Adulteration of Gabus (Channa striata) fish oil with corn oil and palm oil: the use of FTIR spectra and chemometrics

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Abstract

The adulteration practice in fats and oils industry can be in the form of addition or substitution high quality oils such as Gabus Fish oil (GFO) with lower price oils. This research highlighted the application of FTIR spectroscopy combined with multivariate calibrations and discriminant analysis (DA) for quantitative analysis and classification of oil adulterants of palm oil (PO) and corn oil (CO) in GFO. The methods involved preparation of training/calibration and validation samples, scanning of samples using FTIR spectrophotometer using attenuated total reflectance, development of calibration and validation models for quantitative analysis of oil adulterants assisted with multivariate calibrations and classification between genuine GFO and GFO adulterated with PO and CO using discriminant analysis. The quantitative analysis of PO in ternary mixtures with CO as an adulterant in GFO was carried out using first derivative spectra at wavenumbers of 3200-600 cm\(^{-1}\) assisted with partial least square (PLS), while quantitative analysis of CO in ternary mixture with PO and GFO was performed using first derivative spectra at wavenumbers region of 3200-2700 cm\(^{-1}\) assisted with principle component regression (PCR). The \(R^2\) values for the correlation between actual and predicted values of PO and CO in ternary mixtures either in calibration or prediction samples were of > 0.97 with low errors. In addition, DA using the same wavenumbers region as used in the quantitative analysis could classify or discriminate genuine GFO and GFO mixed/adulterated with PO and CO with an accuracy level of 100%. FTIR spectroscopy using suitable wavenumbers region combined with PLS, PCR and DA could be proposed as analytical tools for quantification and classification of oil adulterants in GFO.

1. Introduction

The adulteration of food including fats and oils is an emerging issue recently which is motivated by economic reasons to get the profits. This practice involves the addition or substitution of high price oils with lower ones (Baeten and Aparicio, 2000). Gabus fish oil (GFO) is one of the functional oils having some biological activities due to the contents of omega-fatty acids present in GFO. Gabus fish is indigenous in the tropical and subtropical countries including Indonesia and Malaysia (Ikasari et al., 2020). The price of GFO is approximately 10 times higher than some common vegetable oils like corn oil (CO) and palm oil (PO), therefore, GFO may be adulterated by CO and PO. The addition of foreign oils into genuine oils may take the health-related risks such as an allergy to certain people (Rohman et al., 2019), as a consequence, some analytical methods are developed and validated by chemists and food scientists to detect the adulteration of oils, either based on chemical or molecular biology methods intended to quality control and assure the safety of fats and oils (Li et al., 2019).

Due to its sensitivity, instrumental methods from simple instruments into sophisticated ones have been reported for authentication of high price oils including two-dimensional gas chromatography combined with time of flight mass spectrometer (Indrasti et al., 2010), nuclear magnetic resonance spectroscopy (Standal et al., 2010), liquid chromatography combined with mass spectrometer (Zeng et al., 2010), differential scanning calorimetry (Miklos et al., 2013), electronic nose or fast gas chromatography (Man et al., 2005), Raman spectroscopy (Gómez-Caravaca et al., 2016) and other
spectroscopic methods. Chromatographic-based methods are specific; however, these methods require skillful analyst and extensive sample preparation, therefore, spectroscopic methods such as Fourier transform infrared (FTIR) spectroscopy is continuously developed for rapid screening and detection of the adulteration practice of fats and oils.

Combined with multivariate analysis or chemometrics, FTIR spectroscopy is widely applied for authentication of edible oils in binary mixtures including the authentication of olive oil with some vegetable oils (Lerma-García et al., 2010), authentication of Patin fish oil (Putri et al., 2019), detection of adulteration in red fruit oil (Rohman et al., 2014), analysis of sesame adulterated with corn oil (Fadzlillah et al., 2014), and authentication of virgin coconut oil with canola oil (Man and Rohman, 2013). Most of these applications are related to binary mixtures, and study on the application of FTIR spectroscopy combined with chemometrics for authentication of high price oils in ternary mixtures is limited. This study highlighted the application of FTIR spectroscopy combined with chemometrics for the simultaneous analysis of palm oil (PO) and corn oil (CO) in ternary mixtures with Gabus fish oil (GFO) intended for authentication of GFO from PO and CO.

2. Materials and methods

Gabus fish oil (GFO), corn oil (CO) and palm oil (PO) were obtained from the local market in Yogyakarta, Indonesia. To assure that the used oils are not previously added or substituted with other oils, the fatty acid compositions were determined using gas chromatography using flame ionization detector (FID), and the results revealed that the used oils are authentic. Some solvents and reagents were of pro-analytical grade.

