-squares support vector machine (LS-SVM) was used for calibration. MIRS obtained the better results for both CP (The coefficients of determination for prediction (r_p^2)=0.989) and SPP (r_p^2 =0.986) adulterant quantification. But the results of Vis-NIR spectra were still acceptable (r_p^2 =0.963 for CP and r_p^2 =0.934 for SPP). In order to eliminate useless variables and reduce calculation time, successive projections algorithm (SPA) was used to select effective variables. Less than 1% of the full range spectral variables at either Vis-NIR or MIR range were respectively selected for CP and SSP adulterant quantification. The Effective variables based LS-SVM models obtained similar results of full range spectra for both CP and SPP analysis, which shows that SPA is a useful tool to select the Effective variables. The overall results demonstrate that it is feasible to rapidly quantify SPP and CP contents in CYP using both Vis-NIR and MIR spectra.

Keywords: food quality, visible and near infrared (Vis-NIR) spectroscopy, mid-infrared (MIR) spectroscopy, Chinese yam powder, adulterants

INTRODUCTION Chinese yam (*Dioscorea opposita*) is a root that is used in traditional Chinese medicine. The Chinese pharmaceutical name for this herbal is *Rhizoma dioscoreae*. Chinese yam is native to China, Japan, and Korea, where it can be found growing wild on hill slopes and in valleys. It is also propagated for medicinal and dietary uses. Chinese yam contains protein, fiber, polysaccharides, vitamins A, B1, B2, E, C,

nicotine acid, trace elements and minerals. Traditional Chinese medicine classifies Chinese yam as neutral and sweet. It serves to tonify and augment the spleen and stomach; augment the lung yin and tonify the lung qi; and stabilize, tonify, and bind the kidneys.

Chinese yam powder (CYP) enjoys an excellent reputation as a good source of nutrition and is of great economic importance. Nowadays, authentication of food products is of primary importance for both consumers and industries in both economic and public health terms. Food authentication is also of concern to food processors. They do not wish to be subjected to unfair competition from unscrupulous processors who would gain an economic advantage (Cordella et al., 2002, Reid et al., 2006). Many foods have the potential to be deliberately adulterated. Particularly, when one food is expensive and produced under wide fluctuations in culturing and harvesting conditions (Gallardo-Velazquez et al., 2009). CYP is just one of such susceptible foods.

There are two typical adulterants namely sweet potato powder (SPP) and cassava powder (CP) which are usually added into CYP, because of their low prices compared to CYP. The sweet potato (*Ipomoea batatas*) is a dicotyledonous plant that belongs to the family Convolvulaceae. Its large, starchy, sweet tasting tuberous roots are an important root vegetable. Cassava (*Manihot esculenta*), is a woody shrub of the Euphorbiaceae (spurge family) native to South America that is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous root, a major source of carbohydrates.

Because of the similar colors between these three kinds of powders, it is hard to tell if or how much the SPP or CP are added into SYP through naked eyes. One widely used technique for fast monitoring food quality is visible and near infrared (Vis-NIR) spectroscopy (Cen and He, 2007), where visible spectra mainly contain the pigment information and near infrared spectra are mainly correspond to C-H, O-H, and N-H vibrations. It has the advantages of being rapid, low cost and non-invasive technique. There are some studies on agricultural or food powders using Vis-NIR spectroscopy. We did research on the quantifications of the fat and protein contents in powdered milk using short-wave NIR, NIR and mid-infrared spectroscopy (Wu et al., 2007, Wu et al., 2008a, Wu et al., 2008b, Wu et al., 2008c). Borin and co-workers quantified the common adulterants in powdered milk by NIR spectroscopy (Borin et al., 2006). Shi et al. utilized NIR spectroscopy to characterize powder blending. A ternary powder mixture was used, including lactose, Avicel and acetaminophen fine, and coarse powder (Shi et al., 2008). However, NIRS also has several disadvantages such as wide absorption bands with overlaps, weak absorption and low sensitivity. The results have much noise and other unrelated information arises from overtones and combinations of such vibrations, rendering them more difficult to interpret (Reid, et al., 2006).

