Rapid and undamaged analysis of crude and processed *Radix Scrophulariae* by Fourier transform infrared spectroscopy coupled with soft independent modeling of class analogy

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ABSTRACT

Objective: The main objective of this work is to determine the feasibility of identification of crude and processed *Radix Scrophulariae* using the Fourier transform infrared spectroscopy couple with soft independent modeling of class analogy (FT-IR-SIMCA). **Materials and Methods:** A total of 50 different crude *Radix Scrophulariae* was used to product processed ones. The spectra were acquired by FT-IR spectroscopy using a diffuse reflectance fiber optic probe. For the multivariate analysis, SIMCA was used. Results showed that FT-IR-SIMCA was useful to discriminate the processed *Radix Scrophulariae* samples from crude samples. These samples could be successfully classified by SIMCA. **Results:** In all cases, the recognition and rejection rates were 97.8% and 100%, respectively. When testing with the blind sample that was picked out from the chosen samples, the accuracy was up to 90%. **Conclusion:** It means that the methodology is capable of accurately separating processed *Radix Scrophulariae* from crude samples.

Key words: Fourier transform infrared, quality control, *Radix Scrophulariae*, soft independent modeling of class analogy, traditional Chinese medicine

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INTRODUCTION

Traditional Chinese medicine (TCM) has a robust history with roots dating back thousands of years for medicinal practice in China and some East Asian countries. The processing of Chinese materia medica with excipients has a long history and the efficacy of treatment can be enhanced by using a combination of excipient treatments.[1,2] The purposes of processing Chinese medicinal herbs are briefly summarized to strengthen the effect, eliminate or reduce the toxicity, facilitate the preparation and storage of drugs.[3] During processing, secondary plant metabolites are transformed, thus helping to increase potency and reduce toxicity, and altering their effects. It is also used for preserving active constituents, facilitating administration, improving flavor and increasing purity of Chinese materia medica.[4] In China, the processing methods for crude TCM have been practiced since the Tang Dynasty and well-documented in the Chinese Pharmacopoeia.[5] *Radix Scrophulariae* (Xuanshen in Chinese) is the dried root of *Scrophularia ningpoensis Hemsl*. This root is an essential drug in TCM and has been used for thousands of years.[6] The herb is widely distributed in Zhejiang Province and has a wide range of pharmacological effects. It is commonly used to treat various diseases such as including anti-chronic inflammatory, antihypertensive, abirritative, antispasmodic, anti-hepatitis B virus and immunological enhancement.[7,8] The crude *Radix Scrophulariae* and its processed products of zheng zhi pin (ZZP) are used clinically. The crude *Radix Scrophulariae* was used to treat pathogenic toxic of heat, swelling and pain of eye, superficial and swelling infection syndromes. Although the efficacies of ZZP were cooling blood and replenishing yin and ZZP was used to treat consumption of yin caused by febrile disease, crimson tongue thirst, hectic fever and cough and constipation by different prescription.[9,10] Therefore, consuming the wrong form of *Radix Scrophulariae* may lead to undesirable clinical outcomes. Thus, whether the constituents of a

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ZZP changed or not is a very important issue for not only the efficacy, but also the safety of the herb application. Development of a rapid and specific approach to determine the potential chemical changes is the key to the quality control of these crude and processed herbs.

Fourier transform infrared (FT-IR) spectroscopy is a simple, rapid technique with marked characteristics and high reproducibility, it has been widely used in authentication studies involved plants, food additives, pollen, as well as herbal medicines and drug preparations.[11,12] An IR spectrum contains features arising from vibrations of molecular bonds and specially the mid-IR region (4000-400/cm) is highly sensitive to the precise composition of the crude and processed *Radix Scrophulariae* being analyzed. Furthermore, via FT-IR spectroscopy, herbal medicines can be controlled and identified directly and the samples do not to be separated.[13]

The identification of the FT-IR spectroscopy from crude and processed *Radix Scrophulariae* requires a suitable chemometric classification method which leads to the correct identification of unknown samples. SIMCA is a supervised pattern recognition classification technique and one of the most commonly used class-modeling tools in chemometrics.[14-16] In SIMCA, there is a training set which is modeled by principal component analysis. Subsequently, SIMCA requires a previous knowledge about the category membership of samples.