2.1 The calibration and validation samples

The calibration samples were prepared by taking an appropriate sample (GFO, CO and PO) to form the ternary mixtures with a total percentage was 100% with total samples of 30. For example, the composition of sample 1 is GFO: PO (14: 10: 76 vol/vol); sample 2: (45: 10: 45 vol/vol); sample 3: (7: 54: 39 vol/vol); sample 4: (56: 24: 20 vol/vol); sample 5: (46: 14: 40 vol/vol); sample 29: (38: 7: 55 vol/vol); and sample 30: (41: 52: 7 vol/vol). Then, the validation samples were independently prepared with the same manner to calibration samples spanning concentrations of 0-100%. The selection of these numbers was performed randomly with the aid of Excel (Microsoft Inc., USA). All samples were subjected to FTIR measurement.

2.2 FTIR spectroscopy measurement

The measurement of attenuated total reflectance-FTIR spectra was carried out using Thermo Scientific iS10 spectrophotometer (Thermo Fisher Scientific, USA) equipped with detector of DTGS (deuterated triglycerine sulphate) and beam splitter of KBr/Germanium according to Putri et al. (2019). All samples were measured in the wavenumber region of 4000-650 cm\(^{-1}\). The number of scanning was 32 for each sample measurement with the resolution of 8 cm\(^{-1}\). Each sample was measured in three replicates as absorbance mode to follow Lambert-Beer law. Background spectra were measured before each sample measurement. The ATR crystal was cleaned using n-hexane two times and acetone after each measurement.

2.3 Quantification of PO and CO in ternary mixtures

The quantitative analysis of PO and CO as adulterants in GFO in ternary mixtures was facilitated by the multivariate calibrations of partial least square (PLS) and principle component regression (PCR). The selection of PLS and PCR was based on the capability to predict the prediction levels close to actual levels. Besides, the wavenumbers regions which provide the best statistical parameters namely highest $R^2$ and lowest root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) was also optimized.

2.4 Discriminant analysis

The discrimination between genuine Gabus Fish Oil (GFO) and GFO adulterated with palm oil (PO) and corn oil (CO) was carried out using discriminant analysis (DA) using variables of absorbance values at selected wavenumbers. The selection was based on the capability of variables capable of discriminating both classes with the highest accuracy level.

2.5 Data analysis

Data analysis including multivariate calibrations (PLS and PCR) as well as the discriminant analysis was performed using TQ Analyst® software included in FTIR spectrophotometer. The studied sample spectra were introduced into software and then some statistical parameters were automatically calculated.

3. Results and discussion

FTIR spectra of studied oils, namely Gabus Fish Oil (GFO) along with adulterant oils of palm oil (PO) and corn oil (CO) were scanned in wavenumber region of 4000-650 cm\(^{-1}\). The selection of PO and CO as oil adulterants was based on two reasons, firstly is the fact that the price of PO and CO is much lower than that of
PO and CO and secondly is based on the similarity of PO and CO to GFO in the score plot of first principle component and second principle component during principal component analysis (PCA) (Rohman et al., 2011). Each shoulders and peaks in these spectra corresponded to functional groups composed of GPO, PO and CO namely triacylglycerols. Therefore, some functional groups such as methyl-, methylene-, carbonyl, and ether groups dominate (Fadzillah et al., 2017).

Peaks at 3007 cm\(^{-1}\) was coming from \([\text{cis} -\text{C} = \text{H} \ (\text{stretching})]\), 2953 cm\(^{-1}\) \([-\text{CH}_3, \ \text{asymmetric stretching}]\), 2922 cm\(^{-1}\) \([-\text{CH}_2, \ \text{asymmetric stretching}]\), 2852 cm\(^{-1}\) \([-\text{CH}_2, \ \text{symmetric stretching}]\), 1744 cm\(^{-1}\) \([-\text{C}=\text{O} \ (\text{ester}), \ \text{stretching}]\), 1652 cm\(^{-1}\) \([\text{cis} -\text{C}=\text{C}, \ \text{stretching}]\), 1463 cm\(^{-1}\) \([-\text{CH}_2, \ \text{bending}]\), 1377 cm\(^{-1}\) \([-\text{CH}_3, \ \text{bending}]\), 1238, 1117, 1163, 1098, 1031 cm\(^{-1}\) \([-\text{C}=\text{O}, \ \text{stretching}]\), 966 cm\(^{-1}\) \([-\text{trans} \ \text{HC}=\text{CH} \ -\text{out of plane}]\), 913 cm\(^{-1}\) \([-\text{cis} \ \text{HC}=\text{CH} \ -\text{out of plane}]\), and peak at 722 cm\(^{-1}\) corresponded to \([\text{cis} -\text{HC}=\text{CH}]\); \([\text{cis} -\text{HC}=\text{CH}]\) (Che Man et al., 2011). By detailed investigation, three oils exhibited a bit difference in terms of peak intensity (absorbance values), therefore, some optimization was carried out to select some wavenumbers during quantification of PO and CO in GFO as well as during classification between GFO and GFO adulterated with PO and CO.

