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Fast Detection of White Pepper Adulteration using FTIR-ATR Spectroscopy and Chemometrics

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ABSTRACT

White pepper is a significant spice in Indonesia; however, powdered white pepper is vulnerable to adulteration, particularly with ingredients such as mung bean flour. Consequently, there is a need for a reliable analytical method to ascertain the authenticity of ground white pepper. This research seeks to establish an approach for authenticating white pepper and detecting the presence of mung bean flour utilizing Fourier Transform Infrared (FTIR) spectroscopy combined with chemometric techniques. The findings indicate that the adulteration patterns of white pepper and its contaminants can be effectively identified using the Partial Least Squares (PLS) method and chemometrics. Partial Least Squares (PLS) is a statistical method employed for regression analysis and modelling complex relationships between independent variables (X) and dependent variables (Y). It is one of the primary methods used in chemometrics because of its effectiveness in handling datasets with numerous variables, which is often the case in chemical analysis and spectroscopy. The second derivative FTIR spectrum in the range of 3712-650 cm^{-1} was identified as the optimal calibration model. The coefficient of determination (R^2) for calibration was 0.9535, while for prediction it was 0.9943. Furthermore, the root mean square error of calibration (RMSEC) was calculated at 4.32, and the root mean square error of prediction (RMSEP) was determined to be 1.38. The PLS Discriminant Analysis (PLS-DA) method also successfully classified pure white pepper samples from those adulterated with various concentrations of mung bean flour.

1. INTRODUCTION

Food fraud is prone to be found in various regions. One action of food fraud includes food adulteration, which is carried out by adding other additives, changing the ingredients' composition, hiding the composition, changing the code or expiration date, etc. These actions reduce consumer trust and damage the reputation of producers who do not commit food counterfeiting. This deed can also threaten consumers' health when using counterfeit ingredients that are allergenic or have a toxic effect. Thus, there is a need for fast, accurate, and analytical techniques that are reliable to ensure the authenticity of food ingredients circulating in the market [1].

The authentication analysis method can be carried out in a non-destructive and minimal use of chemicals. Spectroscopy is one of the most frequently used instruments used in this case. Spectroscopy, such as Near-Infrared (NIR) and Fourier Transform Infrared (FTIR), are commonly used to test the authentication of food ingredients. Testing using instruments can identify the pattern of functional groups of each sample tested. The resulting spectra are then combined with chemometrics to find out the equations and related parameters so that we can evaluate the authentication analysis method of a food ingredient.

White pepper is an indigenous spice in Indonesia. Its role as a source of flavor is distinctive because it contains piperine,

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which is the hallmark of this white pepper. For easier usage, it has now developed white pepper powder. Characters with a distinctive flavor and a reasonably high price can be adulterated with materials of lower value but with characteristics similar to physiques. Some research has been conducted to determine adulteration possibilities between white pepper and others. Starch is often used to counter herbs and spices such as garlic, ginger, and onion powder. Apart from that, corn and wheat flour can also be a part of the model of pepper forgery [2], [3]. This action increased the seller's economic motives by mixing herbs and spices with cheaper ingredients such as starch or flour. In addition, the selection of adulterated materials is also based on easy access and does not affect the falsified material's color, smell, and flavor.

Chemometrics is a branch of science that formally applies the theories of mathematics, statistics, and logic to process chemical data. Wu et al. (2017) have successfully developed a method for quantitative analysis of Sichuan pepper powder using near-infrared (NIR) spectroscopy with Partial Least analysis Squares (PLS) [4]. The other researcher have also successfully used spectra from NIR spectroscopy combined with chemometrics to analyze black pepper and adulteration models cumin rapidly [5]. Another spectroscopy often used for testing in this regard is Fourier Transform Infrared (FTIR). FTIR spectroscopy combined with chemometrics discriminant analysis and Partial Least Square (PLS) calibration models are known to have been used for classification and quantification of the presence of soybean oil in VCO [6]. In addition, analyze by using FTIR is quick and does not involve the use of reagents and hazardous solvents [6], [7], [8].

Mung bean and soybean powder possess physical characteristics that closely resemble white pepper powder, rendering them potential adulterants. Existing research on spice adulteration, particularly concerning white pepper, has predominantly concentrated on identifying contaminants such as starch-based fillers or lower-quality substitutes. There has been no specific investigation into detecting white pepper adulteration using mung bean and soybean powder through vibrational spectroscopy techniques. This study addresses this research gap by developing a robust analytical method for authenticating white pepper using Fourier Transform Infrared (FTIR) spectroscopy combined with chemometric modeling. By employing this approach, we enhance the accuracy of adulteration detection and introduce a rapid, non-destructive, and cost-effective method suitable for quality control applications in the spice industry.

