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Halal authentication study of the chicken *siomay* with the adulteration of pork using FTIR spectroscopy combined with chemometrics

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Abstract

Indonesia has a predominantly Muslim population, resulting in strong demand for halal products. *Siomay*, a type of dimsum originating in China, was initially made with pork but was later made with chicken to be marketed in Muslim countries. The large number of *siomay* produced by home industries is detrimental to Muslim consumers because the products are not halal-labeled, so their halal status is unknown. This study, thus, aims to identify chicken *siomay* and to analyze potential pork adulteration for halal verification using Fourier Transform Infrared (FTIR) spectroscopy coupled with chemometric techniques. The samples analyzed in this study consist of chicken *siomay* mixed with different concentrations of pork, which were used as training and testing sets. The animal fatty acid components in the samples were extracted using the Folch method, followed by spectral data acquisition by FTIR spectroscopy at wavenumbers of 4000-500 cm-1. The spectra results were used as chemometric modeling variables. The PCA (Principal Component Analysis) was performed to determine the most influential principal components in sample grouping, namely Dimension 1 (82.8%) and Dimension 2 (10.1%), with a wavenumber of 1744 cm-1 as the variable that contributed most to building the PCA model. Meanwhile, the PLS-DA (Partial Least Squares-Discriminant Analysis) was used to differentiate chicken fatty acids, pork fatty acids, and their binary mixture, with AUC-ROC values of 1.000, 0.9889, and 0.9630, respectively. These results indicate the quality of the discrimination model with high sensitivity and specificity.

Keywords: authentication; chemometrics; halal; pork; *siomay*

INTRODUCTION

Indonesia is the world's largest consumer of halal products, accounting for 12.6% of global halal food consumption[1]. Consequently, Indonesia needs many halal products to be recognized by the state through certification by the Halal Product Assurance Organizing Agency (BPJPH), in accordance with the fatwas issued by the Indonesian Ulama Council (MUI). Indonesia has exported halal products to various countries while also importing foreign goods from both large-scale and home-based industries[2]. One of the products accepted on the market is siomay. Siomay, or Siu may, is a type of dim sum originating in China that uses pork as its main ingredient. However, siomay has been adapted for the Muslim consumer market by using chicken instead of pork as its main ingredient[3].

Specifically, siomay produced by the home-based industry is packaged in plastic packaging and labelled with the producer's name and the brand name only, without displaying an official halal label issued by Indonesian Ulama Council (MUI) based on the certification issued by the Halal Product Assurance Organizing Agency (BPJPH), as regulated under the Law of the Republic of Indonesia No. 33 of 2014 concerning Halal Product Assurance

(JPH). However, there have been some reported cases of food adulteration involving the deliberate addition of non-halal ingredients, including pork. Siomay is particularly vulnerable to such fraud, as pork may be added to increase the profits. This condition may harm consumers who purchase siomay products, as they are unable to determine the halal status of the products they buy [2]. In Islam, in addition to being prohibited, pork has negative health effects when consumed because it has higher levels of saturated fatty acids than chicken, thereby causing an increase in cholesterol levels and Low-Density Lipoprotein (LDL), as well as diastolic blood pressure in the body, which may increase the risk of stroke, heart disease, and vascular disorders [4]. Several analytical methods, such as DNA-based methods using polymerase chain reaction, Enzyme-Linked Immunosorbent Assay (ELISA), gas chromatography, and mass spectrometry for fatty acid analysis of meat, are analytical methods that have been implemented for the authentication of meat-based food products, including protein-based methods [5]; however, these methods require expensive and complex sample preparation [6].

This study, therefore, aims to perform a halal authentication of chicken *siomay* adulterated with pork using Fourier Transform Infrared (FTIR) spectroscopy combined with chemometric techniques, namely Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA). This study offers a new approach to halal authentication of chicken *siomay* products by applying FTIR spectroscopy combined with chemometric analysis to detect and quantify pork adulteration, using a quick, non-destructive technique.

