Quantitative detection of pepper powder adulterated with rice powder using Fourier-transform near infrared spectroscopy

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Abstract. Near infrared (NIR) spectroscopy model was developed for detecting pepper powder adulterated with rice powder. The adulterated pepper powder samples were prepared by mixing rice powders with pure pepper powder to 19 levels of concentrations (w/w) from 5-95%w/w. Two hundred ten NIR spectra of pure and adulterant pepper powders were recorded using Fourier-transform near infrared spectrometer. The NIRs quantitative model for detecting adulterant pepper were established using partial least squares regression (PLS). The optimum model was established from NIR spectra treated by constant offset elimination with the $R^2_{val}$ of 0.99. These results show that the NIR spectroscopy could be a modern method for monitoring adulteration of pepper powder with rice powder.

1. Introduction

Pepper (Piper nigrum Linn) is most important herb and spice of the world. The pepper is well known as “King of Spice” [1]. Pepper is often used to be flavouring because the taste is hot and spicy. Pepper contains high chemical constituents such as starch, protein, vitamin (A, C and K) essential oils, polyphenols, and piperrine [2, 3, 4]. The health benefits and the satisfactory flavour are the reasons for high cost of pepper. Otherwise pepper is consumed with more formula such as berries and powder with black, white and green colour. Due to white pepper taste is softer, several consumers often prefer white pepper than black pepper. White pepper is product from milling of black pepper [2]. Recent years, adulteration of pepper powder with other cheaper materials widely obtained in the markets such as rice powder, job’s tear powder and so on. Broken rice is one of the common adulterants due to its very low value. In addition, visual characteristic of powder from broken rice is same pepper powder. The adulteration of pepper powder affects to satisfaction of consumer, agricultural economy, Therefore, detection of adulterated pepper by rapid and simple technique is important issue for pepper producers, exporter, engineer and researcher.
Several analytical methods such as sensory evaluation, microscopic recognition, and iodide test for indicating starch material have been used to evaluate adulteration of powder material [5, 6, 7]. Nevertheless, these techniques are long-time to test, expensive, destructive and skilled analysts. One of interesting technology which has been used for detecting adulteration and identification authentication of agricultural product is the near infrared (NIR) spectroscopy. NIR spectroscopy is technique base on recording the spectral bands mainly relate with vibration of hydrogen bond (C-H, O-H and N-H) in overtone and combination characteristic [8]. This technique is easy, non-destructive, fast and environmentally friendly. However, the NIR spectroscopy cannot directly be used for qualitative and quantitative analysis but has been always combined with chemometric technique. The most popular multivariate technique for quantitative analysis is partial least square regression (PLS). Satisfied results from using combination of NIR spectra and PLS for quantification of adulterated powder material obtained in several previous research such as Sichuan pepper [7], Shanyao [8], notoginseng [9], cocoa [10], Gleditsia [11], chili [12] and milk powder [13, 14] so on. All above information show possibility of NIR spectroscopy for detecting pepper powder adulterated with rice powder.

In the present research, the aim of this study is to develop NIR spectroscopy quantitative model for detecting pepper powder adulterated with rice powder. The results from this work could be useful to guarantee the pepper powder product in the local and exported market.

2. Methodology

2.1. Pure and adulterated pepper sample

White pepper ball was collected from Bannumjon community enterprise stay in Chanthaburi province, Thailand. Adulterants, i.e., broken rice (Sao Hai variety) were purchased from local market. The pepper ball and broken rice were milled using the hammer mill (FRITSCH, 14.3000/10857, Germany) combined with perforated screen (hole sizes of 0.2 mm). After milling process, moisture content of pepper and rice powder sample were determined by a drying oven (105 °C ± 1 °C) for 48 h and reported on wet basis (%wb). Moisture content of pepper and rice powder sample were 9.33 and 8.81%wb respectively. One hundred ninety adulterated pepper powder samples were prepared by mixing rice powders with pure pepper powder to 19 levels of concentrations (%w/w) from 5-95%w/w. Each level of pepper powder samples was blended using the laboratory cube mixer. Mixing process was performed with speed of 10 rpm and mixing times of 30 minutes. All samples were kept in plastic zipper bag until NIR spectra scanning. The Figure 1 is pure pepper powder, pure rice powder and adulterated pepper powder (25%w/w, 50%w/w and 75%w/w).