In the present work, FT-IR spectroscopy couple with SIMCA method was used to rapidly identify the crude and processed *Radix Scrophulariae* and with this result, the SIMCA method classified 100% of the crude and processed samples. The established approach was applied to discriminate rude and processed *Radix Scrophulariae*, which indicated that the proposed approach is rapid and specific and should also be useful for the quality control of medicinal herbs.

EXPERIMENTAL

Materials, methods and reagents

A total of 50 crude *Radix Scrophulariae* from different areas in China were investigated and collected. These herbal samples were authenticated by Professor Baochang Cai (Research Center of TCM Processing Technology, Zhejiang Chinese Medical University). The relevant specimens were deposited at the Research Center of TCM Processing Technology. The ZZP samples were processed according to the Chinese Pharmacopoeia edited in 2010 through pilot-scale experiment. In the processing procedure, the four relevant factors-processing temperature, processing time, wheat bran dosage and rotational speed of stir-frying machines were investigated by the L_9 (3^4) orthogonal design [Table 1].[17] According to the scheme in the orthogonal list, nine samples were obtained. The crude *Radix Scrophulariae* samples are luridity, but the processed one is brown. The coarse powders of the nine processed samples were pretreated in the same way as mentioned above. The ethanol employed here was analytical reagent.

FT-IR spectroscopy

Perkin-Elmer spectrum 100 FT-IR spectrometer (American Perkin-Elmer) equipped with a mid-IR deuterated triglycine sulfate detector, a resolving powder of 16/cm, spectrum range of 4000-400/cm, and scanning accumulative limitation of 32/times, was used for the analysis of all the samples in the experiments. Collection and analysis of IR images by using Spectrum V6.0 software.

Sample preparation and spectrum measurement

About 1.5 mg of the dried crude and processed *Radix Scrophulariae* powders (100 mesh) were taken and grinded with 300 mg KBr under IR light till evenly mixed, respectively. Then, the mixture was crushed in a mechanical mold to form a tablet with a diameter of 3 mm and a thickness of 0.6 mm. Finally, the spectra of *Radix Scrophulariae* could be gained by scanning the sample tablets immediately.

RESULTS AND DISCUSSION

Comparison of crude and processed *Radix Scrophulariae* by IR spectrum

Representative spectra of crude and processed *Radix Scrophulariae* are illustrated in Figure 1. The curves have been offset for clarity. Because the processed drugs were all prepared from crude samples and the crude and processed *Radix Scrophulariae* had closed relationships and similar components with each other, the obtained IR spectra entirely exhibited a great consistency in general. However, some absorption peaks have obvious differences; each of them still bore its own characters such as different peak shapes, numbers, positions and intensity. It is drawn from Figure 1a, processing procedure of the *Radix Scrophulariae* mostly take place in the transformation of sugar. Therefore, we select a section of sugar fingerprint feature area 850-750/cm to analyze. After crude *Radix Scrophulariae* processing, the IR spectra of crude *Radix Scrophulariae* were significantly changed. The absorption peak at 771/cm in the crude drug is the mixed spectral

Table 1: The interclass distances of crude and processed *Radix Scrophulariae* samples