METHOD

Tools and Materials

Chicken *siomay* is prepared *in-house* according to the previous research by Christiantio et al. [7], with modifications. Boneless chicken thighs and additional ingredients, such as tapioca flour and dim sum wrappers, were obtained from Progo Supermarket in Yogyakarta City, Special Province of Yogyakarta. Pork used as an adulterant was obtained from a pork vendor from Porker Store, Yogyakarta City, Special Province of Yogyakarta. Other materials used for lipid extraction by the Folch method included methanol (technical grade), 1 N hydrochloric acid (HCl), chloroform (technical grade), anhydrous sodium sulfate (Na_2SO_4), and distilled water. The tools used in this study were an FTIR Spectrophotometer (Bruker ALPHA II) equipped with an Attenuated Total Reflectance (ATR), RStudio (version 4.1.1), and RStudio (version 2024.04.2 Build 764).

Preparation of *Siomay* Sample and Adulterant Materials

Equipment preparation involved inspecting all tools to ensure they were in good condition and could work properly throughout the study. The *siomay* used was prepared in-house following the previously described method. The additional ingredient in the *siomay* was tapioca flour, and the *dimsum* wrapper was used to wrap the *siomay*. The *siomay* sample for the training set used replicates 1 and 2, while replicate 3 was used for the test set. *Siomay* samples (20 g) were prepared from a mixture of 90% (w/w) meat and 10% (w/w) additional ingredients (tapioca flour) and steamed for 10-20 minutes at 100 °C [6]. To prepare *siomay* samples for training and test sets, pork was added as an adulterant to chicken *siomay* at varying percentages (Table 1), and the samples were prepared to a total weight of 18 g.

Table 1. Pork content as an adulterant in chicken *siomay* samples for the training set and test set

No.	Sample Code	Pork content (w/w)	Replication
1	Mixture 1	0 %	
2	Mixture 2	10 %	
3	Mixture 3	20 %	
4	Mixture 4	30 %	
5	Mixture 5	40 %	3 times
6	Mixture 6	50 %	
7	Mixture 7	60 %	
8	Mixture 8	70 %	
9	Mixture 9	80 %	
10	Mixture 10	90 %	
11	Mixture 11	100 %	

Acid Hydrolysis

Siomay samples were subjected to acid hydrolysis to improve extraction efficiency by releasing fatty acids bound to glycerol esters. A total of 20 g of samples was homogenized using a chopper and hydrolyzed with 200 mL of 1 N HCl for 30 minutes at 60 °C in a water bath, followed by filtration.

Folch Extraction

The acid-hydrolyzed samples were extracted with 400 mL of chloroform and methanol (2:1 v/v) according to the previous study[7]. The mixture was homogenized using a magnetic stirrer at 300 rpm for 30 minutes at 60 °C and then filtered. The filtrate was transferred into a separatory funnel, mixed with 80 mL of distilled water, and gently shaken. The mixture was allowed to stand until two phases formed; the upper phase, containing methanol and water, was discarded, while the lower phase (chloroform) was collected. The chloroform phase was evaporated using a rotary evaporator at 40 °C until the fatty acid extract was obtained. The fatty acid extract was transferred into vials and stored in the refrigerator at 4-8 °C [8].

FTIR Absorbance Measurement and Spectral Evaluation

FTIR spectra were obtained using an FTIR-ATR spectrophotometer. The FTIR prism was cleaned with ethanol, then dried with a tissue. Background scans were performed before measurement and at each change of sample. The sample was scanned at a resolution of 4 cm^{-1}. The FTIR spectra were recorded in absorbance

mode over the mid-IR region (4000-500 cm^{-1}) with three replicate measurements.

Principal Component Analysis Model Construction

Statistical analysis using the PCA model was performed to analyze the principal components by identifying new 'hidden' variables or latent variables derived from linear functions of the original data set. The PCA model was developed based on eigenvalues and Singular Value Decomposition (SVD). The data were analyzed using the 'factoextra' and 'FactoMineR' packages, resulting in scree plot, individual plot, and variable plot, [9,10].