![Figure 1. Pure pepper powder (a), adulterated pepper powder 25%w/w (b), adulterated pepper powder 50%w/w (c), adulterated pepper powder 75%w/w (d) and pure rice powder.](image)

2.2. NIR spectra scanning

NIR spectra of pure and adulterant pepper samples were scanned using the Fourier-Transform Near Infrared (FT-NIR) spectrometer (Bruker Ltd., Germany) with diffuse reflectance mode. The sample was loaded on the vial (20 mm diameter and 43 mm height). Total of 64 sequent scans were done on wavenumber range of 12500-4000 cm⁻¹ (800-2500 nm) with resolution of 8 cm⁻¹. The NIR spectra
collection was done under controlled room temperature of 25 °C. Data analysis was performed using averaged spectra.

2.3. Overall precision test

Precision of the measurement was studied and shown in term of repeatability and reproducibility. Repeatability was tested under the same measurement condition for presenting the variation of an instrument. For this research, repeatability was performed with 10 replicates of NIR spectra scanning on pure pepper powder under the same condition. The descriptive statistical value, i.e. average and standard deviation (SD) of absorbance peak at 4760 cm\(^{-1}\) (absorbance peaks of starch [15]) from repeated measurement were agent to report repeatability. The wavenumber of 4760 cm\(^{-1}\) was absorbance peaks of starch which is main chemical composition of rice. Reproducibility which defines about an experiment can be reproduced in its completeness. In this work, the reproducibility was analysed to report homogeneity of adulterated pepper powder sample under mixing process. Therefore, reproducibility was tested with scanning NIR spectra on different 10 parts of each adulterated pepper powder sample at all mixing levels. Then average and SD of absorbance peak at 4760 cm\(^{-1}\) from 20 mixing levels were calculated to explain reproducibility.

2.4. Quantitative modelling

The NIRs quantitative model for detecting adulterant pepper were established using partial least squares regression (PLS). Further, 80% of samples (168 samples) were used in the calibration group whilst the remainders (42 samples) were defined to the validation group. The calibration group was applied to develop NIRs model with full cross-validation method. The calibration model was then used to detect level of adulteration rice powder in pepper powder. Spectra pre-treatment and model establishment were performed with multivariate analysis software (OPUS, v. 7.0.129, Germany). Raw spectra and pre-treatment spectra ware used to create the PLS models. Pre-treatment methods consisted of constant offset elimination, straight line subtraction, vector normalisation (SNV), min-max normalization, multiplicative scatter correction (MSC), first derivatives, second derivatives, first derivatives + straight line subtraction, first derivatives + SNV and first derivatives + MSC. The default optimization command of OPUS software was performed for selecting the optimum model based on combination of the number of PLS factors, the wavenumber ranges and the pre-treatment methods. The potential of the model was evaluated as the coefficient of determination of the calibration and validation sets (\(R^2_{cal}\), \(R^2_{val}\) respectively), root mean square error of estimation (RMSEE), root mean square error of prediction (RMSEP), bias, and ratio of standard deviation of validation data to RMSEP (RPD).

3. Results and discussions

3.1. NIR spectra

The Figure 2 show the raw and second derivative spectra of pure pepper powder, pure rice powder and 19 levels of adulterated pepper powder. The evident absorbance peaks obtained on wavenumber of 8230 cm\(^{-1}\), 6896 cm\(^{-1}\), 5665 cm\(^{-1}\), 5155 cm\(^{-1}\), 4760 cm\(^{-1}\) and 4300 cm\(^{-1}\) (1215nm, 1450 nm, 1765 nm, 1940 nm, 2100 nm, and 2325 nm). The three peaks around 8230 cm\(^{-1}\), 5665 cm\(^{-1}\) and 4300 cm\(^{-1}\) are the overtone and combination of CH\(_2\) stretching [15]. The peaks of starch appeared at about 6896 cm\(^{-1}\) and 4760 cm\(^{-1}\) which are vibration band of the O-H first overtone starching and the O-H and C-O combination mode [15]. The peaks located at 6896 cm\(^{-1}\) and 5155 cm\(^{-1}\) associated with first overtone and combination of O-H stretching of H\(_2\)O (water) [15]. From the raw and second derivative spectra of all samples, they did not significantly differ according to adulteration levels, which indicates that it is difficult to detect adulteration level of rice powder in pepper powder directly with NIR spectra. It is necessary to develop PLS model for detect adulteration level.
3.2. Overall precision test