	Crude samples	Processed samples
Crude samples	-	35.4

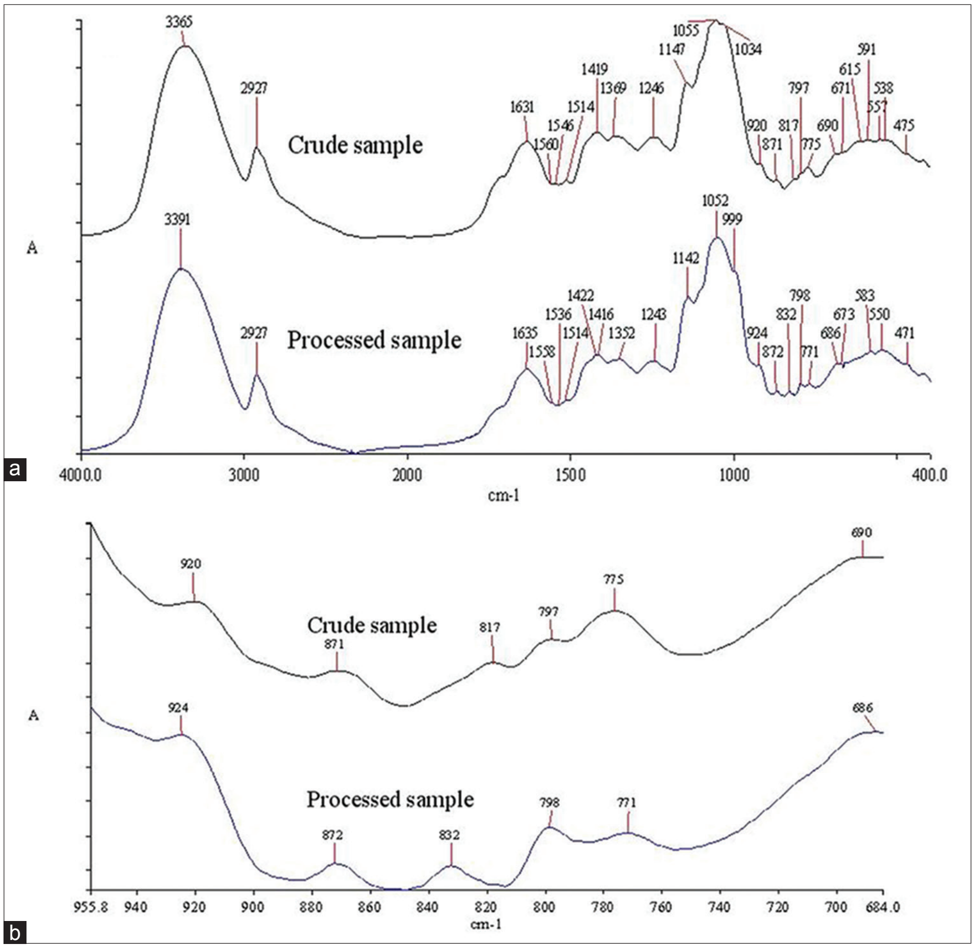

Figure 1: The representative (a) and partial enlargement (b) spectra of crude and processed Radix Scrophulariae

peak of a variety of carbohydrates contained in *Radix Scrophulariae* before hydrolysis, it appeared a blue shift phenomena at 775/cm after processing. Reports in the literature showed that fructose or 5-hydroxymethyl furfural produced by the hydrolysis of a variety of carbohydrates contained in *Radix Scrophulariae* could react with the amino acids into the melanoidins and therefore blackened *Radix Scrophulariae* after steaming. Figure 2 showed the principal sample analysis score plots generated by the optimized SIMCA models for the different crude and processed *Radix Scrophulariae*, and helps to visualize the class separation among them. The boundary ellipse around each cluster represents the 100% confidence interval and each data point inside the cluster represents one sample spectrum. Furthermore, good separation among crude and processed *Radix Scrophulariae* samples was achieved. It was observed that crude and processed samples classify correctly into their respective classes.

