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Rapid authentication measurement of cinnamon powder using FT-NIR and FT-IR spectroscopic techniques

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RESEARCH ARTICLE

Abstract

Owing to the worldwide demand for cinnamon spices, true cinnamon (*Cinnamomum verum*) powder is often being adulterated with another inferior quality of cinnamon known as cassia cinnamon (*Cinnamomum aromaticum*). This study employed Fourier transform near-infrared (FT-NIR) and Fourier transform infrared (FT-IR) spectroscopic analysis to determine the spectral differences in assorted percentages after mixing with adulterants. The absorbance spectra of total 195 samples were collected of true, cassia and various adulterated samples (5-50 wt % adulterate) with 15 replicates for each sample. Independent component analysis was integrated with FT-NIR and FT-IR spectroscopic data for the detection of an unknown cinnamon mixture as a blind source separation tool that revealed distinctive peak difference. The partial least square regression (PLSR) models with spectral preprocessing methods were applied to predict the presence of cassia cinnamon in true cinnamon powder. The PLSR model for the FT-NIR and FT-IR data predicted adulteration with an R^2_p of 0.97 with a root-mean square error of prediction (RMSEP) of 2.2% and an R^2_p of 0.96 with an RMSEP of 2.5% respectively after applying Savitzky-Golay 2nd derivative as preprocessing. Thus, the predictive value of FT-NIR data was greater than the FT-IR data. In addition, the ratio of standard error of performance to standard deviation (RPD>2.5) and the range error ratio (RER>10) values were also calculated for both of the spectroscopic techniques to predict model accuracy for the independent validation set. The β coefficient curve from the PLSR models also revealed the spectral peak differences among the samples concentrated with various amount of cassia cinnamon powder. These methods proved that FT-NIR and FT-IR spectroscopic techniques combined with multivariate analysis could be utilised as a controlled procedure or as an alternative rapid detection method to identify adulterated cinnamon powder.

Keywords: food adulteration, powder food, cinnamon, Fourier transform infrared spectroscopy, Fourier transform near-infrared spectroscopy

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1. Introduction

Cinnamon has been used as a spice for thousands of years, earning worldwide popularity for of its taste, smell, and Aesculapian value. It is now one of the most consumed spices in the USA and Europe, second only to black pepper (Medicinal News Today, 2017). The cinnamon tree belongs to the genus *Cinnamomum*, which includes evergreen aromatic trees in the 'Lauraceae' family and incorporates 250 types of spice plants (Parthasarathy *et al.*, 2008). Cinnamon was found in Sri Lanka (formerly named as Ceylon, giving true cinnamon the name Ceylon cinnamon). The International Standards Organisation (ISO) has indexed 109 spices based on flavouring, anti-inflammatory, antidiabetic, astringent, and diuretic activities (Lee *et al.*, 2010), and separately includes six types of cinnamon: *Cinnamomum verum syn. C. zeylanincum, Cinnamomum aromaticum, Cinnamomum burmanii, Cinnamomum* *loureirii, and Cinnamomum tamala* (Swetha *et al.,* 2014a). Except for Ceylon cinnamon, all the other types of cinnamon are commonly termed as cassia cinnamon. Among all the types of cassia cinnamon, *C. burmanii,* or Korintje or also termed as Indonesian cinnamon that is very popular for bakeries due to its flavour and pretty low price (Uses & Benefits.com, 2016). In this study, the term 'Korintje or Indonesian cinnamon' will be replaced by cassia cinnamon.

Cinnamon can be used as a spice either whole or in powdered form and is widely used in baked goods, applesauce, stewed fruits, puddings, pastries, and even toast. In Europe and Canada, it is common practice to add cinnamon powder to mulled wines, pasta, soups, meats, pies, buns and even vegetable dishes. Cinnamon also contains active compounds, with longstanding use in herbal medicine. 'Cinnamon capsules' are most commonly used for medical purposes to reduce the risk of type 2 diabetes and various cardiovascular diseases (Allen *et al.,* 2013; Lu *et al.,* 2012; Whitfield *et al.,* 2016). Cinnamon is also commonly used to produce cosmetic products. In texture and taste, cassia cinnamon and Ceylon (true or pure) cinnamons are quite different. Cassia has strong peppery and pungent taste whereas the flavour of Ceylon is sweet and delicate.

Cassia cinnamon contains aromatic compound termed as coumarin (1-benzopyran-2-one, $C_9H_6O_2$) in large amount compared to true cinnamon (Sproll *et al.,* 2008), at nearly 1% (Woehrlin *et al.,* 2010), a chemical substance used for making rat poison that causes liver and kidney failure, which may affect the human body (Lungarini *et al.,* 2008). European Union set 2 mg/kg of coumarin limit as a possible genotoxic carcinogen (Sproll *et al.,* 2008) in 1980th and 1990th and European Food Safety Authority established a tolerable daily intake (TDI) of 0-0.1 mg of coumarin per 1 kg body weight (European Council, 1988). The coumarin content in ground cinnamon was reported to a range between 2,650-7,017 mg/kg (European Food Safety Authority, 2004). In 2006-2007, coumarin containing food products were investigated and showed that 85% of all cinnamon flavoured cookies exceeds the EU limit of 2 mg/kg coumarin (Sproll *et al.,* 2008), exceeding the TDI value of many individuals.