Mid-infrared (MIR) spectroscopy technique can detect the compositional differences between samples on the basis of vibrations of various chemical groups at specific wavelengths in the mid-infrared region between 400 and 4000 cm^{-1} (Reid et al., 2006). MIRS provides more information of frequencies and intensities which are richer and stronger than NIRS does as bands in the MIR spectra are fundamental bands of NIR spectral bands (Chung, et al., 1999). So MIRS can monitor low levels of key analytes and functional groups (Roychoudhury et al., 2006). However, the MIRS measurement needs

to add KBr into sample which means the process is destructive. On the contract, NIRS with fiber optic diffuse reflectance probe can be executed with little sample preparation and can be remotely controlled which make the whole operation more convenient (Wang et al., 2006). Thus two techniques have their advantages and disadvantages and need to be considered in different applications. Up to our knowledge, there is not much study on SYP using Vis-NIR spectroscopy, let alone the adulterant quantification of SYP.

Nowadays, the spectroscopy instruments usually have a high resolution, therefore the obtained spectral data often contain hundreds of variables. With these numerous variables and mass samples, spectral data are too complicated to be calculated directly, and the calibration is time-consuming. Therefore is not competed for the high speed of industrial application. Thus it is important to select effective variables which contain useful information. More stable model with the lower prediction error can be generated and is superior interpretability.

Successive projections algorithm (SPA) is a new variable selection way.. It selects variables with minimally redundant which can solve the collinearity problems. In SPA process, a simple projection operation is executed in a vector space to select subsets of variables with minimum of collinearity (Breitkreitz, et al., 2003) More reproducible results can be provided by SPA compared to genetic algorithm (Ye, et al.,2008)

The aim of this work is to investigate the feasibility of using Vis-NIR-MIR spectroscopy as an alternative methodology for the fast quantification of SPP and CP in CYP.

MATERIALS AND METHODS

Sample preparation Pure CYP was provided by the country Kin Wai drug Ltd., Jiaozuo City. Adulterant samples were prepared by adding 5%, 10% 15% and 20% weight of SSP and CP, respectively. CYP and adulterants were weighted using an analytical balance with a precision of 0.01 g and the resulting sample was mixed in a mortar.

Spectroscopic instrumentation and measurement Vis-NIR reflectance spectra in the 325-1075 nm region were measured by a FieldSpec Pro FR spectrometer (Analytical Spectral Devices, Boulder, USA). The whole experiment was made at an ambient temperature of 18-20 °C. Each sample was with full of the adulterated powder in a uniform container (30 mm in diameter and 10 mm in height). The probe was placed at a distance of approximately 15 mm and 45° angle away from the measurement area. A light source of Lowell pro-lam 14.5V Bulb/128690 tungsten (Ushio Lighting Inc., Japan) was 150 mm and 45 of horizon plane away from the measurement area. The spectr um of each sample was the average of 30 successive scans. Twenty samples were obtained for each adulterant concentration type. To avoid low signal-to-noise ratio, only the region of wavelengths (376-975 nm) were employed for the calculations. Absorbance spectra were stored as $\log(1/R)$ (R = reflectance) at 1 nm intervals . Therefore 600 spectral variables were obtained in the whole Vis-NIR spectral region.

In this study, the JASCO Model FT/IR-4000 Fourier Transform Infrared Spectrometer (Japan) was used, with a valid range of 4000–350 cm^{-1} and auto scan speed of 2.0 mm/s, to obtain transmittance (% T) spectrum. The software Spectra Manager CFR is used for spectral measurement and analysis. The temperature was kept at about 25°C during the

whole experiment. In order to enhance the transmission rate, each sample was mixed with KBr at the ratio of 1:49. The mixture was compressed into a uniform tablet with a diameter of 5 mm and a thickness of 2 mm, and then scanned by the spectrometer. Each sample was scanned 16 times and the data averaged. Twenty samples were obtained for each adulterant concentration type. To avoid low signal-to-noise ratio, only the region of wavelengths ($700\text{-}4000\text{ cm}^{-1}$) were employed for the calculations. Finally 3424 spectral variables were obtained in the whole MIR spectral region.

There were 100 samples for CP and SPP in both Vis-NIR and MIR spectral analysis respectively. In each analysis, the 100 samples were divided into a calibration set and a prediction set. In order to obtain a 3/1 division of calibration/prediction spectra, the three spectra of every four samples are selected into the calibration set. Finally the calibration set contains 75 spectra and other 24 spectra constitute the prediction set. The descriptive statistics for SSC and pH of samples determined by standard laboratory methods are presented in Table 1. The range of y-value in calibration set can cover the range in the prediction.