Building and training the models of crude and processed *Radix Scrophulariae*

In present study, the training sets were composed of 30 batches crude and processed *Radix Scrophulariae* samples, respectively and they are selected randomly. The models of crude and processed *Radix Scrophulariae* were builded by using Quant + software (American Perkin-Elmer), and combining with training set for training. The cluster analysis in SIMCA, the diagnostics report provides the interclass distances, i.e. the arbitrary distances between each of the classes (crude and processed *Radix Scrophulariae*). The procedure also checks every standard spectrum to ensure that those from a single class fit that class (recognition) and selects those from other classes and rejects them (rejection). The two rate columns should ideally report 100% for each instance. For this data set the two rate columns reported 100% indicating good separation of each class of compound. The class spaces of crude drug and processed product models are shown in Table 1. Recognition rate and rejection rate between two classed of *Radix Scrophulariae* are shown in Table 2 and Figure 3. This procedure classified the spectra and reports the number of misclassifications. The results indicated that there is no overlap between the crude and processed *Radix Scrophulariae* and it was better to separate the two classed of *Radix Scrophulariae*.

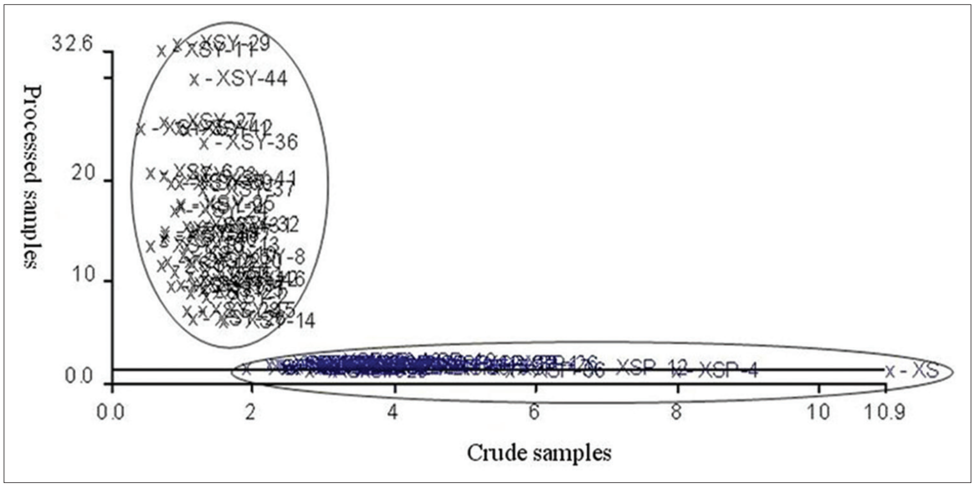

Figure 2: The principal sample analysis score plots generated by the optimized soft independent modeling of class analogy models for the different crude and processed *Radix Scrophulariae* samples

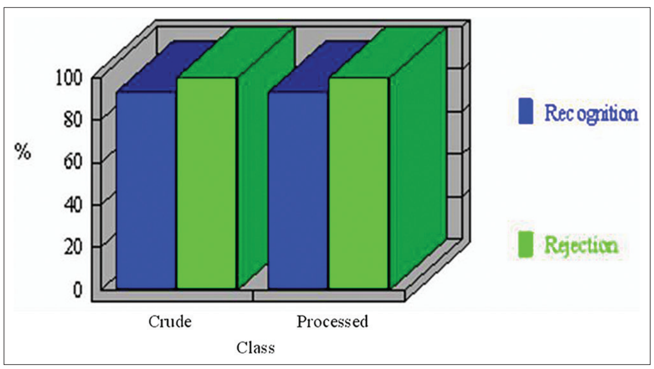

Figure 3: The histogram of recognition rate and rejection rate of crude drug and processed *Radix Scrophulariae* samples

Table 2: The recognition rate and rejection rate of crude and processed samples

Samples	Recognition rate, %	Rejection rate, %
Crude	97.8 (45/46)	100 (45/45)
Processed	97.8 (44/45)	100 (46/46)

Table 3: The class identification of unknown assist *Radix Scrophulariae* samples

Class name	Spectral residuals	Model residuals	Combined residuals	Probability
Crude	3.5	1.42	3.777	0
Processed	0.7834	0	0.7834	0.9464