The German Federal Institute of Risk Assessment warns that consumers who use cinnamon containing products must be careful that the type of cinnamon they consume does not contain high amounts of cassia cinnamon (German Federal Institute of Risk Assessment, 2012). According to the European Food Safety Authority, each teaspoon of cassia cinnamon powder contains between 5.8 and 12.1 mg of coumarin, an amount that exceeds the TDI limit of some individuals (https://tinyurl.com/y2zcsurs). Studies carried out in Germany revealed that cinnamon flavoured products and cinnamon capsules contained cassia cinnamon as a substitute for true cinnamon. All these cases revealed the safety concerns for the presence of high amount of coumarin in cassia cinnamon (Lungarini *et al.,* 2008; Raters and Matissek, 2008; Sproll *et al.,* 2008).

Morphological differentiation is easy for cassia (adulterant commodity) and Ceylon (true) cinnamon in the whole form. But discrimination becomes difficult if the sample is in powdered form as they lose their morphological characteristic and diagnostic features. Spices are often sold in powdered form, is a prime target for substitution or alteration with low-cost imitation products and fillers. The Federal Food, Drug, and Cosmetic Act provides that food is 'adulterated' if it meets the criteria: 'If any substance has been substituted wholly or in part therefor' (US Government, 2011).

Adulterations have recently become common in food substances as well, with spices such as black pepper (Dhanya *et al.,* 2009; Parvathy *et al.,* 2014), chili powder (Haughey *et al.,* 2015; Ko *et al.,* 2014), and saffron (Petrakis *et al.,* 2015) by separate researches. Cassia cinnamon powder is commonly used as an adulterant in true cinnamon powder due to its low cost, though it may cause harm to human health. Distinguishing cassia cinnamon from true cinnamon powder (Ceylon cinnamon) is difficult as they closely resemble one another in appearance. Many new approaches for identifying the presence of adulterants (cassia powder) in true cinnamon powder has been developed, including DNA barcoding (Bhau *et al.,* 2015; Swetha *et al.,* 2014b), HPLC coupled with UV or mass spectrometry (Woehrlin *et al.,* 2010), direct analysis in real time quadrupole time-of-flight mass spectrometry analysis (He *et al.,* 2005), flow-injection mass spectrometric method (Chen *et al.,* 2014). Among all the conventional analytical techniques used for adulterant detection of food including physical methods, chemical or biochemical methods, and immunoassay, they are not suitable for routine analysis due to their complex analysing or time-consuming sample preparation (Qin *et al.*, 2017). To monitor the changes in food quality and the components of food is possible by the use of spectroscopic measurement that is a widely used non-destructive testing method in food analysis (Lim *et al.*, 2017; Lohumi *et al.,* 2016; Seo *et al.,* 2016). The molecular structure of samples with specific absorption bands to functional groups is the primary importance of NIR and IR spectroscopic analysis. Fourier transform near-infrared (FT-NIR) spectroscopy and Fourier transform infrared (FT-IR) spectroscopy are able to screen a large number of samples rapidly containing significant information about the individual components of a complex mixture (Ahmed *et al.,* 2017; Mo *et al.,* 2017). Both techniques include the same type of sample preparation and similar type of data acquisition and analysis methods (Lu *et al.,* 2011).

The objective of this study was to investigate the feasibility of spectroscopic technique to discover the cinnamon purity.

FT-NIR and FT-IR spectroscopic analysis were chosen based on their ability to provide rapid, sensitive results based on the analysis of a single sample. Pure Ceylon (true cinnamon), pure cassia and mixtures of various concentrations were analysed combined with multivariate analysis method to distinguish the spectral differences between the two substances and create a distinctive spectroscopic fingerprint for each.

2. Materials and methods

Multivariate statistical methods

Multivariate statistical process monitoring approaches are basically used for extracting information from large multivariate datasets (Slišković *et al.*, 2012). The key feature of such methods is to handle high dimensional, noisy and highly correlated data. In recent times independent component analysis (ICA) has gained much popularity due to the decomposition of data into linear combinations of statistically independent components (ICs) (Lee *et al.*, 2004). Since it was developed in the 1990s (Delfosse and Loubaton, 1995), it has successfully used in machine monitoring in recent days (Ypma and Pajunen, 1999). ICA is very closely related to blind source separation (BSS) or blind signal separation and most widely used for performing BSS (Hyvärinen and Oja, 2000).

ICA transforms multivariate data into components, which means; it maximises the statistical independence between the components. The method solves the matrices between the source and observed data whereas the observation arises from a linear mixture of unobserved 'source signals'. The matrix that transforms the observation from sources is denoted as 'mixing matrix' (See *et al.*, 2013). The measured variables are assumed to be linear mixtures of some unknown latent variables (ICs) where the coefficients of mixing matrix are also unknown. ICA can separate both the unobserved or independent source variable and observed variables though they were in a combination of matrixes (Liu *et al.*, 2009). The algorithm of ICA to predict the unknown source:

$$W = M^{-1}$$

$$\hat{s} = Wx = M^{-1}Ms$$

Here, ŝ is the estimation of the sources (s) and x represents observed spectra of the object, M is the mixing matrix and W is the separating matrix. Another algorithm for the spectra of the unknown mixture which were in a linear combination of various components' spectra was expressed as:

$$A = MI$$

Matrix $A_{l\times n}$ stands for l samples containing n values; $I_{m\times n}$ stands for the matrix of ICs, including m independent components. $M_{l\times m}$ stands for the mixing matrix, which is related to the component concentration in the mixture.