2. MATERIALS AND METHODS

2.1 Materials

White pepper, mung bean and soybean were collected from Yogyakarta, Indonesia. These materials were grounded separately into powder.

2.2 Adulteration model

The adulteration models were prepared by mixing white pepper powder with mung bean powder at various concentration levels (0%, 3%, 5%, 7%, 10%, 13%, 15%, 17%, 20%, 23%, 25%, 27%, 30%, 33%, 35%, 37%, 40%, 43%, 45%, 47%, and 50% w/w). The mixing process was performed manually by thoroughly blending the powders in a circular motion for approximately 2 minutes to ensure even distribution of the adulterant within the white pepper matrix.

2.3 FTIR analysis

The samples were analyzed using FTIR spectrophotometer (Thermo Scientific Nicolet iS10, Madison, WI) with Omnic software (version 9, Thermo Scientific). FTIR spectrophotometer was equipped with attenuated total reflectance (ATR) sampling technique and Deuterated TriGlycine Sulfate (DTGS) Detector. FTIR spectra acquisition was performed in the 4,000–650 cm^{-1} region by placing the samples directly on the ATR crystal. The resolution used was 8 cm^{-1} with 32 scanning. Each sample was measured in three replicates. Prior to the measurement of each sample, the spectra of the background were recorded using the spectrum of air. After the measurement of each sample, the ATR crystal was cleaned using acetone analytical grade and fine tissues.

This study was conducted following ethical research principles, ensuring that all analytical procedures and sample preparations were performed without causing environmental harm or violating ethical standards in food analysis.

3. RESULTS AND DISCUSSION

3.1 FTIR Spectra Analysis

FTIR spectroscopy with Attenuated Total Reflectance (ATR) is highly sensitive when analyzing the sample. Meanwhile, it analyzes the surface of the sample. Therefore, the samples should be homogenous or evenly distributed. Sometimes, two or more samples can have similar spectra. Consequently, we must meticulously identify and maintain the internal factors (moisture content of sample) and external factors (humidity and temperature laboratory).

FTIR can analyze the white pepper, mung bean, and soybean very well (Fig. 1). The white pepper spectra look similar to the mung bean, especially at region 1200-1000 cm^{-1}. Several functional groups have been successfully identified in the samples (Table 1). White pepper has more peaks at 995-675 and 1300-1050 cm^{-1} regions. These regions showed alkenes, alcohol, ether, carboxylic acid, and ester more than in soybeans and mung beans. Meanwhile, white pepper also has aliphatic fluoro compounds at 1015-1000 cm^{-1}. Both soybeans and mung beans have protein and lipid more than white pepper at 1900-1000 cm^{-1}. O-C-O and C-H from alkenes were found in all samples at 995-606 cm^{-1} regions. The stretching vibration of C-F from aliphatic fluoro compounds was only found in the white pepper at 1014,4 cm^{-1}. Some variations in the type of proteins were also found in all samples at 1632-1000 cm^{-1}. The stretching vibrations of C=O from lipid was not found in the white pepper. Meanwhile, all of the samples containing lipid and protein by C-H (symmetric and asymmetric stretching vibration) at 2800 and 2900 cm^{-1}, C=H (stretching vibration) at 3000 cm^{-1}, and the stretching vibrations of N-H at 3300 cm^{-1} [9]. In the white pepper, some signals were specifically found at 1632.9; 1583.72; 1251.85; 995.89 cm^{-1}, which was thought to be a sign of the presence of piperine [10]. Piperine is a compound specifically found in pepper [10], [11].

Table 1. FTIR spectral identification of functional groups present in white pepper, mung bean, and soybean powders, along with their corresponding wavenumbers.

