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Implementation of Fourier Transform Infrared Spectroscopy Combined with Chemometrics for the Authentication of Patin (*Pangasius micronema*) Fish Oil Emulsion

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ABSTRACT

Patin fish oil (PFO) contains a high level of polyunsaturated fatty acid that has beneficial effects on the human body, such as preventing various cardiovascular diseases, maintaining body fat composition, and aiding in human brain development. PFO can be developed as patin fish oil emulsion (PFOE) and used as a dietary supplement. Given its beneficial value, PFOE is at risk of being adulterated with low-quality oils. Therefore, authentication is crucial to guarantee its quality and safety. However, authenticating PFOE is difficult because its physical appearances are masked by the emulsion component. This study aims to authenticate PFOE using Fourier transform infrared (FTIR) spectroscopy combined with chemometrics. Adulteration models were prepared using palm oil (PO) as an adulterant. All samples were analyzed using ATR-FTIR spectroscopy at 4000–650 cm^{-1}. Chemometric techniques such as discriminant analysis (DA), partial least square regression, and principal component regression (PCR) were adopted for quantitative analysis. Results showed that DA successfully discriminated PFOE from the adulterant. PCR within the normal spectrum of 1004–2936 cm^{-1} produced the best values of 0,9846 highest R^2_{cal}, 0,9073 R^2_{pred}, 0,0565 lowest root mean square error of calibration, and 0,1330 root mean square error of prediction. Therefore, FTIR spectroscopy combined with chemometrics is a rapid, accurate, and suitable method for distinguishing pure PFOE from PO adulterant.

Keywords: adulteration, fish oil emulsion, chemometrics, principal component regression, discriminant analysis

INTRODUCTION

Patin fish oil (PFO) contains a high level of polyunsaturated fatty acid (Sugata *et al*., 2019), which can alleviate various cardiovascular diseases (Fernandez *et al*., 2021; Golanski *et al*., 2021; Wang *et al*., 2021), maintain body fat composition (Monnard & Dulloo, 2021), and aid in brain development (Ramaswami *et al*., 2016; Wen *et al*., 2021). Long-chain omega 3 fatty acids which can be found in PFO have been accepted as food supplements (Singh *et al*., 2022). These beneficial effects render PFO as a potential food supplement, particularly when prepared in patin fish oil emulsion (PFOE) form to decrease the fishy odor and taste. Moreover, lipid oxidation of polyunsaturated fatty acid especially omega 3 fatty acid has been successfully reduced using several polymers in emulsion form compared with

unwrapped fish oil (S. X. Liu *et al*., 2015; Padial-Domínguez *et al*., 2020; Yesiltas *et al*., 2021).

Owing to its benefits, PFOE is at risk of being adulterated with low-quality oils. Adulteration is difficult to investigate because the emulsion component masks the odor and taste of the oils. Hence, PFOE authentication is crucial to guarantee the quality and safety of its product (Bansal *et al*., 2017; Danezis *et al*., 2016). Authentication is an act of determining whether an object is. Commonly, the authentication process is performed using several chemical analysis which surely confirms that the product quality meets technical specifications which stated or documented previously (Rodionova *et al*., 2016).

PFO is mainly adulterated using palm oil (PO) because of their similarities and the relatively low price of the latter (Putri *et al*., 2019). PFO adulterated with PO exhibits reduced quality. For authentication, various methods have been developed, such as Fourier transform infrared (FTIR) spectroscopy combined with chemometrics. Oils can be discriminated from each other on the basis of their fingerprint profiles (Poonia *et al*., 2017; Valand *et al*., 2020). This technique is ideal for the authentication of different oils, such as milkfish fish oil from PO, PFO from PO (Putri *et al*., 2020), Gabus fish oil from corn oil and PO (Irnawati *et al*., 2021), meat products (Candoğan *et al*., n.d.), *Rosa damascena* essential oils, (Cebi *et al*., 2021), and Maltese extra virgin olive oil (Lia *et al*., 2021).