Table 1 and Table 2 reveal the optimization of multivariate calibrations of partial least square regression (PLS) and principle component regression (PCR) as well as wavenumbers region for quantitative analysis of PO in ternary mixtures with CO and GFO. Based on the capability for providing the highest values of coefficient of determination (R\(^2\)) and the lowest values of root mean square error of calibration (RMSEC) and prediction (RMSEP), the first derivative FTIR spectra at wavenumbers region of 3200-600 cm\(^{-1}\) combined with PLS was selected for quantification of PO in ternary mixtures with CO and GFO. Derivative spectra could resolve the overlapping peaks; therefore, they could increase the model performance. However, derivative spectra could decrease sensitivity. In this condition, R\(^2\) calibration, RMSEC, R\(^2\) prediction, and RMSEP were of 0.9972, 0.014%, 0.9818 and 0.036%, respectively (Table 1). In addition, CO in ternary mixtures with PO and GFO was determined using PCR using variables of absorbance values of first derivative spectra 3200-2700 cm\(^{-1}\). In this condition, R\(^2\) calibration, RMSEC, R\(^2\) prediction, and RMSEP were of 0.9731, 0.050%, 0.9703 and 0.051%, respectively (Table 1). Figure 1 revealed the correlation between actual and predicted values of oil adulterants of PO [A] and CO [B] ternary mixtures with GFO. The errors occurring during modelling were randomly distributed along the correlation line between actual values of PO and CO and predicted values, therefore, the errors were not systematic (Figure 2). The high values of R\(^2\) either in calibration and validation and low values of RMSEC and RMSEP indicated that FTIR spectroscopy and multivariate calibrations (PLS and PCR) offered the accurate and precise results for quantification of PO and CO in ternary mixtures with GFO (Miller and Miller, 2010).

Figure 3 reveals linear discriminant analysis (LDA) for the classification of GFO and GFO adulterated with...
Table 1. The optimization of multivariate calibrations of partial least square regression (PLSR) and principle component regression (PCR) as well as wavenumbers region for quantitative analysis of palm oil as adulterant in corn oil in ternary mixtures with Gabus Fish Oil (GFO)*

| Multivariate calibrations | Wavenumber (cm\(^{-1}\)) | Spectra          | Calibration          | Validation          |
|---------------------------|---------------------------|-------------------|----------------------|---------------------|
|                           |                           |                   | R\(^2\) | RMSEC | R\(^2\) | RMSEP |
| **PLS**                   | 3200-660                  | normal            | 0.9899 | 0.027 | 0.9828 | 0.035 |
|                           |                           | derivative 1      | 0.9972 | 0.014 | 0.9818 | 0.036 |
|                           |                           | derivative 2      | 0.9964 | 0.016 | 0.9806 | 0.037 |
|                           | 1850-660                  | normal            | 0.9701 | 0.046 | 0.9482 | 0.063 |
|                           |                           | derivative 1      | 0.9963 | 0.016 | 0.9805 | 0.038 |
|                           |                           | derivative 2      | 0.9932 | 0.022 | 0.9770 | 0.040 |
|                           | 3200-2700                 | normal            | 0.9816 | 0.036 | 0.9844 | 0.034 |
|                           |                           | derivative 1      | 0.9889 | 0.028 | 0.9839 | 0.034 |
|                           |                           | derivative 2      | 0.9831 | 0.034 | 0.9826 | 0.035 |
|                           | 3200-2700 and 1510-660    | derivative 1      | 0.9930 | 0.022 | 0.9827 | 0.035 |
|                           |                           | derivative 2      | 0.9930 | 0.022 | 0.9856 | 0.032 |
|                           | 3200-660                  | normal            | 0.9906 | 0.026 | 0.9855 | 0.032 |
|                           |                           | derivative 1      | 0.9877 | 0.029 | 0.9865 | 0.031 |
|                           |                           | derivative 2      | 0.9857 | 0.032 | 0.9849 | 0.033 |
|                           | 1850-660                  | normal            | 0.9896 | 0.027 | 0.9861 | 0.032 |
|                           |                           | derivative 1      | 0.9887 | 0.028 | 0.9861 | 0.031 |
|                           |                           | derivative 2      | 0.9883 | 0.029 | 0.9843 | 0.033 |
|                           | 3200-2700                 | normal            | 0.9816 | 0.036 | 0.9804 | 0.039 |
|                           |                           | derivative 1      | 0.9839 | 0.034 | 0.9855 | 0.032 |
|                           |                           | derivative 2      | 0.9820 | 0.036 | 0.9813 | 0.037 |
|                           | 3200-2700 and 1510-660    | derivative 1      | 0.9999 | 0.027 | 0.9823 | 0.036 |
|                           |                           | derivative 2      | 0.9902 | 0.026 | 0.9850 | 0.033 |