Vibration type	Functional group	Wavenumber (cm^{-1})	Reference	Wavenumber (cm^{-1})		
				White pepper	Mung bean	Soybean
O-C-O (bending vibration)	CO_2	600-900	[9]	569,26	564,73	613,6
C-H	Alkenes	675-995	[16]	606,94	612,36	663,44
				702,04	701	697,21
				716,07	763,57	796,83
				763,74	799,94	
				786,4	859,08	
				804,28	994,82	
				830,71		
				847,27		
				927,98		
				995,89		
C-F (stretching vibration)	Aliphatic fluoro compounds	1000-1015	[16]	1014,4		
C-O (stretching vibration)	Starch	1040-1050	[9]			1046,7
PO_2-(symmetric stretching vibration)	Nucleic acid					
C-O	Alcohol, Ether, Carboxylic acid, Ester	1050-1300	[16]	1077,04	1074,94	
				1103,99		
				1134		
				1150	1147,32	
				1195,18		
C-N (stretching vibration)	Amide III bonds (Protein)	1200-1400	[15]	1251,85	1240,25	1240,19
				1365,41		1315,11
CH_3 (bending vibration)	Protein	1390	[9]		1394,08	1394,34
COO- (symmetric stretching vibration)	Fatty acids, Amino acids		[9]			
CH_2 (bending vibration)	Lipid	1450		1446,73		1441,92
				1491,4		
C-N (stretching vibration)	Amide II bonds (Protein)	1500-1580	[15]	1509,33	1538,14	1537,89
N-H (bending vibration)				1583,72		
C=O (stretching vibration)	Amide I bonds (Protein)	1600-1700	[15]	1612,11	1635,36	1633,26
				1632,9		
C=O (stretching vibration)	Lipid	1745	[9]		1743,33	1743,5
					1805,9	1815,36
					1853,16	1836,95
					1913,76	1853,06
					1983,48	
O-C-O (stretching vibration)	CO_2	2200	[9]	2076,18	2114,65	2106,89
C-H (symmetric stretching vibration)	Lipid	2800	[9]	2855,46	2855,37	2855,48
C-H (asymmetric stretching vibration)	Lipid	2900	[9]	2938,44	2924,98	2925,5
C=H (stretching vibration)	Lipid	3000	[9]	3009,93		
N-H (stretching vibration)	Protein	3300	[9]	3288,01	3281,01	3279,05
O-H (stretching vibration)	Water	3500	[9]			

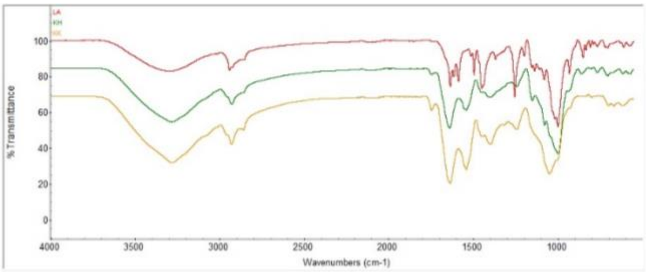

Fig. 1. The FTIR spectras of white pepper (red-line), mung bean (green-line), and soybean (yellow-line) showing characteristic absorption peaks of each component

3.2 Pattern recognition chemometrics for mung bean identification in the white papper

In this study, mung bean powder (MBP) were combined with white pepper powder (WPP) as adulteration models. To achieve the best prediction models, multivariate calibration optimization were done. Two calibration method, Partial Least Square (PLS) and Principal Component Regression (PCR) were used in this case. The other researcher revealed that FTIR followed by multivariate data analysis has been successfully used to investigate adulteration or authentication. FTIR on Mid-Infrared (MIR) has the advantage of being able to work quickly and reliably [12]. The use of FTIR is increasing for the detection of food adulteration due to saving analysis costs and increasing the desirability of high resolution analyzes coupled with a demand for a lower instrumental limit of detection. The results of analysis using FTIR are then processed using multivariate analysis. The two methods most often used are partial least squares (PLS) and principal component regression (PCR). These two statistics are normally used for calibration. The main difference between PLS and PCR is that PCR looks at a high variance distance between the space of the covariates. Meanwhile, PLS seeks the space between covariates that will predict the best outcome, using the concentration information [12], [13]. Partial Least Squares Regression (PLS) also can employ minor sources of variance to increase group separation, as well as predict percentages of adulteration for each fraud using multivariate calibration [5].

Table 2. Optimization of Partial Least Squares (PLS) and Principal Component Regression (PCR) models for adulteration detection using FTIR Spectroscopy, based on different spectral preprocessing methods and wavenumber ranges

Calibration method	**Wavenumber (cm^{-1})**	**Spectra**	**Calibration**		**Prediction**	
			RMSEC	**R^2**	**RMSEP**	**R^2**
PLS	**3712-650**	Normal	5.41	0.9262	1.81	0.9784
		1st derivative	5.12	0.9340	1.73	0.9856
		2nd derivative	**4.32**	**0.9535**	**1.48**	**0.9943**
	3712-3009	Normal	5.67	0.9186	3.24	0.9759
		1st derivative	5.57	0.9215	3.10	0.9727
		2nd derivative	4.03	0.9596	4.69	0.8184
	3010-2824	Normal	5.43	0.9256	2.74	0.9734