Chemometrics uses analytical data to establish a measurement model and applies multivariate calibration for complex analysis in a mixture (Miller & Miller, 2018). Multivariate data from FTIR combined with chemometrics serve as a basis to differentiate fish oil from various adulterants (Rohman *et al*., 2021). This technique is rapid, easy, and supports green chemistry (Ikhsan *et al*., 2021). Discriminant analysis (DA) is a supervised pattern recognition method that discriminates various authentication models according to their FTIR spectra (Mustafidah *et al*., 2021; Putri *et al*., 2020; Xagoraris *et al*., 2021).

This study aims to authenticate PFOE adulterated with PO by using FTIR spectroscopy combined with chemometrics. DA was performed to discriminate among PFOE, PO emulsion (POE), and mixture oil emulsion (MOE). Partial least square regression (PLSR) and principal component regression (PCR) calibrations were also adopted to quantitatively analyze the adulteration models.

MATERIAL AND METHODS

Patin fish is obtained from a local fish market in Pati, Central Java, Indonesia. Species determination is performed at Aquaculture Laboratorium, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada to confirm sample species. Fish cleaned, filleted, and dried for 24 hours to remove moisture. The dried sample then pressed using hydraulic press to extract patin fish oil. PO and other emulsion components were purchased from Brataco (Yogyakarta, Indonesia)

Preparation of falsification models

A falsification model was prepared by mixing PFO and PO within the concentration of 0%–100% b/b, followed by adding Tween 80, Span 80, xanthan gum, sorbitol, and distilled water (aquadest) as shown in Table 1. Each sample was homogenized using a vortex mixer to obtain a homogenous mixture. Oil percentages in Table 1 were randomly obtained using Excel software (Microsoft Inc, USA).

FTIR spectroscopy analysis

Twenty-two samples were analyzed using FTIR spectrophotometer (Thermo Scientific Nicolet iS10, Madison, WI) (Putri *et al*., 2019). Spectral data were obtained and processed using Omnic software. All samples were measured in various wavelengths between 4000 and 650 cm^{-1} at 16 cm^{-1} resolution. Horizontal attenuated total reflectance (HATR) composed of ZnSe crystal was used as the sampling accessory. Prior to sample analysis, correction was performed by scanning a background spectrum of air under the same condition. Measurements were conducted in three replicates. The HATR crystal was cleaned using n-hexane and acetone after each FTIR test.

Chemometrics analysis

Chemometrics analysis including multivariate calibration and DA was performed using TQ Analyst 9 (Thermo Fisher Scientific Inc., USA) and Minitab 19 (Minitab Inc., USA). PLSR and PCR were applied for quantification.

RESULT AND DISCUSSION

Fish oils are mainly composed of fatty acids either in triacylglycerol or free fatty acid form differentiated by their profiles. Adulteration is performed by mixing high-price and low-price oils that could not be physically distinguished from

Table I. Adulteration model composition of patin fish oil and palm oil as adulterants.