Sample collection and preparation

Two different cinnamon powders (Ceylon and cassia) were mixed in assorted concentrations to prepare different adulterated samples. Powder formed samples of cassia cinnamon (Burma Spice, West Milford, NJ, USA) and Ceylon cinnamon (KIVA, Honolulu, HI, USA) were used to prepare sample mixtures which were purchased from local markets. Cassia cinnamon was originated from Peoples' Republic of China and the true cinnamon was from Sri Lanka. Prior to experimental evaluation, all the samples were dried at 80 °C for 1 h. To avoid the errors occurred due to moisture content all the experiments were done on the same day after drying. Afterward, the powders were sieved manually using a 250-μm sieve to ensure homogenous particle sizes, in total 5 mg of each sample was prepared. In the United States, there are no limits on the amount of coumarin permitted in cinnamon-flavoured baked goods (The People's Pharmacy, 2013). Moreover, in North America 75% used cinnamon is cassia cinnamon (Cinnamon Vogue, 2018). Thus, samples of eleven different concentrations of cassia cinnamon (in true cinnamon) were prepared in this manner: 5, 7, 10, 12, 15, 18, 20, 25, 30, 40 and 50% (*w/w*) along with pure (100%) cassia and pure (100%) Ceylon (true cinnamon) powder. The sample preparation of this experiment was aimed to detect a small amount of adulteration of cassia cinnamon in true cinnamon powder. A high-speed shaker (Vortex-Genie 2, Scientific Industries, Inc., model G560, Bohemia, NY, USA) was used on every sample for 20 s to mix the two different cinnamon powders evenly. Fifteen replicates of each concentrated sample were used for both of the spectroscopic measurements.

FT-NIR and FT-IR spectral acquisition

The spectra of total 195 (13 set samples × 15 replications) samples were collected using an FT-NIR spectrometer (Antaris II FT-NIR analyser, Thermo Scientific Co., Waltham, MA, USA) equipped with an InGaAs detector. Data acquisition was performed by a computer using Thermo Scientific™ OMNIC™ Series Software. Due to collecting spectra, a furnished sample holder with a unique accessory was used. During the spectra collection, an accessory (containing a central hole with 20 mm thick × 20 mm diameter) was used over the irradiated surface to maintain the uniform thickness and the shape of the powder samples. Scanning of all the samples was taken after performing the reference scan with a golden slit. The diffuse reflectance was measured during the FT-NIR spectra measurements and for data analysis, a total successive 32 scans were used for each sample. Average spectra were generated using preprocessing and were used for the analysis while the wavelength range between 1000-2,500 nm (4,000-10,000 cm^{-1}) at 8 cm^{-1} spectral resolution.

For FT-IR spectroscopic analysis, an FT-IR spectrometer (Nicolet 6700 FT-IR analyser, Thermo Scientific Co.) was used to measure single sample spectral absorbance controlled by OMNIC software. This spectrometer was designed with an attenuated total reflectance (ATR) sampling technique which containing a diamond crystal internal reflective element with the angle of 45° for the IR beam, a KBr beam splitter, and a deuterated triglycine sulphate detector. Due to taking spectral data of powder sample, every single time sample was placed on the diamond crystal sampling plate and clamped with a pointed tip. Before every scan of the sample, always an empty sample plate scan was performed as background scan and cleaning of ATR crystal and pointed tip. Spectrum for the cleaned blank crystal was taken as a reference spectrum. The measured FT-IR spectra used a total successive 32 scans for every sample and a wavelength range of mid-infrared region (650-3,500 cm^{-1}) at an interval of 4 cm^{-1}. For both instruments, FT-NIR and FT-IR each spectrum for every single sample was stored against the corresponding background spectrum as absorbance values.

Spectral preprocessing and chemometrics

The raw spectra directly captured form FT-NIR and FT-IR were comprised of broad, overlapping peaks, and thus contained unexpected scattering noise. These scattered variations may have been generated from various physical phenomena such as; baseline shifts, slop variations, light scattering, or instrumental drift (Lohumi *et al.,* 2017). To obtain useful spectra, these variations should be removed by using spectral preprocessing methods, such as three normalisation techniques (maximum, minimum and range); standard normal variate (SNV); multiple scatter correlation and Savitzky-Golay derivatives. The primary objective of preprocessing the spectra is to remove aforementioned effects and improvement of multivariate regression analysis (Rinnan *et al.,* 2009). Among the preprocessing methods, Savitzky-Golay derivatives perform a local polynomial regression on a series of value to determine the smoothed value (Savitzky and Golay, 1964).