		1st derivative	5.44	0.9254	2.35	0.9713
		2nd derivative	6.61	0.8874	6.83	0.6396
	1805-349	Normal	5.48	0.9241	1.73	0.9734
		1st derivative	5.29	0.9294	1.75	0.9750
		2nd derivative	4.66	0.9459	2.43	0.9649
	1340-650	Normal	5.21	0.9317	2.21	0.9818
		1st derivative	5.28	0.9297	2.65	0.9878
		2nd derivative	4.62	0.9468	1.76	0.9774
PCR	3712-650	Normal	5.08	0.9350	1.51	0.9874
		1st derivative	4.98	0.9377	2.17	0.9903
		2nd derivative	5.16	0.9330	2.18	0.9713
	3712-3009	Normal	4.98	0.9377	2.24	0.9779
		1st derivative	5.18	0.9326	2.34	0.9783
		2nd derivative	6.91	0.8761	5.73	0.7371
	3010-2824	Normal	5.37	0.9272	2.30	0.9688
		1st derivative	5.33	0.9284	2.28	0.9698
		2nd derivative	5.19	0.9322	0.82	0.9944
	1805-1349	Normal	4.94	0.9387	1.61	0.9933
		1st derivative	4.88	0.9404	2.22	0.9785
		2nd derivative	4.94	0.9388	2.67	0.9701
	1340-650	Normal	5.09	0.9349	2.33	0.9678
		1st derivative	5.01	0.9370	1.60	0.9901
		2nd derivative	5.72	0.9170	3.05	0.9285

The FTIR spectra were optimized at normal, derivative 1 and 2 spectra. Table 2 showed the RMSEC following the R^2 of calibration equation and RMSEP following the R^2 of prediction equation at each level spectra by PLS and PCR method. The method selected was the method with the highest of coefficient determination (R^2) and the lowest error score. PLS at 3712-650 cm^{-1} wavenumber with second derivative spectra were chosen as the best. It has the lowest of RMSEC and RMSEP, also has the highest R^2 in calibration and prediction equation, respectively. To evaluate the model fitting, the R^2 value was used, a model with R^2 closer to 1 had the goodness of fit [8]. The high R^2 value and low RMSEP and RMSEC values can indicate that the model developed for authentication can be categorized to be quite accurate and precise [7]. The R^2 for calibration and prediction were 0.9535 and 0.9943, respectively. Root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) were 4.32 and 1.38, respectively. This result showed that the model for authenticating white pepper was accurate and precision. Fig. 2 and 3 also showed the actual concentration and calculated concentration by using FTIR combined with chemometrics. The Fig. 3 illustrated the difference between actual and predicted values in the PLS model, assessing the model's accuracy.

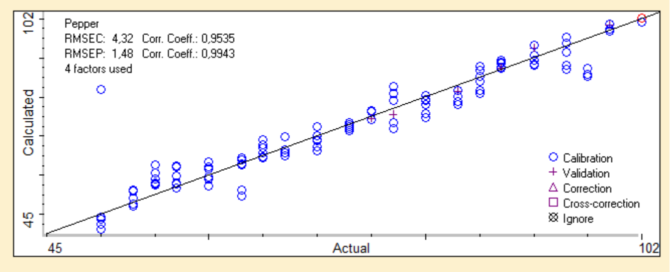

Fig. 2. Calibration and validation plots of pepper adulteration models using Partial Least Squares (PLS) regression, demonstrating model performance in predicting adulterant concentration

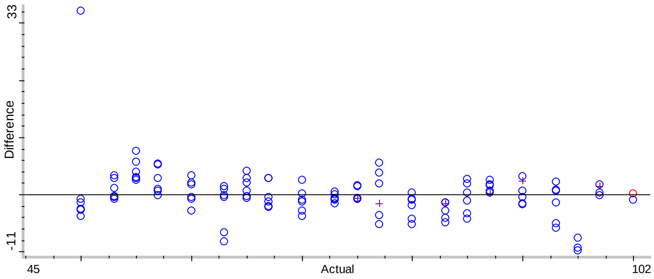

Fig. 3. Residual analysis plot

In this study, we used three types of pattern recognition analysis, PCA, PLS-DA and sPLS-DA. By PCA, PLS-DA and sPLS-DA; we found that all adulterated samples can be clearly separated from the pure sample (WPP). The red ones indicating as pure WPP, the green and blue ones as WPP containing middle and high of MBP, respectively (Fig. 4). Jiang et al. (2020) also found that PCA-classification by Metaboanalyst 3.0 can separate the adulterated *Dendrobium officinale* stem powder with different proportions of it from 10% to 100% [14]. This study indicated that this pattern recognition analysis were effective method to classify the pure and adulterated samples.