The selection condition was marked with italicized bold

Table 2. The optimization of multivariate calibrations of partial least square regression (PLSR) and principle component regression (PCR) as well as wavenumbers region for quantitative analysis of corn oil as adulterant in palm oil in ternary mixtures with Gabus Fish Oil (GFO)*

| Multivariate calibrations | Wavenumber (cm\(^{-1}\)) | Spectra          | Calibration          | Validation          |
|---------------------------|---------------------------|-------------------|----------------------|---------------------|
|                           |                           |                   | R\(^2\) | RMSEC | R\(^2\) | RMSEP |
|                           |                           |                   | R\(^2\) | RMSEC | R\(^2\) | RMSEP |
| **PLS**                   | 3200-660                  | normal            | 0.9716 | 0.051 | 0.9517 | 0.065 |
|                           |                           | derivative 1      | 0.9688 | 0.053 | 0.9707 | 0.051 |
|                           |                           | derivative 2      | 0.9706 | 0.052 | 0.9707 | 0.051 |
|                           | 1850-660                  | normal            | 0.9663 | 0.055 | 0.9602 | 0.059 |
|                           |                           | derivative 1      | 0.9645 | 0.057 | 0.9651 | 0.056 |
|                           |                           | derivative 2      | 0.9645 | 0.057 | 0.9667 | 0.054 |
|                           | 3200-2700                 | normal            | 0.9719 | 0.051 | 0.9666 | 0.056 |
|                           |                           | derivative 1      | 0.9735 | 0.049 | 0.9617 | 0.059 |
|                           |                           | derivative 2      | 0.9755 | 0.047 | 0.9609 | 0.060 |
|                           | 3200-2700 and 1510-660    | derivative 1      | 0.9673 | 0.055 | 0.9690 | 0.052 |
|                           |                           | derivative 2      | 0.9703 | 0.052 | 0.9715 | 0.050 |
|                           | 3200-660                  | normal            | 0.9669 | 0.055 | 0.9630 | 0.057 |
|                           |                           | derivative 1      | 0.9684 | 0.054 | 0.9710 | 0.051 |
|                           |                           | derivative 2      | 0.9682 | 0.054 | 0.9705 | 0.052 |
|                           | 1850-660                  | normal            | 0.9658 | 0.056 | 0.9518 | 0.065 |
|                           |                           | derivative 1      | 0.9639 | 0.057 | 0.9672 | 0.054 |
|                           |                           | derivative 2      | 0.9634 | 0.058 | 0.9709 | 0.051 |
|                           | 3200-2700                 | normal            | 0.9693 | 0.053 | 0.9479 | 0.068 |
|                           |                           | derivative 1      | 0.9731 | 0.050 | 0.9703 | 0.051 |
|                           |                           | derivative 2      | 0.9705 | 0.052 | 0.9715 | 0.052 |
|                           | 3200-2700 and 1510-660    | derivative 1      | 0.9665 | 0.055 | 0.9639 | 0.056 |
|                           |                           | derivative 2      | 0.9672 | 0.055 | 0.9737 | 0.048 |

The selection condition was marked with italicized bold

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PO and CO based on Coomans plot. LDA is one of the supervised pattern recognition techniques intended to classify/discriminate among objects. Compared with partial least square-discriminant analysis (PLS-DA), LDA is simpler in terms of the algorithm used. Using absorbance values at whole wavenumber regions (4000-650 cm\(^{-1}\)), both groups could be classified according to its classes with an accuracy level of 100%, indicating that LDA is successful for classification. The misclassification may occur if the selected variables are not appropriate which resulted in the lower accuracy levels of classification (Marina et al., 2009).

4. Conclusion

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy in combination with multivariate calibrations of PLS and PCR as well as discriminant analysis has been successfully used for quantification and classification of oil adulterants, namely palm oil (PO) and corn oil (CO) in Gabus Fish Oil (GFO). The quantification of PO was performed using variables of absorbance values of first derivative spectra at 3200-600 cm\(^{-1}\) while quantification of CO was performed using first derivative spectra at 3200-2700 cm\(^{-1}\). In addition, discriminant analysis using normal FTIR spectra at 4000-650 cm\(^{-1}\) could discriminate genuine GFO and GFO adulterated with PO and CO with an accuracy level of 100%.

Conflict of interest

The authors declare no conflict of interest.
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