Development of the PLS-DA Discriminant Model

The PLS-DA discriminant model was developed to classify siomay samples into non-adulterated (chicken-only) and pork-adulterated samples. The PLS-DA model was developed using the 'mixOmics' package in R. In the PLS-DA model, the X variables represented absorbance values at each wavenumber, while the Y variable represented the group labels [9]. The FTIR spectra obtained from the measurements were further evaluated to identify the functional groups represented in the sample. The quantification of pork in chicken siomay was performed using PCA and PLS-DA. PCA was analyzed based on the resulting scree plot, individual plot, and variable plot, from which the grouping of chicken fatty acids and pork fatty acids in the samples could be distinguished [11]. PLS-DA is a discriminant modeling technique that incorporates a validation process to evaluate predictive results. Spectral data were analyzed based on Area Under the Curve-Receiver Operating Characteristics (AUC-ROC). A good AUC-ROC value approaches or equals 1, indicating strong model performance in discriminating between chicken fatty acids and pork fatty acids [12].

RESULT AND DISCUSSION

Home-made siomay samples were separated from the wrappers, homogenized using a chopper, and subjected to acid hydrolysis with HCl 1 N for 30 minutes at 60 °C in a water bath, followed by filtration. The acid-hydrolyzed samples were extracted using the Folch method with chloroform-methanol (2:1 v/v). The analysis using FTIR spectroscopy was performed over the range of 4000-500 cm^{-1} at a resolution of 4 cm^{-1} in absorbance mode, and the measurements were performed in triplicate. The FTIR spectra of chicken and pork siomay were quite similar. However, careful inspection of the

sample's FTIR spectrum revealed several differences in the peak profile. It was found that 13 major spectral peaks were identified at 3007, 2922, 2853, 2361, 1744, 1463, 1377, 1236, 1160, 1118, 1098, 967, and 721 cm^{-1}, corresponding to chicken siomay, pork siomay, and their mixture (Figure 1). The identified functional groups by FTIR Spectroscopy, along with their absorbance intensities, are presented in Table 2.

Table 2. Functional group identification using FTIR spectroscopy

| Wavenumber (cm^{-1})	Functional Groups	Absorbance intensities			Vibrational modes	Ref.
		Pork Siomay	Chicken Siomay	Mixture Siomay		
3006	=C-H (cis)	0.0230	0.0257	0.0237	Stretching	[13]
2922	-CH$_2$ (methylene)	0.2592	0.2533	0.2589	Stretching (asymmetric)	[14]
2853	-CH$_3$ (methyl)	0.1792	0.1750	0.1792	Stretching (symmetric)	[14]
2361	-C-O (ester)	0.0169	0.0027	0.0099	Stretching	[15]
1744	-C=O (ester)	0.2715	0.2489	0.2753	Stretching	[14]
1463	-CH (methylene; methyl)	0.0845	0.0852	0.0847	Bending (scissoring)	[16]
1377	-CH$_3$ (methyl)	0.0491	0.0499	0.0489	Bending (symmetric)	[14]
1236	-C-O; -CH$_2$-	0.0805	0.0802	0.0803	Stretching; bending	[17]
1160	-C-O; -CH$_2$-	0.1809	0.1706	0.1822	Stretching; bending	[16]
1117	-C-H	0.1019	0.0966	0.1024	Bending	[14]
1098	-C-O	0.0998	0.0935	0.1003	Stretching	[14]
967	-HC=CH-(trans)	0.0328	0.0359	0.0325	Bending (out of plane)	[13]
721	-(CH$_2$)$_n$; -HC=CH-(cis)	0.0888	0.0899	0.0892	Bending (rocking)	[17]
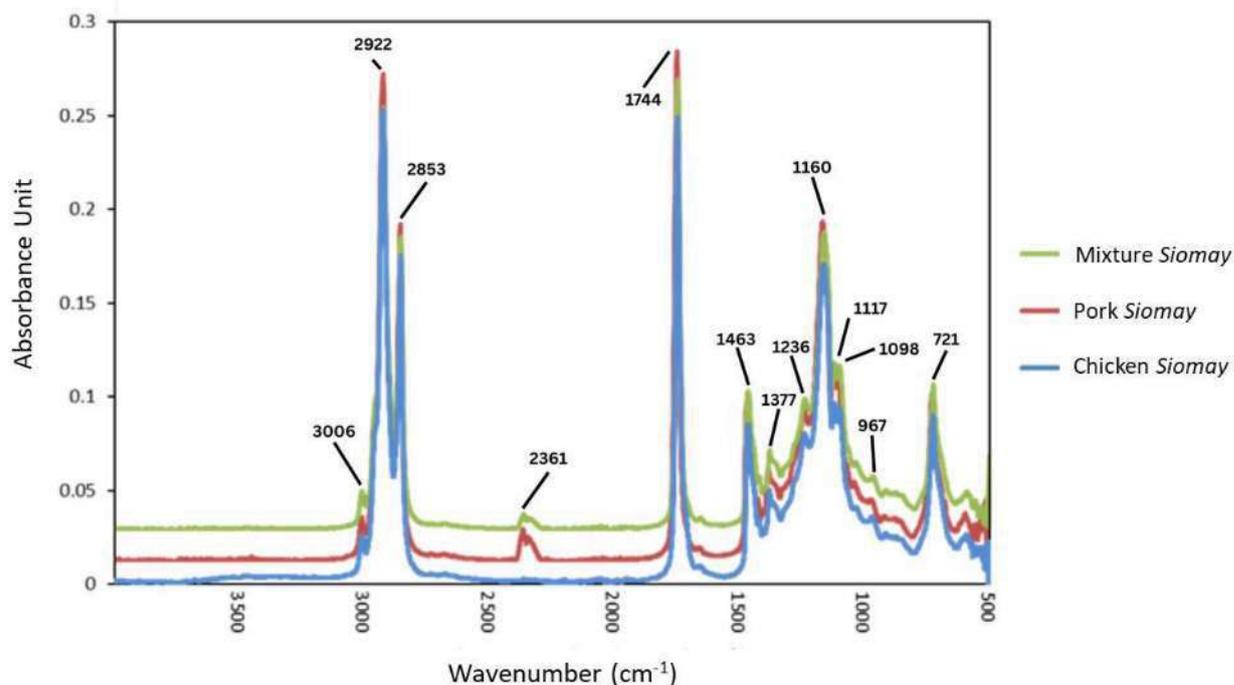
Figure 1. FTIR Spectral profile of chicken *siomay*, pork *siomay*, and their mixture.