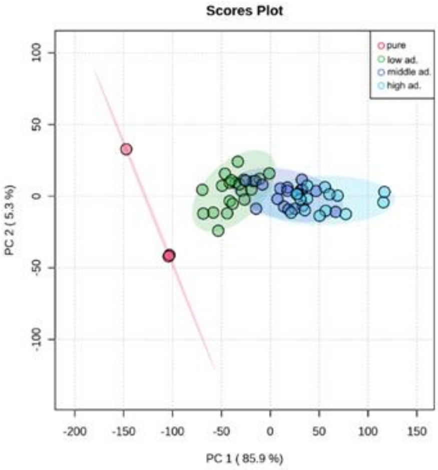

Fig. 4. Principal Component Analysis (PCA) score plot for sample classification. Red represents pure ssamples, while green, purple, and blue indicate low, middle, and high levels of adulteration, respectively.

The Partial Least Square-Discriminant Analysis or PLS-DA was further used to analyze the differentiation between authentic and adulterated samples. PLS-DA could be used to differentiate pure white pepper and adulterated with variation concentrations of mung bean powder more clearly. This study aligned with another study that reported that PCA demonstrated an excellent ability to differentiate tuna fish oil from palm oil. Still, it couldn't differentiate authentic tuna fish oil samples from those adulterated with palm oil [8]. However, both PLS-DA (Fig. 5) and (Fig. 6) and sPLS-DA (Sparse Partial Least Square-Discriminant Analysis) showed some overlapping at some concentrations of adulterants. PLS-DA was still better than the sPLS-DA. Compared to other research, this study was the easiest to detect the adulteration of white pepper powder.

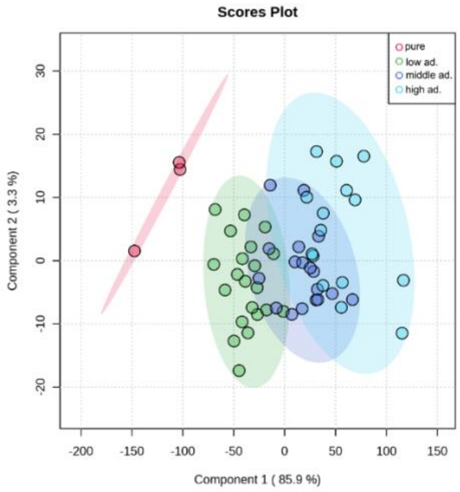

Fig. 5. Partial Least Squares Discriminant Analysis (PLS-DA) score plot for sample classification. Red represents pure samples, while green, purple, and blue indicate low, middle, and high levels of adulteration, respectively.

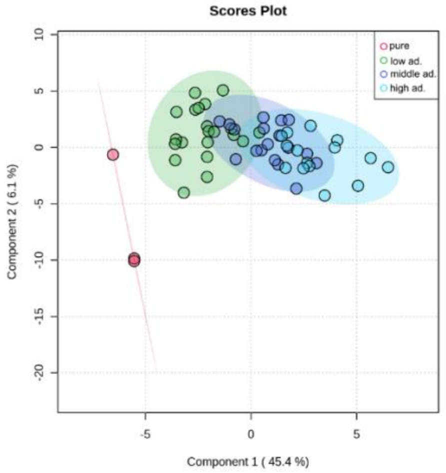

Fig. 6. Sparse Partial Least Squares Discriminant Analysis (PLS-DA) score plot for sample classification. Red represents pure samples, while green, purple, and blue indicate low, middle, and high levels of adulteration, respectively.

The PLS-DA and OPLS-DA discrimination methods were also renowned for their ability to discriminate between contaminated samples by up to 90-100%. The main advantage of OPLS-DA modeling is its ease of interpretation, particularly in multiclass cases. This is accomplished by separately modeling predictive and class-related variation in the X-matrix by identifying Y-orthogonal variance [5].

4. CONCLUSION

The adulteration pattern of white pepper and its adulterants was successfully identified using the PLS technique using FTIR-ATR spectroscopy combined with chemometrics. PLS-DA can also classify pure white pepper samples from those adulterated with mung bean flour at various concentrations. The second derivative FTIR spectrum at wavelengths 3712-650 cm^{-1} was selected as the calibration model. The R^2 for calibration and prediction were 0.9535 and 0.9943, respectively. Root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) were 4.32 and 1.38, respectively. For future work, further studies can explore other potential adulterants in white pepper, such as flour, etc. Additionally, FTIR-ATR spectroscopy combined with chemometrics can also be used for broader applications in food authentication, especially for herbs and spices.

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