The partial least square regression (PLSR) models used these following equations:

$$X = TP^T + E \tag{1}$$

$$Y = UQ^T + E \tag{2}$$

The matrix X relates to the spectral variable of the pure (true) cinnamon spectra and is decomposed into the score matrix T, loading matrix P, and error matrix E. Conversely, the Y score matrix relates to the dependent variable of the true cinnamon spectra and is decomposed into the score matrix U, loading matrix Q, and error matrix E. To compose and corroborate the regression models, all data for both spectral analysis were randomly split into two subsections: a calibration set and a prediction set. The model was composed based on the calibration set and then tested with the prediction set. To minimise the risk of model inaccuracies, an optimum number of latent factors were tabbed on the lowest value of the root-mean-square error (RMSE) and the coefficient of determination for calibration (R_c^2) and prediction (R_p^2). The RMSE value measures the accuracy of model prediction and evaluate model performance in regression analysis.

$$R^2 = 1 - \frac{\sum_{i=1}^{z}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{z}(y_i - \bar{y}_i)^2} \tag{3}$$

$$RMSE = \sqrt{\frac{1}{z}\sum_{i=1}^{z}(y_i - \hat{y}_i)^2} \tag{4}$$

Here, y_i is the actual spectra of true cinnamon powder, $\hat{y}_i$ is the predicted spectra of adulterated cinnamon powders from the PLSR models, and z is the total number of spectra.

The analysis of chemometrics with the multivariate analysis in the combination of ICA and PLSR was performed by the MATLAB (The MathWorks, Natrick, MA, USA) software. The spectra acquired from these samples were divided on the basis of their concentrations. As the first step, ICA was performed as BSS tool, applied to a cinnamon mixture and true cinnamon powder. In this study, ICA has revealed spectral peak difference at a specific waveband region for both of the instrumental analysis. The ratio of standard error of performance to standard deviation (RPD), the range error ratio (RER), limit of detection (LOD) and limit of quantification (LOQ) were calculated which also evaluates the predictive ability of the model. The RPD and RER values also measure the goodness-of-fit of the calibration model (Lohumi *et al.*, 2017).

$$RER = \frac{y_{max} - y_{min}}{RMSEP} \tag{5}$$

$$RPD = \frac{SD}{RMSEP} \tag{6}$$

Where, y_{max} and y_{min} are the maximum and minimum reference values for the data in prediction set. SD is the standard deviation of the values obtained in the reference analysis.

The LOD provides the lowest analyte concentration information that can be reliably distinguished from a sample with no analyte. The LOQ value gives the limit value at which the difference between two different concentration values can be determined. LOD was calculated following the equation estimated as the three times of the ratio of the SEP of the absorbance divided by the slope whereas, the LOQ was estimated as ten times of the ratio (Scholl *et al.*, 2014).

$$LOD = 3 \times \frac{SEP}{m} \tag{7}$$

$$LOQ = 10 \times \frac{SEP}{m} \tag{8}$$

Where, SEP is the standard error for prediction and m is the slope of the regression line.

In the final classification model, the shorter and longer wavelength regions were removed as they contained less information and reader noise. To interpret the presence of adulterant in true cinnamon powder, PLSR model was developed after data preprocessing. From the total 195 samples, 165 samples (excluding pure (100%) cassia cinnamon powder and pure (100%) Ceylon cinnamon powder) were used for calibration and validation. For calibration 5, 10, 15, 20, 30, 50% adulterated (total 90 samples) and for prediction 7, 12, 18, 25, 40% adulterated (total 75 samples) were taken. The calibration and validation data sets were created using MATLAB (The MathWorks, Natrick, MA, USA) software. The same calibration and prediction strategy was applied to both spectroscopic data analysis.

3. Results and discussion

Blind source separation using independent component analysis

BSS refers to a problem where both the mixing methodology and the sources are unknown. Though for cinnamon adulteration only cassia cinnamon powder is used throughout the world, still BSS is necessary for some reasons. In real life scenario, to make a general model the complication begins with the input signal where most of the parameters are unknown. Researches revealed that BSS does not look for specific pre-defined components, but rather allows the data to determine the components (Clifford, 2007). In this research ICA was applied for blind separation that finds the ICs to constitute the observed variables (Chen and Wang, 2001).

According to the definition of ICA, the number of ICs would be equal to the number of samples (Hyvärinen and Oja, 2000). The various numbers of ICs were applied to find out the lowest value of SEC and SEV. In this research, initial 4 ICs improved the ability of making model rather than the application of more ICs. A combined preprocessing method of SNV, Savitzky-Golay 2nd derivative and 4 points of smoothing was applied (Figure 1). For FT-NIR spectroscopic analysis, a specific peak for cassia cinnamon was highlighted at 4,000 and 5,400 cm^{-1}. For FT-IR spectroscopic analysis, distinctive peaks appeared between 675 and 1,800 cm^{-1}. The reason for using SNV due to the effective removal of any multiplicative inferences caused by scattering for particle size differences (Savitzky and Golay, 1964). ICA loading plot has revealed a huge number of spectral peak difference between true cinnamon (Ceylon) powder and a cinnamon mixture for both of the spectroscopic analysis within this region. As a very first observation, ICA loading plots for both spectroscopic analysis revealed unique peaks for true cinnamon (Ceylon) powder and cinnamon mixture. If the true cinnamon powder is mixed with adulterant commodity, it can be detected applying the region between 4,000 and 5,400 cm^{-1} for FT-NIR and between 675 and 1,800 cm^{-1} for FT-IR spectroscopic analysis. Therefore, to yield results for the detection of adulterant in true cinnamon powder,