Spectra pre-processing technique The implement of spectra pre-processing might can improve the performance of spectral analysis. Several typical spectral pre-processing techniques, including Savitzky-Golay smoothing (Savitzky and Golay, 1964), multiplicative scatter correction (MSC) (Helland et al., 1995), 1st and 2nd derivatives, and standard normal variate (SNV) (Barnes et al., 1989), and their combinations were implemented. Savitzky-Golay smoothing is an averaging algorithm that fits a polynomial to the data points. MSC is a transformation method used to compensate for additive and/or multiplicative effects in spectral data. Derivative attempts to correct for baseline effects in spectra. SNV is a row-oriented transformation which centers and scales individual spectra.

Multivariate modeling LS-SVM is an optimized version based on the standard support vector machine. The RBF kernel was adopted here. A grid-search technique was applied to find out the optimal parameter values, which are the regularization parameter γ and the RBF kernel function parameter σ^2 . The optimization value ranges of γ and σ^2 were set as $2^{-1}\text{-}2^{10}$, and $2\text{-}2^{15}$ here. For each combination of γ and σ^2 parameters, the root-mean-square error of cross-validation (RMSECV) was calculated. The optimum parameters were selected when they produced the smallest RMSECV. The details of LS-SVM description can be found in the literature (Wu et al., 2008d). The process of LS-SVM was executed in MATLAB 7.6 (The Math Works, Natick, USA).

Successive projections algorithm (SPA) In SPA process the instrumental response data are disposed in a matrix X of dimensions ($N \times K$) where the k th variable x_k is corresponding to the k th column vector. Let $M = \min(N-1, K)$ be the max number of selected variables (Galvao, et al., 2008). First step consists of projections on the X matrix, where k chains of M variables are generated. Each element in a chain is selected to display the least collinearity with the previous ones. The second step consists of evaluating candidate subsets of variables selected in the first step. The candidate subset of m variables starting from x_k is defined by the index set $\{SEL(1,k), SEL(2,k), \dots, SEL(m,k)\}$. A total of $M \times K$ subsets of variables are tested, and the best variable subset is selected. For this purpose root mean square error (RMSE) is adopted. The SPA was operated in MATLAB 7.6 (The Math Works, Natick, USA).

Model evaluation The performances of the calibration models were evaluated in terms of the root mean square error of calibration (RMSEC). Root mean square error of prediction (RMSEP) and residual predictive deviation (RPD) were used to evaluate the performances in the prediction process. The coefficients of determination (r^2) were used for both calibration (r_c^2) and prediction (r_p^2). Generally, a good model should have higher r_c^2 , r_p^2 and RPD values, and lower RMSEC and RMSEP values.

RESULTS AND DISCUSSION

Overview of the spectra The average spectra of different adulterant types are shown in Fig 1. Throughout the Vis-NIR spectral range, the differences between each adulterant types in both CP analysis and SPP analysis are very close. For SPP analysis, the CYP average spectrum is lower than other adulterant types. When more SPP were added, the absorbance increased, except SPP-20% whose spectrum is lower than SPP-15%. For CP analysis, when more CP were added, the absorbance decreased. However, the direct ratio of the added adulterant content and absorbance would not exist when more samples were considered. In the MIR spectral range, the differences between each adulterant types are obvious in CP analysis. The profile of the average spectrum of CYP is different to the adulterant types. The phenomenon is similar in SPP analysis, while the differences between each adulterant types are still small.

