The use of FTIR spectroscopy in combination with chemometrics for the authentication of milk fat from palm oil

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Abstract. This research was aimed to develop Fourier transform infrared spectroscopy (FTIR) combined with chemometrics of linear discriminant analysis (LDA), partial least square (PLS), and principal component regression (PCR) for authentication of milk fat from palm oil adulterant. FTIR spectroscopy and LDA have been successfully used to detect the presence of palm oil in MF. All the adulterated samples were clearly separated with authentic MF shown by the Cooman’s plot. Chemometrics of PLS at the wavenumber of 3033-692 cm\textsuperscript{-1} using first derivative spectra was successfully applied for the quantification of palm oil in MF. The suitability of the model was presented by its high R\textsuperscript{2} value both for calibration and validation models, accounting for 1 and 0.9998 respectively and its lower RMSEC (root mean square error of calibration) and RMSEP (root mean square error of prediction) value, accounting for 0.154 and 0.743 respectively. Quantification of palm oil was also successfully performed using chemometrics of PCR. The model showed high R\textsuperscript{2} in both calibration (0.9998) and validation (0.9997) values with lower RMSEC (0.671) and RMSEP (0.905) values. It can be concluded that a combination of FTIR spectroscopy with chemometrics could be used for the authentication of milk fat adulteration.

1. Introduction
Milk fat is one of the high-quality fats which have many applications in food products. It has various compositions of nutrition which are important for human health. It contains essential fatty acids and rich in fat-soluble vitamins including vitamins A, D, E, and K [1]. Moreover, it also provides proteins, minerals, lactose, and triglycerides [2]. Milk fat is considered as the most valuable fat among other fats and it has a higher price compared to other fats and oils. Unethical sellers may take advantage by mixing it with other fats or oils having a lower price for economic reasons. This fraudulent practice is a serious problem, therefore, an analytical method capable of ensuring quality toward the authenticity of milk fat is highly required [3].

Several methods have been developed for fats analysis including milk fat either conventional method or advanced method using certain instruments. Analysis of fatty acid composition in milk fat has been performed using gas chromatography using a detector of flame ionization detector (FID) and
mass spectrometer detector (MS) [4,5]. High-performance liquid chromatography has also been explored for milk fat analysis by determining the triglycerides composition [6]. These methods offer reliable and reproducible analytical methods for milk fat authentication. However, there are some disadvantages of using them for instance, they require a long time, need several steps of sample preparation, and require many solvents. Therefore, a rapid and reliable detection method such as FTIR spectroscopy is important to be investigated.

FTIR spectroscopy is a fingerprint technique that offers several advantages such as rapid analysis [7], it does not require sample preparation meaning that samples can be directly measured using attenuated total reflectance (ATR) technique by directly placing the samples on ATR crystal, it requires less solvent (considered as green analytical chemistry), and it is easy to be coupled with advanced statistical techniques such as chemometrics of multivariate analysis [8,9]. Chemometrics has been widely used for food and pharmaceutical product authentication. Chemometrics is divided into two categories, namely chemometrics of pattern recognition such as discriminant analysis (DA) and principal component analysis (PCA) and chemometrics of multivariate calibration or regression such as multiple linear regression (MLR), partial least square (PLS), and principal component regression (PCR) [10].

FTIR spectroscopy has wide applications in food analysis including milk, butter, cheese, fat, and oil [11,12]. For instance, FTIR spectroscopy using attenuated total reflectance (ATR) technique has been used for the detection and quantification of urea in milk. Combination with chemometrics of SIMCA (soft independent modeling class analogy) perfectly classified between milk samples and urea [13]. Quality analysis of raw milk has been carried out using FTIR spectroscopy by determining some parameters such as fat protein, lactose, and total solids. Based on the results, it showed that FTIR is a fast and reliable analytical technique for authentication of raw milk [14]. The objective of this research was to develop FTIR spectroscopy coupled with chemometrics of multivariate analysis for the authentication of milk fat from palm oil.

2. Materials and methods

2.1. Materials

Three commercial pure milk fat samples were obtained from different markets, namely in Bantul, Sleman, and Yogyakarta. The pure palm oil used was purchased from a market in Yogyakarta. The milk fat samples were high-quality food-grade while the palm oil used was commercially produced by industry. There were 42 adulterated samples and they were prepared manually in binary mixtures of milk fat and palm oil. Acetone and n-hexane were purchased from Mercks (Germany).

2.2. Samples preparation

A pure sample of milk fat and palm oil was prepared in a glass vial. Adulterated samples of milk fat with palm oil were prepared by making binary mixtures of samples with various adulterant concentrations in the range of 0-100% w/w with a total weight of 2 grams for each sample.

2.3. FTIR measurement

Each sample was measured using FTIR spectroscopy (Thermo Nicolet iS10) using ATR (attenuated total reflectance) technique. The acquisition of spectra was performed in the wavenumber region of 4000-650 cm⁻¹ using a resolution of 8 cm⁻¹ and a scan number of 32. Background spectra were carried out prior to each sample measurement.