The results of the overall precision test shown in the Table 1. FT-NIR spectrometer showed high precision with repeatability of 0.6516±0.0027. Reproducibility of mixing process was 0.6472±0.0029. Normally standard deviation value was calculated to explain amount of variation in data set. A low standard deviation indicates that the data points tend to be close to the expected value. In this experiment repeatability and reproducibility showed low standard deviation compared to the average. These results indicate that the experiment was precision under same and different condition.

Table 1. Results of precision tests

| Absorbance values at 4760 cm⁻¹ | Repeatability | Reproducibility |
|--------------------------------|---------------|-----------------|
| Average                       | 0.6516        | 0.6472          |
| SD                             | 0.0027        | 0.0029          |

3.3. Quantitative modelling

The Table 2 show the results of quantitative detection of pepper powder adulterated with rice powder using FT-NIR spectroscopy combined with PLS algorithm. The optimum model obtained from spectra was treated by constant offset elimination method on wavenumber range between 5446 cm⁻¹ and 6102 cm⁻¹. The PLS model demonstrated $R^2_{acc}$ of 0.99, RMSEP of 1.68 %w/w, Bias of 0.059 %w/w and RPD of 18.1. Williams [16] recommended that models with $R^2 > 0.980$ and RPD > 8.10 were
excellent and could be used in any application including quality assurance [17]. The Figure 3 shows scatter plot of prediction and reference from detection pepper powder adulterated with rice powder using NIR spectroscopy combined with PLS regression technique.

**Table 2.** the results of quantitative detection of pepper powder adulterated with rice powder using FT-NIR spectroscopy combined with PLS algorithm.

| Pre-treatment                      | Wavenumber range (cm\(^{-1}\)) | PC | \(R^{2}_{\text{cal}}\) | \(R^{2}_{\text{val}}\) | RMSEE (%w/w) | RMSEP (%w/w) | Bias (%w/w) | RPD |
|-----------------------------------|---------------------------------|----|------------------------|------------------------|---------------|---------------|-------------|-----|
| constant offset elimination       | 5446.3-6102                     | 3  | 0.99                   | 0.99                   | 1.67          | 1.68          | 0.059       | 18.1|

**Figure 3.** Scatter plot of prediction and reference from detection pepper powder adulterated with rice powder using NIR spectroscopy combined with PLS regression technique. (a) calibration set; (b) validation set.

The Figure 4 is regression coefficient plot of PLS model. High regression coefficient values indicated that effect of molecular vibration band at any wavenumber on PLS model [17]. During this test, the permanent peaks appeared at important chemical composition band of 5618 cm\(^{-1}\), 5716 cm\(^{-1}\), 5747 cm\(^{-1}\) and 5935 cm\(^{-1}\) (1780 nm, 1750 nm, 1740 nm and 1685 nm). The prominent features at 5618 cm\(^{-1}\) is C-H first overtone starching of cellulose [15]. The absorbance band around 5716 and 5747 cm\(^{-1}\) related to S-H first overtone starching of sulphur amino acids (as S-allyl-cisteine) [18]. The evident peak at 5935 cm\(^{-1}\) is C-H first overtone starching of aromatic compounds [15].

**Figure 4.** Regression coefficient plot of PLS model.
4. Conclusions
The PLS model for detecting pepper powder adulterated with rice powder was produced using input from FT–NIR spectroscopy. The optimum model was established from NIR spectra treated by constant offset elimination with the $R^2_{\text{val}}$ of 0.99. The findings indicated that the NIR spectroscopy could be a good method for monitoring adulteration of pepper powder with rice powder.

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Acknowledgement
The authors are very gratefully acknowledging the instrument and software support provided by the NIRS Research Center for Agricultural Product and Food, Department of Agricultural Engineering, Faculty of Engineering, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. We also acknowledge the financial support from the King Mongkut’s Institute of Technology Ladkrabang research fund (KREF016006).