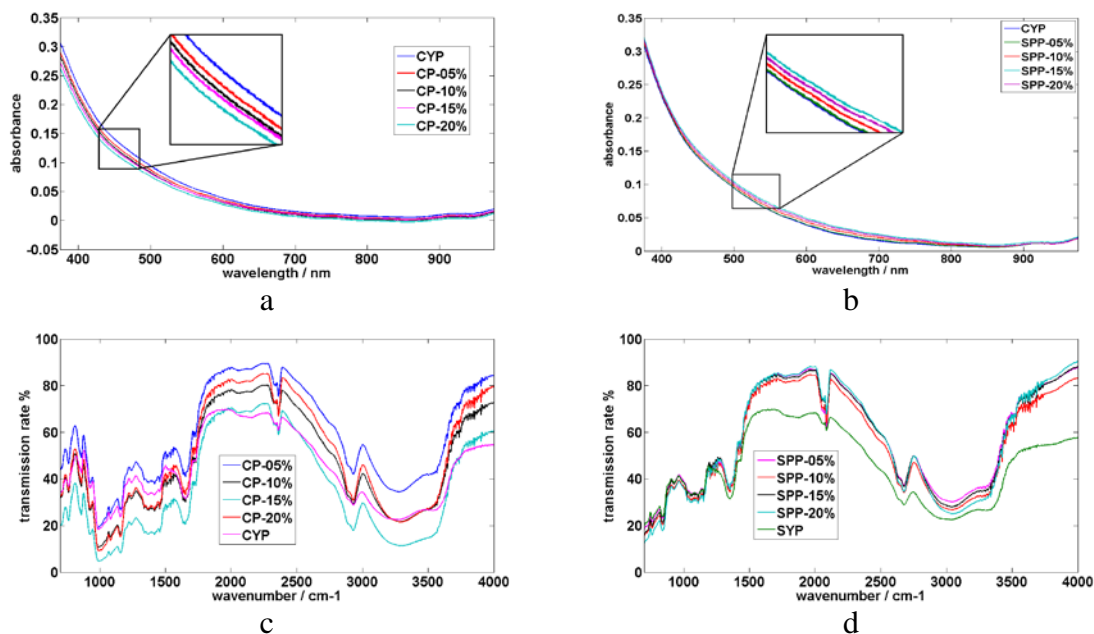


Figure 1. Average spectra of different adulterant types. CP analysis in Vis-NIR range (a), SPP analysis in Vis-NIR range (b), CP analysis in MIR range (c) and SPP analysis in MIR range (d).

Choosing the best pre-processing technique Full spectra LS-SVM models were established based on different pre-processing techniques. The calculated results of calibration and prediction processes are shown in Table 1 and 2. According to the model evaluation standard, the original spectra obtained the best performances at Vis-NIR spectral range for both CP and SPP analysis, which shows that there was no noisy need to be smoothed and outlier needs to be reduced in Vis-NIR spectra. Therefore the pre-

processing is not necessary for the Vis-NIR spectra. High RPD values of 5.161 for CP analysis and 3.730 for SPP analysis show that a good prediction based on Vis-NIR spectra for both CP and SPP adulterant content determinations.

When MIR spectra were used for the analysis, MSC and SNV pre-processing were the best for CP and SPP analysis, respectively. But the differences between pre-processed spectra and original spectra were not large for CP analysis. Only a little improvement were obtained. On the contract, SNV pre-processing much improved the performance for SPP analysis. Higher RPD values of 9.748 for CP analysis and 9.234 for SPP analysis show that MIR spectra can do better determination for both CP and SPP adulterant contents compared to Vis-NIR spectra. The detail determinations are shown by the sample scatter plots in Fig.2, shows that the adulterant contents for both CP and SPP can be well determined based on MIR spectra. Although Vis-NIR spectrum obtained worse performances compared to MIR spectrum, its results were still acceptable. Moreover, considering the prices of MIR spectra measurement instruments usually are much higher than Vis-NIR spectra measurement instruments, Vis-NIR spectra can also be used for the adulterant quantification.

Table 1 Results of LS-SVM models at Vis-NIR spectral range based on different pre-processing techniques in calibration and prediction processes.

pretreatment	Adulterant prediction of CP					Adulterant prediction of SPP				
	r_c^2	RMSEC	r_p^2	RMSEP	RPD	r_c^2	RMSEC	r_p^2	RMSEP	RPD
none	0.974	0.012	0.963	0.014	5.161	0.943	0.017	0.934	0.019	3.730
smooth	1.000	0.000	0.957	0.015	4.756	0.921	0.020	0.920	0.021	3.448
SNV	0.963	0.014	0.933	0.018	3.861	1.000	0.000	0.910	0.022	3.278
smooth+SNV	0.948	0.017	0.924	0.020	3.594	0.965	0.013	0.912	0.021	3.363
MSC	0.963	0.014	0.933	0.018	3.860	1.000	0.000	0.910	0.022	3.258
smooth+MSC	0.955	0.015	0.929	0.019	3.731	0.978	0.011	0.917	0.020	3.458
1 st Der	1.000	0.000	0.899	0.026	2.798	1.000	0.000	0.801	0.034	2.076
smooth+1 st Der	0.996	0.005	0.922	0.022	3.311	1.000	0.000	0.864	0.028	2.546
2 nd Der	1.000	0.000	0.024	0.070	1.012	1.000	0.000	0.029	0.070	1.012
smooth+2 nd der	0.981	0.019	0.090	0.067	1.048	1.000	0.000	0.084	0.069	1.022