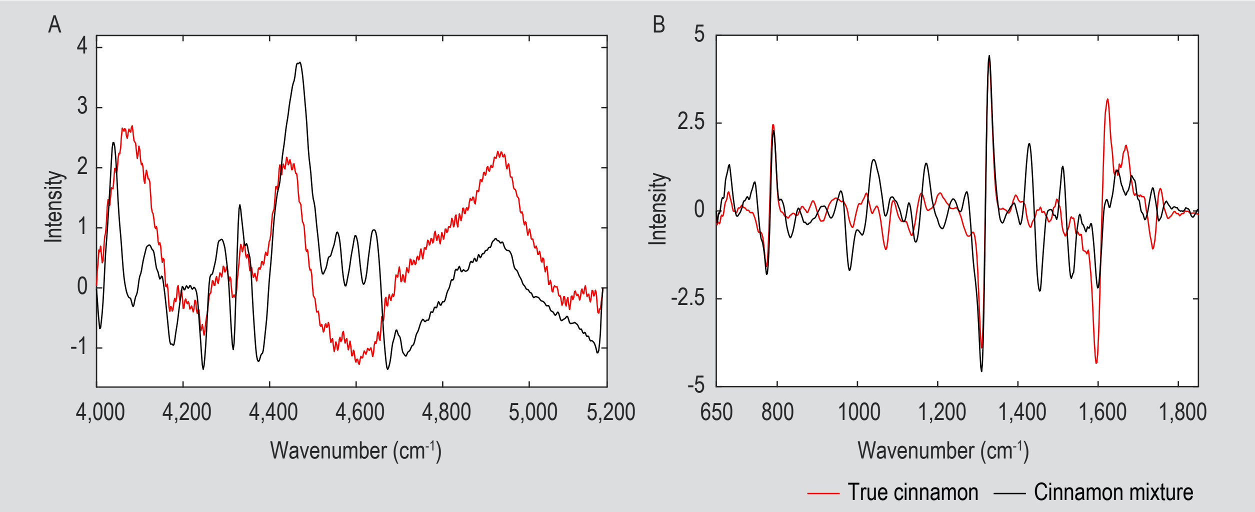

Figure 1. Independent component analysis loading plot after applying a combined preprocessing of standard normal variate, Savitzky-Golay 2nd derivative and 4 points of smoothing (A) Fourier transform near-infrared spectroscopy and (B) Fourier transform infrared spectroscopy.

PLSR was performed using respective regions for FT-NIR and FT-IR spectroscopic analysis.

Quantitative analysis of cinnamon powder concentrations using FT-NIR and FT-IR analysis

A wide variety of resinous compounds are present in cinnamon, including cinnamate ($C_9H_7O_2$), essential oil, cinnamic acid ($C_9H_8O_2$), and cinnamaldehyde (C_9H_8O) (Senanayake *et al.,* 1978). The spicy taste and redolence of cinnamon depend on the level of cinnamaldehyde (C_9H_8O), which occurs due to oxygen absorbance (Gende *et al.,* 2008). Though Ceylon and cassia are both types of cinnamon, they displayed notable spectral peak differences in the FT-NIR and FT-IR spectroscopic analysis. For, FT-IR spectroscopic analysis; it uses smaller ATR cell scanning point than FT-NIR. Therefore, the spectra caused by the physical variation as well as background effect sensitive region (2,280-2,400 cm^{-1}) reflects the CO_2 artifact which is not related to the samples, were removed (Cho *et al.,* 2016). Thus, during multivariate analysis for FT-IR spectroscopic analysis, this specific region was omitted.

The true cinnamon powder and cassia cinnamon powder has shown spectral peak differences in both of the spectroscopic analysis. The raw spectra from Near-infrared and Infrared spectroscopy for both the cinnamon powder were obtained for full waveband region. For both FT-NIR and FT-IR spectroscopic analysis, the informative spectral information was observed between 4,000-5,400 cm^{-1} and 800-1,800 cm^{-1} respectively (Figure 2A and 2B) which is also similar to ICA loading plot also. However, after applying Savitzky-Golay 2nd smoothing derivative, data highlighted spectral differences (Figure 2C and 2D) and for further multivariate analysis, these regions for both the spectroscopic analysis were used. It is also a smoothing method based on least-squares polynomial approximation. Fitting a polynomial to a set of input