Sample Id	PFO (g)	PO (g)	Tween 20 (g)	Span 80 (g)	Xanthan gum (g)	Sorbitol (g)	Aquadest (g)
PFOE1	2.00	0.00	0.64	0.28	0.15	4.00	ad 20
PFOE2	2.00	0.00	0.64	0.28	0.15	4.00	ad 20
PFOE3	2.00	0.00	0.64	0.28	0.15	4.00	ad 20
AOE1	1.80	0.20	0.64	0.28	0.15	4.00	ad 20
AOE2	1.78	0.22	0.64	0.28	0.15	4.00	ad 20
AOE3	1.64	0.36	0.64	0.28	0.15	4.00	ad 20
AOE4	1.60	0.40	0.64	0.28	0.15	4.00	ad 20
AOE5	1.36	0.64	0.64	0.28	0.15	4.00	ad 20
AOE6	1.24	0.76	0.64	0.28	0.15	4.00	ad 20
AOE7	1.24	0.76	0.64	0.28	0.15	4.00	ad 20
AOE8	0.84	1.16	0.64	0.28	0.15	4.00	ad 20
AOE9	0.78	1.22	0.64	0.28	0.15	4.00	ad 20
AOE10	0.72	1.28	0.64	0.28	0.15	4.00	ad 20
AOE11	0.66	1.34	0.64	0.28	0.15	4.00	ad 20
AOE12	0.60	1.40	0.64	0.28	0.15	4.00	ad 20
AOE13	0.56	1.44	0.64	0.28	0.15	4.00	ad 20
AOE14	0.22	1.78	0.64	0.28	0.15	4.00	ad 20
AOE15	0.20	1.80	0.64	0.28	0.15	4.00	ad 20
AOE16	0.12	1.88	0.64	0.28	0.15	4.00	ad 20
POE1	0.00	2.00	0.64	0.28	0.15	4.00	ad 20
POE2	0.00	2.00	0.64	0.28	0.15	4.00	ad 20
POE3	0.00	2.00	0.64	0.28	0.15	4.00	ad 20

**PFO: patin fish oil; PO: palm oil; PFOE: patin fish oil emulsion; AOE: adulterated oil emulsion; POE: palm oil emulsion.*

each other. Pure and adulterated products in emulsion form are particularly difficult to discriminate because the emulsion component masks their odor and taste. FTIR spectroscopy has the ability to distinguish pure oil from adulterated products according to their fingerprint spectra. In addition, molecular bonds and structures can be determined using this method.

Patin fish are obtained, selected, and confirmed based on species determination to make sure its authenticity. Fish are processed and the fish oil extracted based on method which previously developed (Mustafidah *et al*., 2021).

However, adulteration models are built using palm oil (PO) because its low price and easiest to find. PO is an edible oil which safe for human consumption. Nevertheless adulteration practice using PO still bring detrimental for producer and consumer because the quality of the product didn't met.

Fish oil emulsion is a alternative way to consume fish oils because its bad taste and odor may be covered, so the consumption become convenient. Basicly, surfactant such as Tween 80 and Span 80 are used to form stable emulsion. However, another components such as Xanthan Gum and Sorbitol also used as viscousity enchancer and sweetener to improve emulsion stability and provided sweet taste. In this reseach, emulsion is stable and perfectly covered fishy odor and fishy taste. Total 25 emulsion samples are prepared, the oil composition were randomly mixed. However, the other components such as tween 80, span 80, xanthan gum, sorbitol, and aquadest are kept constant. From these samples, there is no physical instability observed. The whole sample is completely physically homogenous and stable. Sample homogeneity is crucial to obtain the best analysis results. Emulsion easily separated if the surfactant wasn't unsuccesful stabilize oil and water phase. The separated emulsion tends to give wrong results and directing to false sample determination.

Fish oil consists of various components such as fatty acid, protein, and vitamin. Each functional groups from these components has capability to absorb light radiation energy in the infrared area. All samples are scanned using FTIR-ATR spectrophotometry at 4000-650 cm^{-1} wavenumbers. The FTIR spectra are collected and analyzed based on the difference between peak pattern, peak intensity, and peak wavenumber.

Spectra analysis

Angeline *et.al.* (2019) was performed rapid analysis to authenticate similar turmeric powder from adulterant. However due similar color and aroma, physical discrimination between sample was difficult. The alternative way was using vibrational spectroscopy to authenticate samples based on FTIR spectra profiles. Same as this research, physically discrimination between pure and adulterated emulsion is arduous because the color and the odor are similar. FTIR spectra will provided specific spectra which can be used to discriminate between pure samples, adulterated samples, and the adulterants.

