

AUTHENTICATION OF PATIN (*PANGASIUS MICRONEMUS*) FISH OIL ADULTERATED WITH PALM OIL USING FTIR SPECTROSCOPY COMBINED WITH CHEMOMETRICS

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Received: 23 Nov 2018, Revised and Accepted: 06 Apr 2019

ABSTRACT

Objective: The goal of this research was to perform authentication of patin (*Pangasius micronemus*) fish oil (PFO) adulterated with palm oil (PO) using FTIR spectroscopy combined with chemometrics method.

Methods: Patin fish oil (PFO) and PFO adulterated with palm oil (PO) were measured using FTIR instrument at wavenumbers region of 4000–650 cm⁻¹. The chemometrics methods, namely multivariate calibration of partial least square (PLS) and principal component regression (PCR) were used to make calibration and validation models during quantification. Discriminant analysis (DA) was used to make grouping pure PFO and PFO adulterated with PO.

Results: The results showed that PLS and PCR could be used to quantify PO as adulterant in PFO, either in calibration or validation models. FTIR spectroscopy combined with multivariate calibration offered accurate and precise method for quantitative analysis with R² value of >0.999 and low RMSEC and RMSEP. DA was capable of grouping PFO and PFO adulterated with PO with an accuracy level of 100%.

Conclusion: FTIR spectroscopy combined with chemometrics could be reliable technique for quantification and discrimination of PFO and PFO adulterated with PO.

Keywords: Patin fish oil, Palm Oil, Authentication, Partial least square, Principle component regression, Discriminant analysis

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DOI: <http://dx.doi.org/10.22159/ijap.2019v11i3.30947>

INTRODUCTION

Patin fish (*Pangasius micronemus*) is the one of the most popular freshwater fish in Indonesia. Patin fish oil (PFO) has the potency to be used as a source of omega-3 fatty acids, which are beneficial to human health. Omega-3 fatty acids are unsaturated fatty acid which consist of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA). Omega-3 fatty acids can be used to prevent some diseases such as hypertension, eczema, psoriasis, osteoporosis, breast cancer, allergic [1], cardiovascular [2], and Alzheimer [3].

Oils might have the same composition with other oils, therefore it is possible to adulterate high priced oil with lower ones [4]. Authentication is important to protect the consumer from adulteration practices. Patin fish oil (PFO) has the possibility to be adulterated with other oils which are cheaper like palm oil so it will decrease the quality of PFO. Some authentication methods based on physicochemical properties and molecular biology have been developed for authentication analysis, and among this method, fourier transform infrared (FTIR) spectroscopy combined with chemometrics is the most used ones. Because of its nature as a fingerprint technique, meaning that every oils have different FTIR spectra, FTIR spectroscopy is an idela method [5].

Chemometrics is the method in chemical that using mathematical, statistical, and other methods to make a measurement model by analysing chemical data [6]. FTIR Spectroscopy that combined with chemometrics are widely used to detect adulteration product like adulteration virgin olive oil with palm oil [5], adulteration in virgin coconut oil [7], sesame oil with corn oil [8], adulteration sunflower and corn oils in extra virgin olive oil [9], detection of rice bran oil as adulterant in extra virgin olive oil [10], and candlenut adulterated with palm oil [11]. FTIR spectroscopy was also used for monitoring oxidative stabilities of frying oils [12]. Chemometrics usually use multivariate calibration for analysis complex mixture. This method is rapid, no time consuming, easy, and simple preparation sample [13]. Partial least square (PLS) and principle component regression (PCR) were commonly used in multivariate calibration techniques [14]. Another method in chemometrics is discriminant analysis (DA). This method was used to find the relationship between descriptive variable

and qualitative variable [15]. The goal of this research is to authenticate patin (*Pangasius micronemus*) fish oil (PFO) adulterated with palm oil (PO) using FTIR spectroscopy combined with chemometrics. In this research, PLS and PCR calibration techniques was used for quantitative analysis, while DA was explored for classification between pure PFO and PFO adulterated with PO.

MATERIALS AND METHODS

Sample preparation

Patin Fish was obtained from freshwater fish cultivation in Tulungagung, East Java, Indonesia. PFO was extracted with maceration process using *n*-hexane solvent. Palm oil was obtained from market in Yogyakarta, Indonesia. Twenty (21) samples containing PFO and PO with the concentration range of 0-100% v/v were prepared for quantitative analysis using PLS and PCR, and 13 samples containing pure PFO and PFO adulterated with PO was prepared for DA.

FTIR analysis

Samples were analysed using FTIR spectrometer (Thermo Scientific Nicolet iS10, Madison, WI). This instrument was controlled with Omnic software. The measurements were done in middle infrared region of 4000 - 650 cm⁻¹ with 32 scanning and at a resolution of 16 cm⁻¹. The background scanning has to do to reduce the effect of the reference spectrum of the air. Before and after analysis sample, ATR crystal has to be cleaned with acetone p.a. Replications were done with scanning same samples for 3 times.

Chemo metrics

Chemo metrics analysis including multivariate calibration and discriminant analysis was performed using TQ Analyst software version 9 (Thermo Fisher Scientific, Inc.). For quantification, multivariate calibrations used were PLS and PCR.