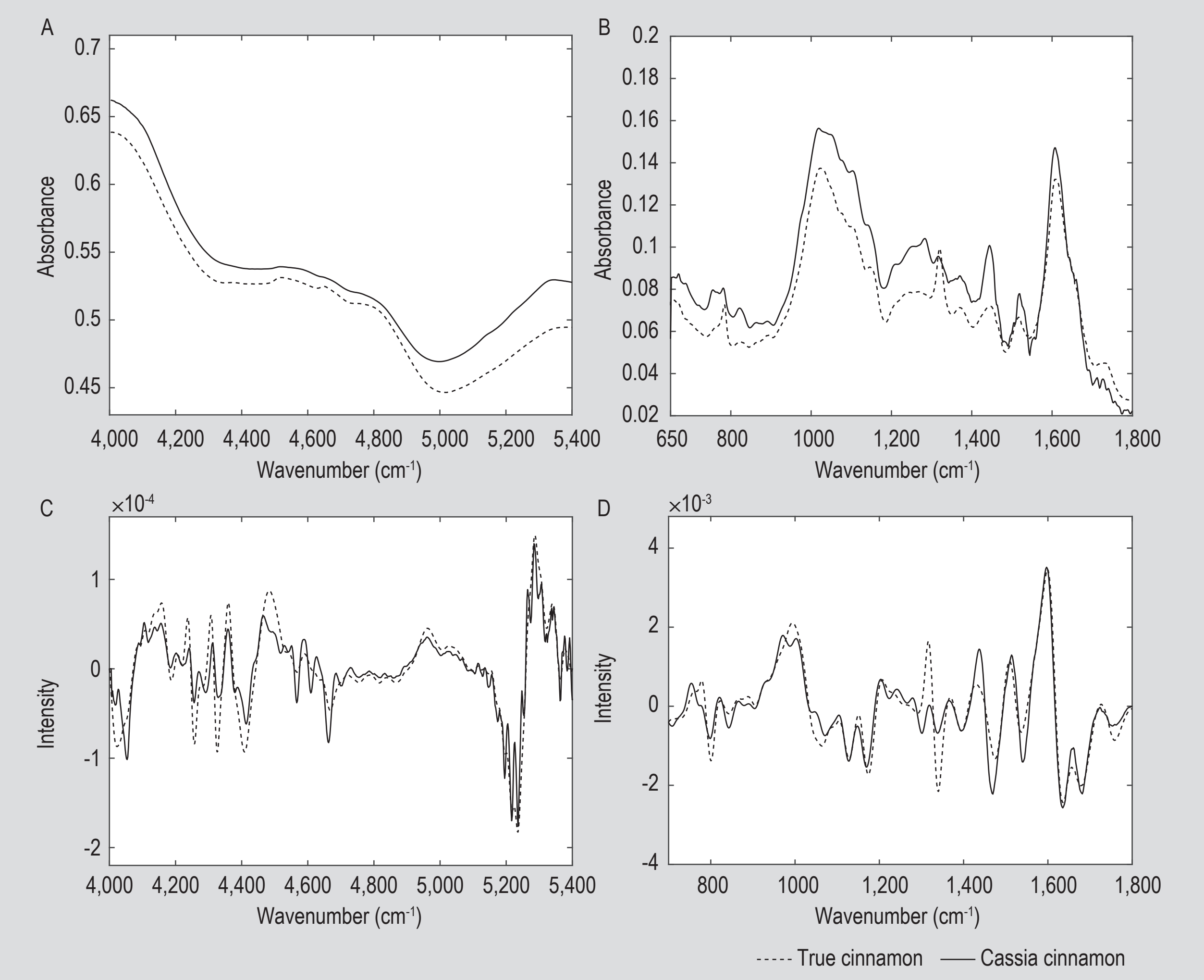

Figure 2. Spectral peak differences between true cinnamon, and cassia cinnamon of original spectra form (A) Fourier transform near-infrared spectroscopy (FT-NIR) and (B) Fourier transform infrared spectroscopy (FT-IR); preprocessed spectra using Savitzky-Golay 2nd derivative (C) FT-NIR and (D) FT-IR.

samples that evaluates the resulting polynomial at a single point within the approximation interval can be shown by Savitzky-Golay derivatives. This interval is equivalent to discrete convolution with a fixed impulse response as low pass filters and known as Savitzky-Golay filters.

For FT-NIR spectroscopic analysis, a specific peak at 5,284 cm^{-1} for cassia cinnamon was highlighted, reflected for $-CO_2R$. Here, R represents the alkyl group which is presumably for coumarin. Appendino *et al.* (1991) proved that coumarin contains an alkyl group. For FT-IR spectroscopic analysis, a spectral peak for cassia cinnamon observed at 756 cm^{-1} reflected strong C–H bending for benzene derivative which may represent coumarin.

The Partial Least-Squares Regression (PLSR) models for both the spectral analysis were constructed after the application of various preprocessing methods on raw data which were collected from the spectroscopies. The PLSR models reveal the prediction of added cassia cinnamon powder concentration in true cinnamon powder. To make calibration and prediction model, only the concentrated samples were used. The number of factors (latent variables) used to develop PLSR models were selected based on lowest RMSE values in cross-validation set.

The highest correlation coefficient value (R^2_p) was obtained with a lowest root-mean square error of prediction (RMSEP) by using Savitzky-Golay 2nd derivative in both spectroscopic analysis. The PLSR result obtained from spectroscopic data shows a strong relationship (for FT-NIR, R^2p=0.97; FT-IR, R^2p=0.96) between the actual and predicted concentration values.

The obtained values for RER and RPD were regarded as the measurement of the precision and the accuracy of prediction assess the model performance. From the previous researches (Williams and Norris, 2001) it was established that, if the RER value is around or above 10 and RPD is greater than 2.5, then it is the indication of a good model. In this research, both the RER and RPD values has given good result after the application of Savitzky-Golay 2nd smoothing derivative for both the spectroscopic analysis which means the model was properly developed. The calculated RPD of 4.8 and RER of 13.7 for FT-NIR spectroscopic analysis indicated that the developed model produces very accurate estimations rather than FT-IR spectroscopic analysis (RPD of 4.4 and RER of 12.5). Higher values for RPD suggests the increased model accuracy. For RER>10; the calibration is acceptable for quality control (Celignis Analytical, 2018). Therefore, both the spectroscopic analysis can be used for further research. Previous published journal proved that, a combination of RPD (3.1-4.9) and RER (13-20) values gives fair classification model (Starr *et al.*, 1981). It proves that the model developed from FT-NIR spectroscopic analysis gave more accurate estimation for the independent validation set than FT-IR spectroscopic analysis. RPD and RER values with PLSR models developed from FT-NIR and for FT-IR spectroscopic analysis shown in Table 1. Our results indicate that, if true cinnamon powder is adulterated with inferior quality cassia cinnamon powder which contains high amount of coumarine, is suitable to detect as an analytical screening tool. Results obtained from FT-NIR and FT-IR spectroscopic technique with multivariate analysis is a good alternative for determining adulteration in true cinnamon powder to traditional methods. The LOD and LOQ values were also summarised in Table 1. The LOD and LOQ values were higher due to the reason for high range of concentrated samples (5-50% *w/w*). But the obtained regression and residual plot for both of the spectroscopic analysis (Figure 3) showed that all the concentrated samples were very close to the regression line which indicated that the model can be used to detect cassia powder adulteration in true cinnamon powder.