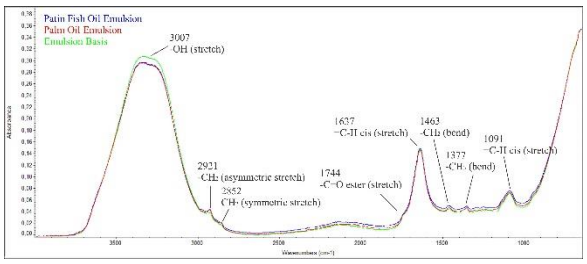

Figure 1. Fourier transform infrared spectra of between Palm Fish Oil Emulsion, Palm Oil Emulsion, and Emulsion Basis at 4000-650 cm^{-1} wavenumber regions.

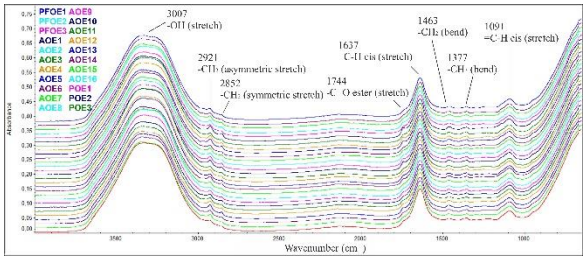

Figure 2. Fourier transform infrared spectra of 22 emulsion adulteration models at 4000-650 cm^{-1} wavenumber regions.

FTIR spectra and the corresponding functional groups and/or bonds are shown in Figure 1. Each peak is attributed to a functional group and/or bond: 3007 cm^{-1} is the (-OH) stretch and indicates water presence; 2921 cm^{-1} is the ($-CH_2$) asymmetrical stretch, 2852 cm^{-1} is the ($-CH_2$) symmetrical stretch, 1744 cm^{-1} is the (-C=O) ester stretch, 1637 cm^{-1} is the (=C-H) cis stretch, 1463 cm^{-1} is the ($-CH_2$) bend, 1377 cm^{-1} is the ($-CH_3$) bend, and 1091 cm^{-1} is the (=CH) stretch.

From figure 1 we can observe several spectral differences between these 3 samples. In Patin Fish Oil Emulsion, peak 1744 cm^{-1}, 1637 cm^{-1}, 1463 cm^{-1}, 1377 cm^{-1}, dan 1091 cm^{-1} has higher absorbance intensity compared with Palm Oil Emulsion and Emulsion basis. The highest 1744 cm^{-1} and 1637 cm^{-1} indicates unsaturated bonds from fatty acid. Patin fish oil contains higher polyunsaturated fatty acid compared with palm oil. Otherwise, in 2921 cm^{-1} and 2852 cm^{-1}, Patin Fish Oil Emulsion has lower absorbance compared with others. Palm oil contains higher level of saturated bonds which may be observed in 2921 cm^{-1} and 2852 cm^{-1}. Moreover in Emulsion Basis spectra, 3007 cm^{-1} has the highest absorbance intensity compared with others. The 3007 cm^{-1} correspond to water presence which is higher in Emulsion Basis. These several peak differences may become a critical factors to discriminate patin fish oil emulsion from palm oil emulsion and emulsion basis.

Discriminant analysis

Discrimination pure samples (PFOE) and adulterated samples (POE and MOE) is carried out using Discriminant Analysis (DA). DA can discriminate PFOE from adulterated models (POE and MOE) according to their FTIR spectra. The wavenumber regions used for DA is 1744 cm^{-1}, 1637 cm^{-1}, 1463 cm^{-1}, 1377 cm^{-1}, 1091 cm^{-1}, 2921 cm^{-1}, 2852 cm^{-1}, and 3007 cm^{-1} based on spectral characteristic which explains in the previous section. DA discriminates the whole samples into 2 groups which we can observe in the Coomans' plot in figure 3. The first group are pure samples, however the next group are adulterated samples (POE and MOE). From both groups, some samples are used for validation to evaluate the DA method.