2.4. Chemometrics analysis

Chemometrics pattern recognition was carried out using discriminant analysis (DA). The variables used for creating the DA model were absorbance in the region of 1500-900 cm⁻¹. DA result was evaluated using the Cooman plot. Chemometrics multivariate calibration for quantitative analysis was carried out using partial least square (PLS) and principal component regression (PCR) techniques.
Samples were divided into calibration and validation models. Wavenumber optimization was performed to obtain the best calibration and validation model in the range of 4000-650 cm\(^{-1}\). The spectra were also derivatized using Savitzky-Golay either the first or second derivative. The models were chosen by evaluating the coefficient of determination (R\(^2\)) and root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP).

3. Results and discussion

3.1. FTIR spectra analysis

FTIR spectroscopy is a fingerprint technique which means that no samples are having identical FTIR spectra. Figure 1 shows the FTIR spectra of pure milk fat and pure PO. General investigation through the spectra showed that the spectra were very similar because the major component in fat and oil is fatty acids [7]. Further investigation revealed some differences in several peaks especially in the fingerprint region (1500-900 cm\(^{-1}\)). The functional groups of milk fat and palm oil observed using FTIR spectroscopy are shown in table 1. Even though deep investigation could differentiate between milk fat and palm oil spectra, however, in adulterated samples of milk fat with PO, it is very difficult to differentiate between authentic milk fat spectra and adulterated milk fat spectra. Therefore, an advanced statistical method such as chemometrics was performed to detect and quantify the adulterant (PO) in milk fat.

![Fig 1](image1.png)

**Figure 1.** FTIR spectra of authentic milk fat and palm oil in the wavenumber region of 4000-650 cm\(^{-1}\).

3.2. Chemometrics analysis

Chemometrics analysis was successfully used for managing the huge data extracted from FTIR spectra. The absorbance values were used for variables in chemometrics models because they are directly associated with concentration. Chemometrics of discriminant analysis perfectly classified between pure milk fat sample and adulterated milk fat with palm oil. All the adulterated samples were correctly separated from a pure sample of milk fat as can be seen in the Cooman plot (figure 2).
Discriminant analysis is one of the supervised pattern recognitions in the chemometrics technique which is widely used in sample differentiation. The x-axis showed the Mahalanobis distance which demonstrated the distance among samples. Samples containing a higher level of adulterants showed higher Mahalanobis distance from pure milk fat samples indicating a greater difference between them.

**Table 1.** Functional groups identification of FTIR spectra observed in milk fat and palm oil [15].

| Wavenumber region (cm$^{-1}$) | Functional groups and vibration modes |
|-----------------------------|--------------------------------------|
| 3005                        | =CH, stretching vibration             |
| 2926 and 2853               | -CH$_3$, -CH$_2$, and -CH asymmetric, and symmetric stretching vibrations |
| 1740                        | carbonyl (C=O), stretching vibration  |
| 1463                        | methylene (-CH$_2$), bending vibration |
| 1412, 1375, 1235, 1161      | bending vibration (wagging, twisting, scissoring) of -CH$_3$, -CH$_2$, and CH |
| 1116                        | C-O, stretching vibrations            |
| 960                         | –HC=CH-(trans), bending out of the plane |
| 715                         | –HC=CH-(cis), bending out of the plane |

![Figure 2](image.png)

**Figure 2.** Discriminant analysis of milk fat adulterated with palm oil.

Chemometrics of multivariate calibration namely partial least square and principal component regression were investigated for palm oil quantification in milk fat. The values of coefficient of determination ($R^2$) both in calibration and validation models were used for model evaluation because they are related to the model accuracy. Moreover, the root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) were used to evaluate the model error. A model with a high value of $R^2$ and low value of RMSEC and RMSEP was chosen for analysis because it showed good accuracy, precision, and predictability [10,16]. PLS model was successfully applied using the first derivative FTIR spectra at the wavenumber region of 3033–692 cm$^{-1}$ (figure 3). Spectra derivatization could provide better resolution in overlapping spectra so that it generated a good dataset for creating a model. The obtained $R^2$ and RMSEC values of the PLS calibration model were 1 and 0.154, respectively. The model is then validated using an external validation technique and the $R^2$ and RMSEP of the validation model were evaluated. Validation is important to be carried out to evaluate the validity of the PLS calibration model and to avoid model overpredicting. The result of the validation model demonstrated a high $R^2$ value (0.9998) and a low RMSEP value (0.743).
Another chemometrics model for quantification, namely PCR also demonstrated good accuracy and precision model for milk fat authentication. PCR was successfully used for palm oil quantification in binary mixtures with milk fat. PCR model was created using the first derivative spectra at the wavenumber combination of 3033-2770 cm\(^{-1}\) and 1806-692 cm\(^{-1}\). The model had a high value of \(R^2\) either in calibration (0.9998) or validation (0.9997). The obtained RMSEC and RMSEP values were 0.671 and 0.905, respectively. The difference between PLS and PCR models is in their algorithm for creating a model. PLS search for latent variables to create a calibration model while PCR created principal components (PCs) then directly used them to build a regression model. From the above results, it is suggested that FTIR spectroscopy and chemometrics analysis offer potential advantages for milk fat authentication.

4. Conclusion

FTIR spectroscopy is a fingerprint technique that can be used for the authentication of milk fat. Combination with chemometrics of multivariate analysis such as linear discriminant analysis, partial least square, and principal component regression offered a rapid and reliable analytical method for adulterant detection and quantification in milk fat. It can be concluded that the combination of FTIR spectroscopy and chemometrics is a promising method for future authentication of fats and oils.

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