Table 2 Results of LS-SVM models at MIR spectral range based on different pre-processing techniques in calibration and prediction processes.

pretreatment	Adulterant prediction of CP					Adulterant prediction of SPP				
	r_c^2	RMSEC	r_p^2	RMSEP	RPD	r_c^2	RMSEC	r_p^2	RMSEP	RPD
none	0.998	0.0031	0.990	0.0073	9.714	0.997	0.0038	0.982	0.0098	7.532
smooth	0.998	0.0030	0.990	0.0073	9.663	0.997	0.0037	0.983	0.0096	7.607
SNV	1.000	0.0014	0.989	0.0074	9.604	0.999	0.0027	0.989	0.0077	9.234
smooth+SNV	1.000	0.0014	0.989	0.0075	9.546	0.999	0.0027	0.989	0.0078	9.122
MSC	1.000	0.0014	0.989	0.0073	9.748	0.998	0.0029	0.986	0.0084	8.421
smooth+MSC	1.000	0.0014	0.989	0.0073	9.746	0.997	0.0039	0.986	0.0085	8.364
1 st Der	1.000	0.0002	0.939	0.0193	3.664	1.000	0.0001	0.923	0.0227	3.237
smooth+1 st Der	1.000	0.0002	0.941	0.0191	3.712	1.000	0.0001	0.924	0.0225	3.263
2 nd Der	1.000	0.0000	0.811	0.0315	2.254	1.000	0.0000	0.635	0.0450	1.609
smooth+2 nd der	1.000	0.0000	0.816	0.0311	2.279	1.000	0.0000	0.636	0.0449	1.611

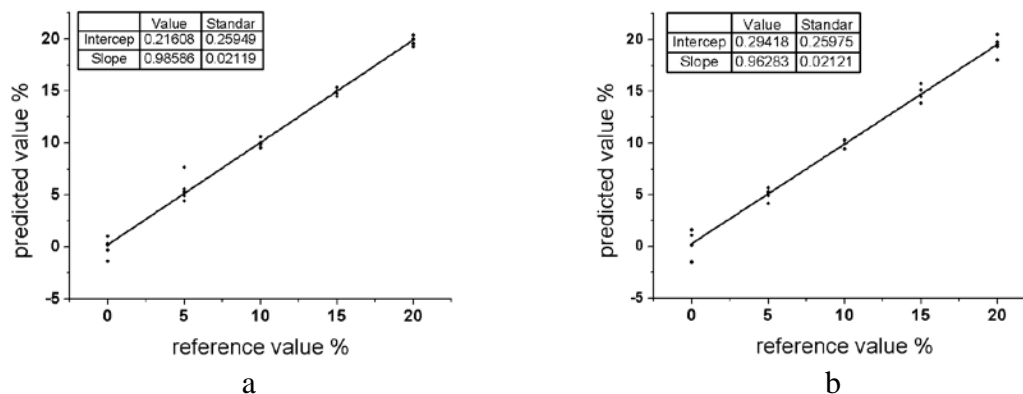


Figure 2. Sample scatter plot for CP (a) and SPP (b) adulterant content prediction in prediction set based on MIR spectra

SPA calculation based on the full-spectrum After choosing the optimal pre-processing techniques, SPA was carried out for selecting effective variables for CP and SPP analysis in Vis-NIR and MIR ranges, respectively. Finally, 2 variables (RMSE=0.015) were selected in Vis-NIR range for CP analysis, 4 variables (RMSE=0.023) were selected in Vis-NIR range for SPP analysis, 17 variables (RMSE=0.006) were selected in MIR range for CP analysis, and 12 variables (RMSE=0.009) were selected in MIR range for SPP analysis. After the variable selection, the variable numbers were much reduced (2 and 4 vs. 600 in Vis-NIR range, 17 and 12 vs. 3424 in MIR range for CP and SPP, respectively). Fig. 3 is the locations of the selected variables by SPA. The selected variables by SPA were set as input variables of LS-SVM, and the results are shown in Table 3. In Vis-NIR spectral analysis, the models' performances were similar compared to the full range spectra. In MIR spectral analysis, SPA can improve the result of SPP analysis, while its performance was not good in CP analysis. However, considering only less than 1% of full range variables were used for the model establishment, the results of SPA selection were still satisfactory.