Mahalanobis distance is important in justifying similarities between an unidentified sample and other identified samples (Mustafidah *et al*., 2021). The x axis indicates the Mahalanobis distance to pure PFOE, and the y axis shows the Mahalanobis distance to the adulterated emulsion. The findings showed that DA successfully discriminated pure PFOE, POE, and MOE with 100% accuracy, it means that there were no samples are falsely discriminate into false group. Missdiscrimination is commonly caused by unsuitable wavenumber selection and unspesific spectra profile. The method tend to false discriminate the sample because the similiarity between sample is high and hard to differentiate. Therefore effective and suitable for distinguishing PFOE from PO adulterant.

Table II. Optimization of multivariate calibration (partial least square regression and principal component regression) at 4000-650 cm^{-1} wavenumber regions.

	Wavelength (cm^{-1})	Spectra	RMSEC	R^2_{cal}	RMSEP	R^2_{pred}
		Normal	0.1250	0.9220	0.1580	0.8649
PLSR	1004–2936	1st derivative	0.2620	0.5863	0.2890	0.3927
		2nd derivative	0.2660	0.5704	0.2930	0.3902
		Normal	***0.0565***	***0.9846***	***0.1330***	***0.9073***
PCR	1004–2936	1st derivative	0.0579	0.9998	0.2990	0.3554
		2nd derivative	0.2580	0.6054	0.3250	0.1660

**The selection method is marked in bold and italic font style. PLSR: partial least square regression; PCR: principal component regression.*

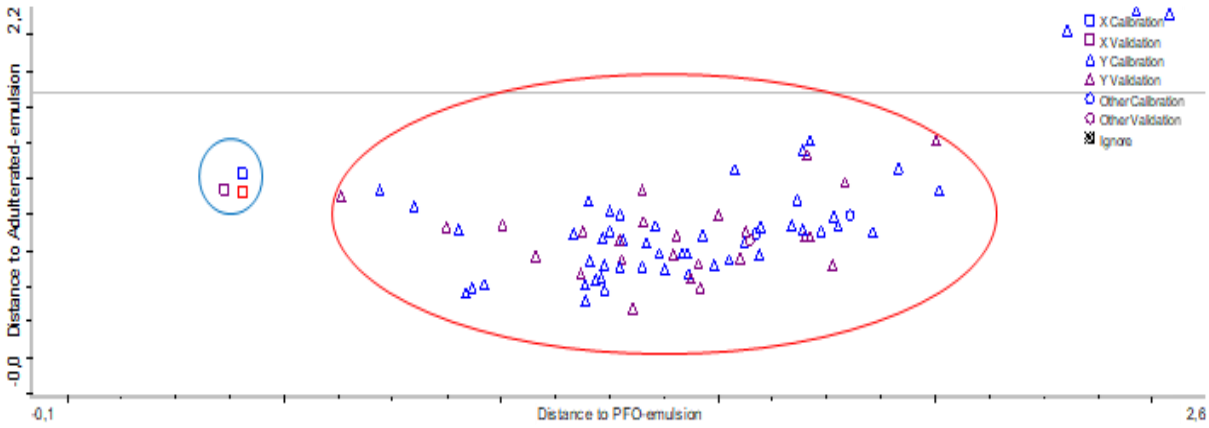

Figure 3. Coomans' plot for pure patin fish oil emulsion (blue circle) and adulterated emulsion (red circle) (palm oil emulsion and mixture oil emulsion).