The predictive classification of unknown samples by model

After validation procedures of the SIMCA models with the data collected from the fiber optic probe, we used the established models to test unknown spectra against each class. In the present study, A and B as the unknown samples were test by the established method. Initially, spectra of crude and processed *Radix Scrophulariae* from the independent validation sets were tested and the results of classification of crude and processed *Radix Scrophulariae* are given in Table 3. The report produced values for the spectrum residuals, which measure the lack of fit of the spectrum to the class model. The smaller the number, the more likely the spectrum belongs to that class. In a similar manner, a number is generated for the model residual, which represents the residual within class space. The critical probability level was set to 0.01. Therefore, any number produced in the probability column, which is bigger than 0.01 is a positive classification and the "unknown" belongs to that class. The probability of the spectrum belonging to the processed *Radix Scrophulariae* class is 0.9464, much bigger than the 0.01 limit, with all other probabilities being zero. Therefore, it can be concluded that this model has positively classified the "unknown" spectrum as the processed *Radix Scrophulariae* class. Therefore, the unknown sample A was most likely to belong to the processed *Radix Scrophulariae* class. This result is presented graphically in Figure 4. However, the unknown sample B had not been presented in any area, but it could clearly found that the unknown sample B was closed to processed *Radix Scrophulariae* class. On the basis of above works, we used the established SIMCA models for predicting classification of five crude and processed *Radix Scrophulariae*, respectively. The accuracy was up to 100% and the SIMCA method provided a powerful tool for classifying products, which are spectroscopically similar and this study showed successful classification of various crude and processed herbal medicines.

CONCLUSION

In the present study, a set of the qualitative method based on FT-IR spectroscopy for the quality control of crude and processed *Radix Scrophulariae* was established and the

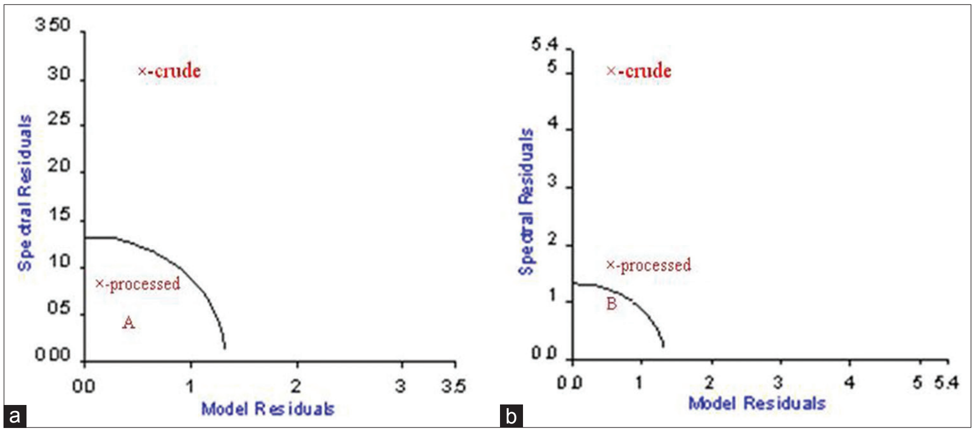

Figure 4: Classification results of unknown sample (a) and (b) by soft independent modeling of class analogy models

processed *Radix Scrophulariae* samples prepared from the crude samples were successfully discriminated using the SIMCA method. Compared with the traditional identification methods, the FT-IR spectroscopy couple with SIMCA method possessed strong characteristic sense, required low quantity of samples, and was also rapid, simple and accurate. The method was developed to rapidly and accurately identify the crude and processed *Radix Scrophulariae*. As components contained in herbal medicines were various and complicated, NIR fingerprint analysis method was proposed for the consistency checking of different batches of herbal medicines, which is beneficial to the industrial production in quality. Therefore, to ensure the efficacy, safety and batch-to-batch uniformity of herbal medicine products, each processing procedure should be standardized from crude drugs, manufacturing processes to final preparations. The established principles and methodology could also been applied to the rapid identification of other natural products.

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