Siomay made from 100% pork exhibited a major peak at 1744 cm^{-1}, related to stretching vibrations with both symmetric and asymmetric modes from the carbonyl (C=O) group, as well as 1160 cm^{-1} a peak at 1160 cm^{-1}, corresponding to bending and stretching vibrations of the -C-O and –CH$_2$- groups in fatty acids[14]. The absorbance intensities at 2922 and 1744 cm^{-1} indicated the presence of aliphatic chains and ester groups in triglycerides [18], and the areas at these frequencies were optimized for analyzing fatty acids in chicken *siomay* using multivariate calibration [19]. Samples containing pork fat exhibited a characteristic peak at 2361 cm^{-1}, indicating the presence of C-O ester functional groups typical of pork fat triglycerides, aligning with previous findings that pork content can be identified by a peak around 2300 cm^{-1} [15].

PCA-Based Data Analysis

PCA is a method used to identify the most significant variables in structuring data [20]. The data were obtained from samples using an FTIR spectrophotometer over the range of 4000 to 500 cm^{-1} at a resolution of 4 cm^{-1}. Thirteen main peaks were successfully identified, and the resulting data were used to construct the PCA model. The results were presented as Scree Plot, Variable Plot, and Individual Plot from the PCA model (Figure 2) to analyze the principal components. The

Scree Plot was used for visual analysis to show the proportion of variation explained by each component, the Variable Plot illustrated which variables contributed to the principal components through the angles between vectors, and the Individual Plot displayed the positions of individual data points in two dimensions for the first two components [21]. The individual plot show the separation of fatty acids from pure chicken *siomay*, chicken *siomay* mixed with pork, and pork-based *siomay*. The two-dimensional PCA model accounted for 92.9% of the total variance, with Dimension 1 accounting for 82.8% and Dimension 2 accounting for 10.1%. The plot variable illustrates the contribution of each variable and its corresponding load. Absorbance at 1744 cm^{-1} was the most important variable in the PCA model.