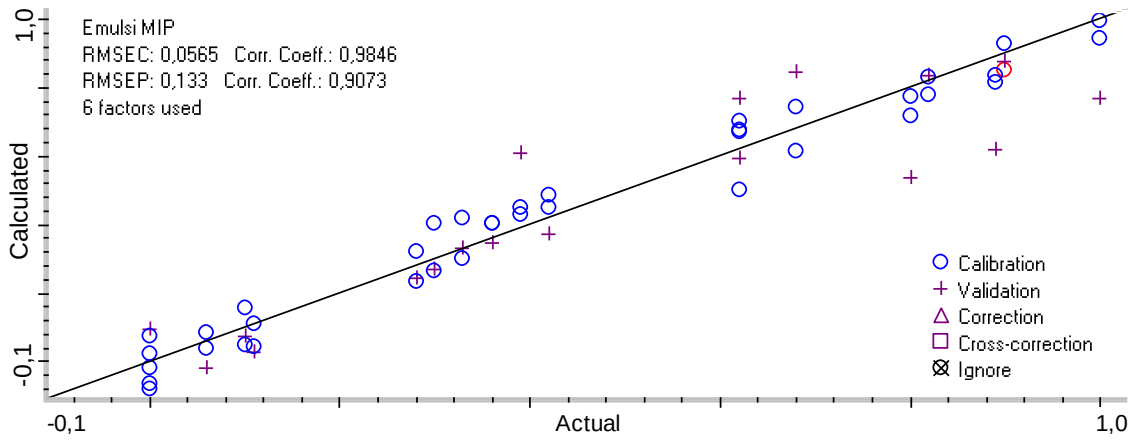

Figure 4. Correlation between the actual and predicted values of pure patin fish oil emulsion from principal component regression within the normal spectrum of 1004–2936 cm^{-1}.

Multivariate calibration

The least-squares technique is generally adopted in fitting various liniear regression models such as Partial Least Square (PLSR) and Principal Component Regression (PCR) (Liu *et al.*, 2003). PLSR was a linear regression technique which is done on scores. PLSR was transforming the original variables to novel variable which

latent and have high correlation with variable response (Geladi & Dåbakk, 2016). PLSR has similarities to Principal Component Regression (PCR) in building and extracting a novel variable from original variables (Liu *et al*., 2003). PCR was used in two stage procedure, first principal component analysis (PCA) was performed, then followed by regression. The selected principal components were considered as a novel explanatory variable in the model (Kawano *et al*., 2018).

The combination of FTIR spectroscopy, PLSR, and PCR was applied for the quantitative analysis of PFO and adulteration models. PLSR was adopted to determine the correlation between spectral absorption changes and sample concentration. PCR combines the spectral data of samples into a one-step model (Putri *et al*., 2019). The results from these analyses are shown in Table 2. The results of PLSR and PCR were optimized using absorbance as a variable at several wavenumber regions. R^2 value (prediction and calibration) and errors (root mean square error of calibration and root mean square error of prediction) were employed to evaluate accuracy and precision.

As shown in Table 2, PLSR within the normal spectrum of 1004–2936 cm^{-1} produced the following results: highest R^2_{cal} value of 0,9220, R^2_{pred} value of 0,8649, lowest root mean square error of calibration (RMSEC) of 0,1250, and lowest root mean square error of prediction (RMSEP) of 0,1580. In addition, PCR using normal spectrum at wavenumbers 2936 cm^{-1} obtained the following values: highest R^2_{cal} of 0,9846, R^2_{pred} of 0,9073, lowest RMSEC of 0,0565, and RMSEP of 0,1330. In this study, PCR showed better efficiency than PLSR because its highest R^2_{cal} and R^2_{pred} values were higher than those of PCR. In addition, the lowest RMSEC and RMSEP values of PCR were smaller than those of PLSR.

The first and second derivative spectra aim to increase the molar sensitivity and separate the peaks overlapping in normal spectra. However, these spectra do not always provide the best results because the peak already had good separation and therefore are unnecessary for this study.

Figure 3 exhibits the correlation between the actual and predicted values for PFOE and adulterant models. As shown in Figure 4, the errors were distributed randomly along the correlation line and were not systematic. In addition, concentration difference had no influence on the analysis results. Therefore, FTIR spectra combined with PLSR/PCR is an accurate and precise technique for quantitative analysis to discriminate PFOE from PO adulterant.

CONCLUSION

FTIR spectroscopy combined with DA and PCR successfully discriminated PFOE from PO adulterant. Adopting the PCR technique within the normal spectrum of 1004–2936 cm^{-1} for quantitative analysis provides a high accuracy and a low error percentage. This method is rapid, accurate, and suitable for distinguishing pure PFOE from PO adulterant.

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