CONCLUSION This paper proposed the application of Vis-NIR and MIR spectroscopy for the fast adulterant quantification of CP and SPP in CYP. Based on LS-SVM for the model calibration, original spectra did the best performances on Vis-NIR spectra for both CP and SPP analysis, while MSC and SNV pre-processing techniques did the best on MIR spectra for CP and SPP analysis, respectively. MIRS obtained the better results for both CP and SPP adulterant quantification. But the results of Vis-NIR spectra were still acceptable. SPA selected less than 1% of the full range spectral variables at either Vis-NIR or MIR range for CP and SPP adulterant quantification, respectively. The results of SPA selection were satisfactory for both CP and SPP analysis.

Table 3 Results of LS-SVM models at MIR spectral range based on different pre-processing techniques in calibration and prediction processes.

spectra	Adulterant prediction of CP					Adulterant prediction of SPP				
	R_c^2	RMSEC	R_p^2	RMSEP	RPD	R_c^2	RMSEC	R_p^2	RMSEP	RPD
Vis-NIR	0.973	0.0116	0.968	0.0131	5.552	0.914	0.0208	0.911	0.0227	3.109
MIR	0.997	0.0036	0.981	0.0098	7.231	0.996	0.0042	0.991	0.0073	10.252

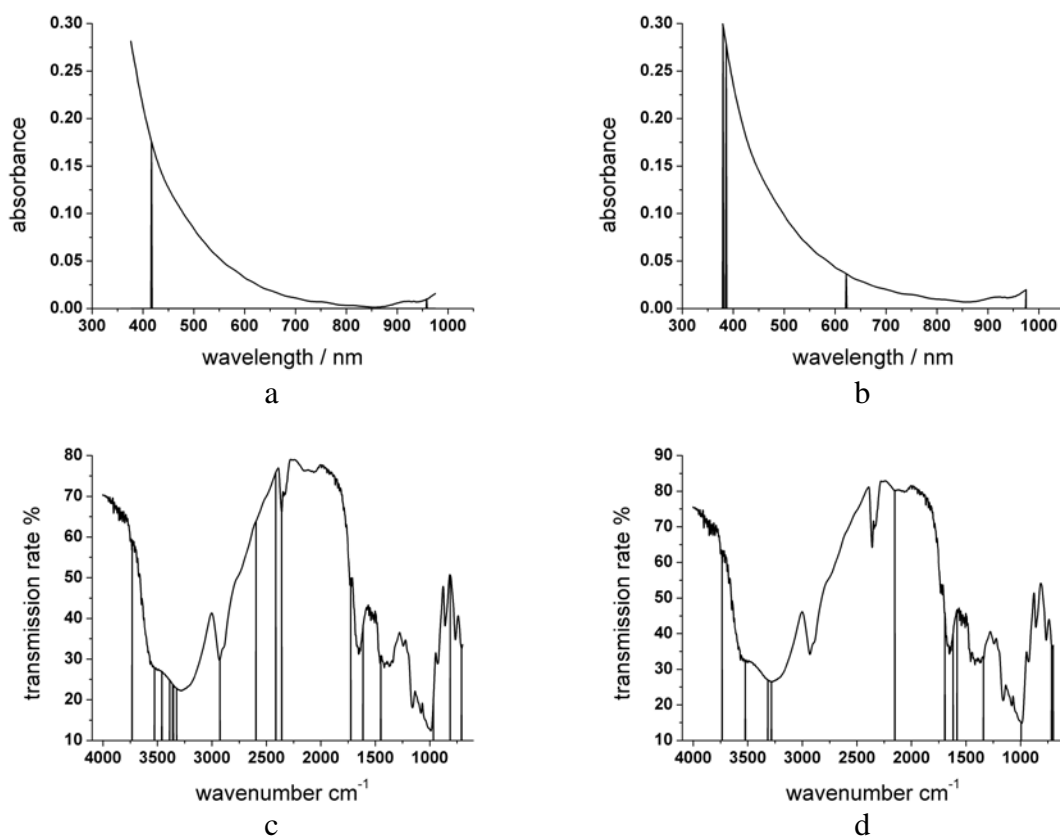


Figure 3. Selected variables by SPA. Specifically, 2 variables were selected in Vis-NIR range for CP (a), 4 variables were selected in Vis-NIR range for SPP (b), 17 variables were selected in MIR range for CP (c), and 12 variables were selected in MIR range for SPP (d). Columns represent selected variables. The curves show the original spectra.

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