Sample Discrimination via PLS-DA

PLS-DA is a discrimination modeling technique that incorporates a validation process to evaluate predictive results [12].
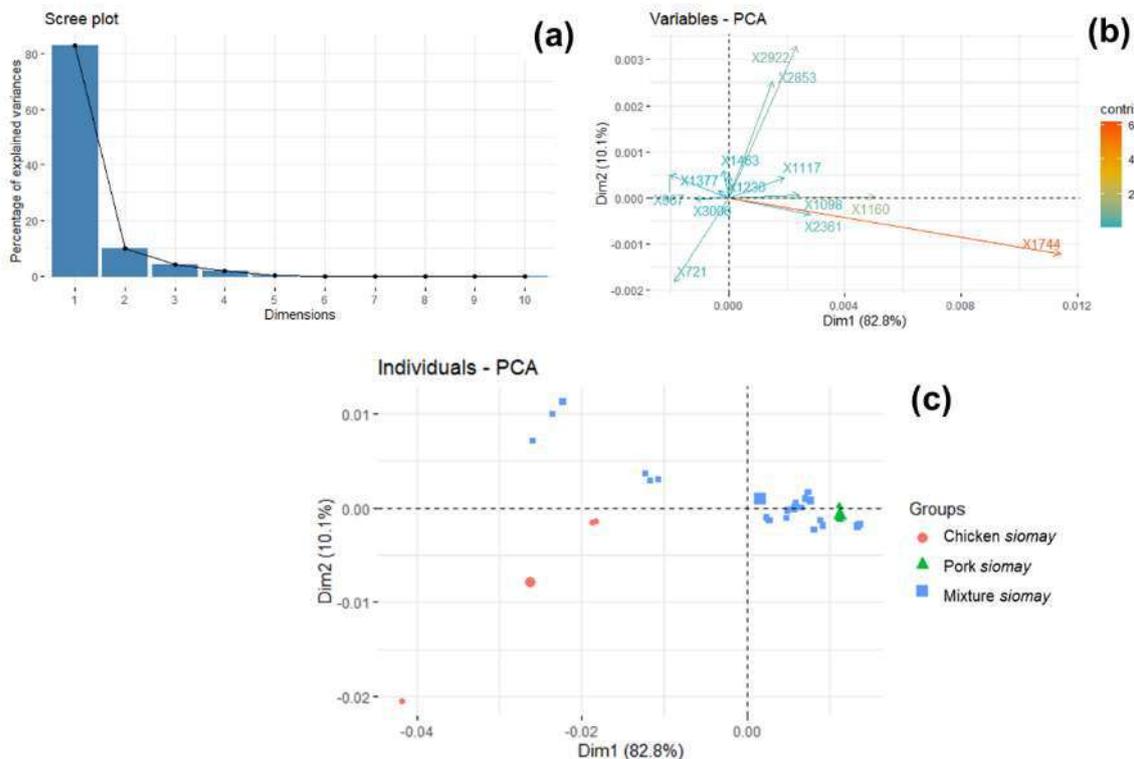