The PLSR results clearly demonstrate that both the spectroscopic analysis contain sufficient information to differentiate between Ceylon, cassia, and their various

Table 1. Regression results of predicted cassia cinnamon powder concentrations in true (Ceylon) cinnamon powder for different data sets by partial least square regression.[1]

Instruments	Pre-processing	Factors	Calibration		Prediction		RPD	RER	LOD (%)	LOQ (%)
			R^2c	RMSEC	R^2p	RMSEP				
FT-NIR	Raw (without pre-processing)	4	0.98	1.9	0.97	2.4	3.6	11.3	5.7	20.0
	Savitzky-Golay 2nd derivative	3	0.98	1.8	0.97	2.2	4.8	13.7	4.9	18.3
FT-IR	Raw (without pre-processing)	4	0.91	3.2	0.90	3.8	3.0	8.6	10.2	35.6
	Savitzky-Golay 2nd derivative	4	0.98	2.2	0.96	2.5	4.4	12.5	6.5	21.8

[1] FT-IR = Fourier transform infrared; FT-NIR = Fourier transform near-infrared; LOD = limit of detection
LOQ = limit of quantification; R^2c = correlation coefficients of calibration; R^2p = correlation coefficients of prediction; RER = range error ratio; RMSEC = the root-mean-square error of calibration; RMSEP = root-mean square error of prediction; RPD = ratio of standard error of performance to standard deviation.

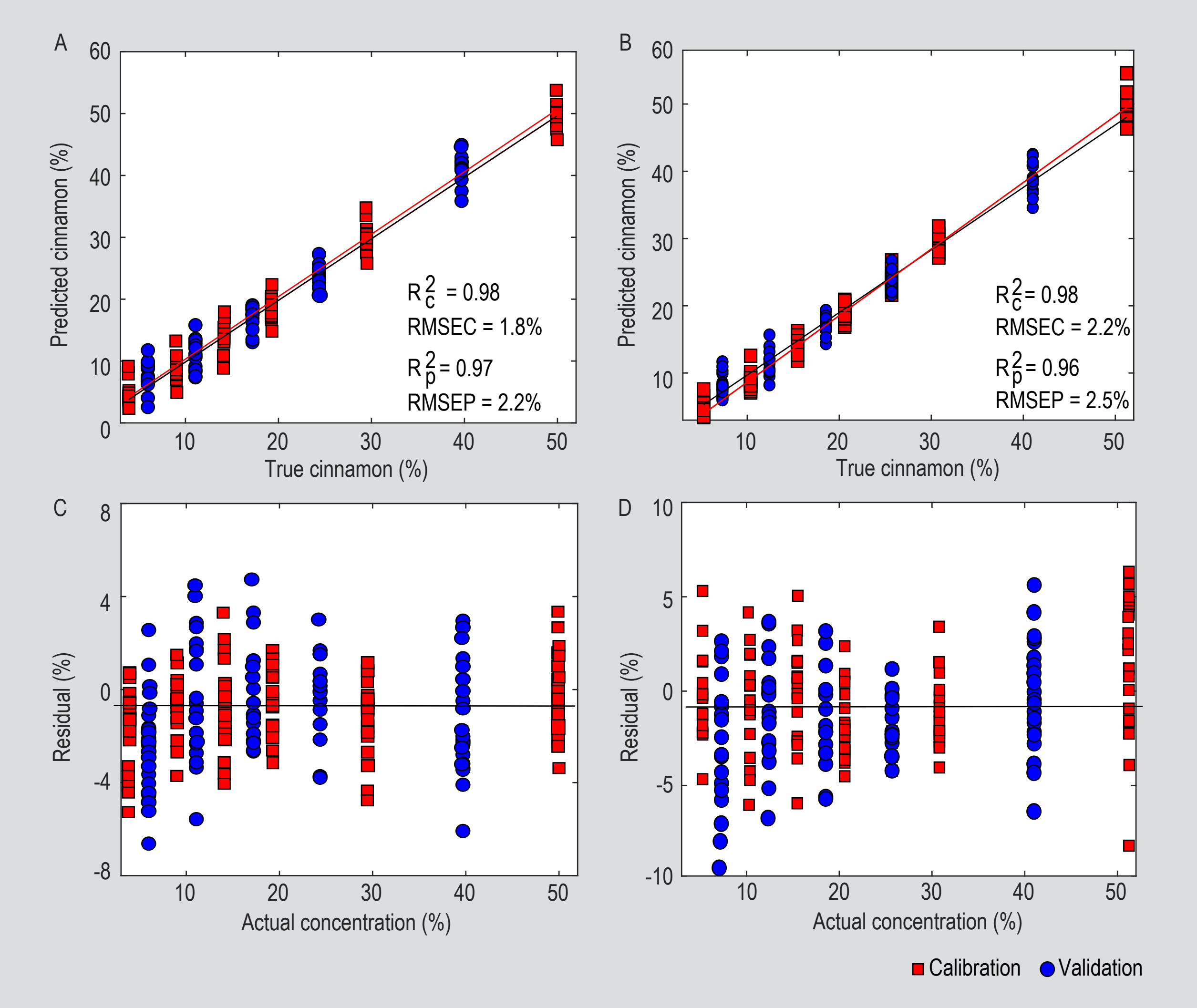

Figure 3. Data plotted from actual and predicted values for (A) Fourier transform near-infrared spectroscopy (FT-NIR) and (B) Fourier transform infrared spectroscopy (FT-IR); corresponding residual value (C) FT-NIR and (D) FT-IR.

amounts of mixtures (Figure 3). The better result has obtained from FT-NIR spectral analysis rather than FT-IR spectroscopic analysis. The results obtained in this study confirmed the presence of qualitative differences between true and cassia cinnamon.

Finding the adulteration in true cinnamon powder

To find the presence of cassia cinnamon powder as adulterated commodity in true cinnamon for the real-time experiment, an unknown percent of cassia cinnamon powder sample was prepared following the similar procedure as aforementioned in 'materials and methods' section. The spectral data of 'cinnamon mixture' is collected as raw data from both the spectroscopic techniques. Savitzky-Golay 2nd smoothing derivative was applied as preprocessing to the spectra (Figure 4). In both spectroscopic analysis, 'cinnamon mixture' revealed peaks for coumarin successfully. The FT-NIR and FT-IR spectroscopic analysis can be used to detect cassia cinnamon adulteration in a rapid and non-destructive way.

4. Conclusions

The arbitrary points are, detection of true cinnamon and cassia cinnamon powder with various concentration (5-50 wt %) of adulterated powder were investigated with two different types of vibrational spectroscopic techniques. By properly mixing the samples, both FT-NIR and FT-IR spectroscopic methods demonstrated potential findings. The BSS method has also proposed unique findings to detect adulterated powder after applying a combined preprocessing method of SNV, Savitzky-Golay 2nd derivative, and smoothing. However, PLSR model combined with FT-NIR spectroscopic technique has given greater prediction accuracy (R^2p=0.97) than FT-IR spectroscopic analysis (R^2p=0.96). The RPD and RER values proved high model accuracy for both of the spectroscopic analysis. The developed method after preprocessing can be

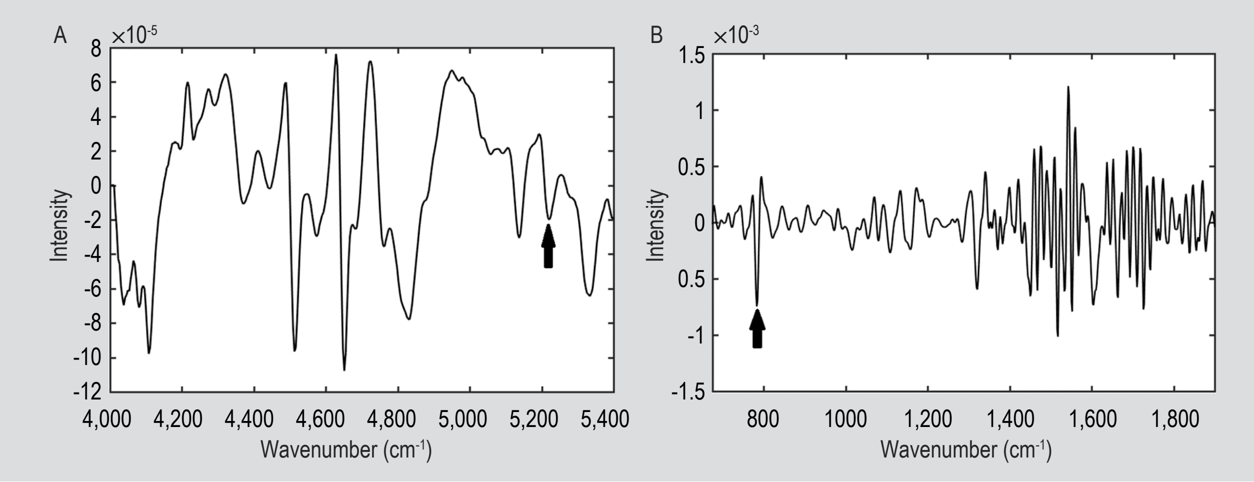

Figure 4. Presence of coumarin peak in 'cinnamon mixture' (A) Fourier transform near-infrared spectroscopy (FT-NIR) and (B) Fourier transform infrared spectroscopy (FT-IR) (Savitzky-Golay 2nd derivative was applied in both cases).

used to detect the adulteration of cassia cinnamon powder in true cinnamon with an adulteration level measured by LOD (FT-NIR: 4.9 and FT-IR: 6.5) and LOQ (FT-NIR: 18.3 and FT-IR: 21.8) values. Savitzky-Golay 2nd derivative improved the overall accuracies which seem to be promising to detect a large amount of the adulterated cinnamon powder in a non-destructive, chemical free, effective, and rapid way for quality control and authenticity detection of cinnamon powder.

Conflict of interest

The authors report there are no conflicts of interest.

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