Figure 2. Principal component analysis of the original spectrum with the selected wavenumber used to generate data (a). Scree plot, (b). Variable plot, dan (c). Individual plot.
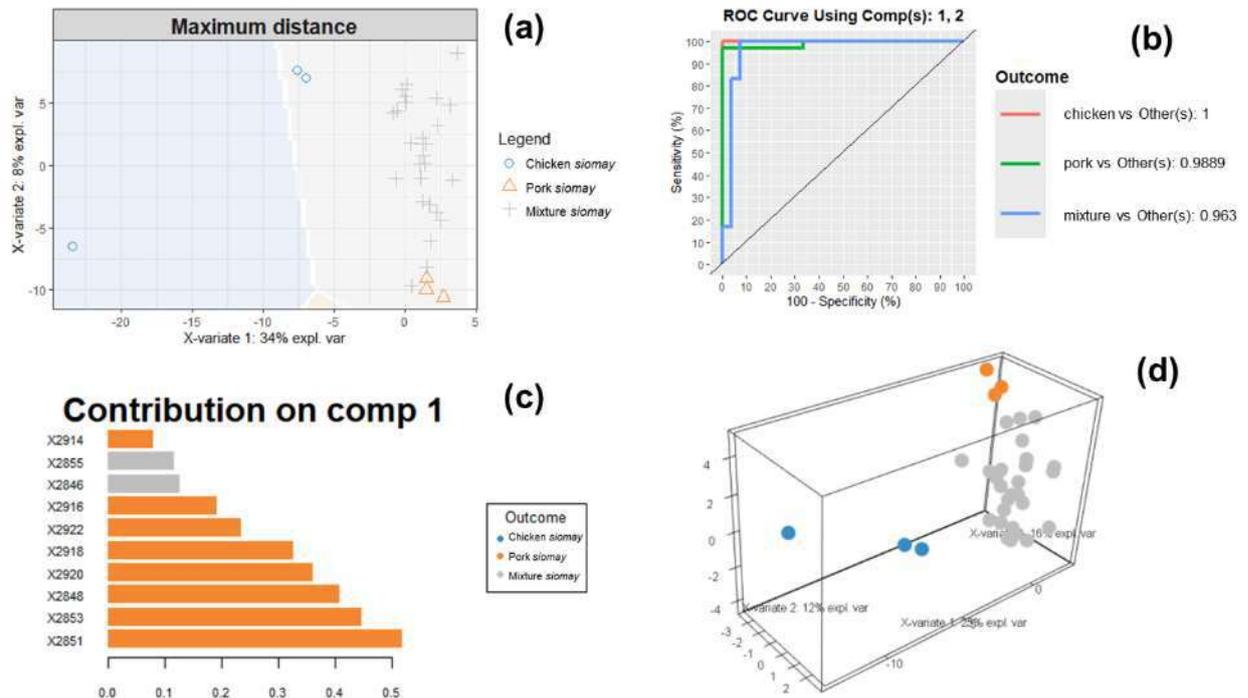
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Figure 3. PLS-DA processing for the halal analysis of chicken *siomay* against pork adulteration included background prediction plot (a), AUC-ROC plot (b), contribution plot on the first component (c), and 3D individual plot (d)

This technique is used to predict a model, which is evaluated based on the AUC-ROC value approaching or equal to 1. The results (Figure 3) were presented as background plot, AUC-ROC plot, contribution plot, and 3D individual plot [22]. The background prediction plot visualizes the discrimination markers for each group. Spectral data were analyzed based on Area Under the Curve-Receiver Operating Characteristics (AUC-ROC). A satisfactory AUC-ROC value approaches or is equal to 1, indicating a better model in discriminating between chicken and pork fatty acids. For chicken fatty acids, pork fatty acids, and the mixture, the AUC-ROC were 1.000, 0.9889, and 0.963, respectively, indicating that the PLS-DA model has a 100%, 98.9%, and 96.3% probability of correctly distinguishing chicken fatty acids, pork fatty acids, and their mixtures from other groups. Absorbance at 2846 and 2851 cm^{-1} were the variables that contributed most strongly to the formation of the PLS-DA model. The variables with the highest contributions originated from narrow bands above 2800 cm^{-1} corresponding to stretching vibrations. The method was successfully developed for *siomay*;

however, it remains limited to the identification of functional groups. In this study, the authors developed a rapid, non-destructive, and solvent-saving halal authentication method for *siomay* products using FTIR spectroscopy combined with chemometrics. Other previous studies using chromatographic and DNA-based techniques generally require longer analysis times, costly reagents, and more complex sample preparation[23−25]. Further identification techniques are required, such as Liquid Chromatography-High-Resolution Mass Spectrometry (LC-HRMS). This method is capable of identifying metabolites present in the sample material[26].

CONCLUSION

A study on the halal authentication of chicken siomay adulterated with pork using FTIR spectroscopy combined with chemometrics has been successfully conducted. PCA was successfully implemented as an exploratory data analysis. It was found that the wavenumber at 1744 cm-1 contributed most to building the PCA model. Computational discrimination using PLS-DA successfully distinguished chicken fatty acids, pork fatty acids, and their binary mixture, with AUC-ROC values of 1, 0.9889, and 0.9630, respectively.

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Conflict of interest

The authors declare that they have no conflict of interest.

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