RESULTS AND DISCUSSION

Spectra analysis

Oils and fats are basically triglycerides which can be differed from the fatty acid composition in terms of length chain and unsaturation

degree. The adulteration practice was usually met in high priced oil having similar color with oil adulterant, therefore, it would be difficult to detect the oil that mixed with another oil, physically [4]. Meanwhile, fats and oils can be differentiated using FTIR spectra due to the property of FTIR spectra as fingerprint technique. FTIR spectra could be used to get information about molecular structure from the vibration of functional groups at the certain wavenumbers [16]. The FTIR spectra of patin fish oil (PFO) and palm oil (PO) were

showed in fig. 1. Each peaks and shoulders correspond to functional groups responsible for the absorption of infrared radiation. Table 1 explain the functional groups corresponding to each peaks/ shoulders. There are some differences in both spectra at wavenumber around 2954 cm^{-1} and at the fingerprint area (1500-650 cm^{-1}). PO spectra have no peak at 2954 cm^{-1} and around 700 cm^{-1} . Thus, these areas can be used for detection and quantification of PFO that adulterated with PO.

Table 1: Vibration of functional groups in patin fish oil (PFO) and palm oil at certain wavenumbers [20]

Wavenumber (cm^{-1})	Functional group
3005	<i>cis</i> -double-bond stretching
2954	-CH ₃ asymmetrical stretch
2922 and 2854	Asymmetric and symmetric vibrations of methylene (-CH ₂) group
1744	-C=O stretch
1460	-CH ₂ bending vibration of an aliphatic group
1374	-CH ₃ bending vibration
1233 and 1157	Stretching vibration of -C-O ester
1113	Bending vibration of -CH
721	Rocking vibration of methylene (-CH ₂)

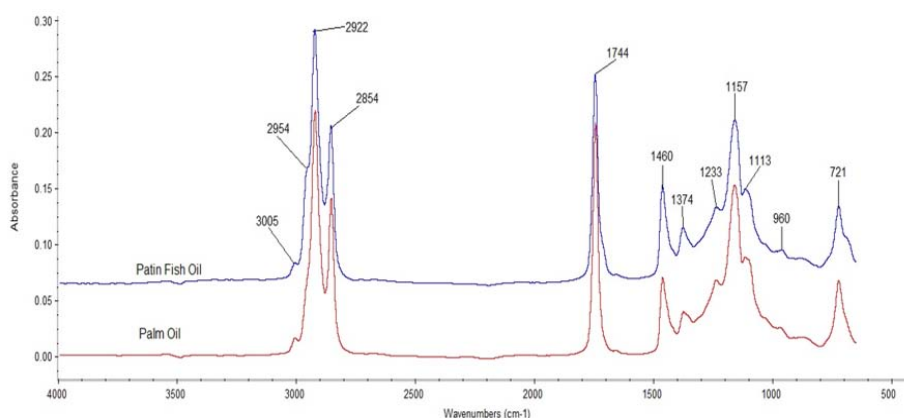


Fig. 1: FTIR spectra of patin fish oil (PFO) and palm oil (PO) at wavenumber of 4000-650 cm^{-1}

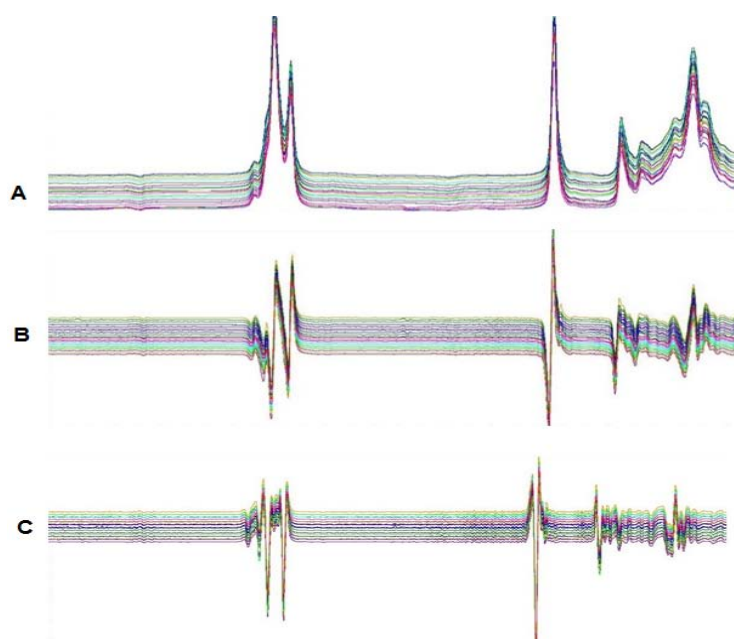


Fig. 2: Overlay FTIR spectra of patin fish oil (PFO) and palm oil (PO). (A) FTIR normal spectra (B) first derivative spectra (C) second derivative spectra

Quantification of patin fish oil

Quantification of patin fish oil (PFO) that mixed with PO was performed with partial least square (PLS) and principle component regression (PCR). For quantification, 21 samples containing PFO that mixed with PO with range concentration 0-100% and 13 samples containing pure PFO. The quantification using spectral regions at 1001-1501, 1776-1712, and 2819-2981 cm^{-1} . These regions was selected by optimization processes that give the highest values for R^2 and the lowest values of error [7]. PLS method using correlation between spectral absorption change and concentration of samples then computing with other spectra that may disturb analyte spectra [15]. While the PCR method combines spectral and data concentration sample into a model in one step [16]. Overlay FTIR

spectra of PFO and PO was shown in fig. 2. In PLS and PCR calibration techniques, linearity was used to evaluate the relationship between absorbance and samples concentration. From the PLS and PCR method, the statistical parameters namely coefficient of determination (R^2) value, root mean square error of calibration (RMSEC), and root mean square error of prediction (RMSEP) in normal, first derivative and second derivative spectra were compiled in table 2.

Root mean square of calibration (RMSEC) is the value that used to evaluate error in calibration model and the root mean square of prediction (RMSEP) is used to evaluate the validation model [7].

Table 2: The performance of partial least square (PLS) and principle component regression (PCR) for quantitative analysis of patin fish oil (PFO) in palm oil (PO)

Model	Spectra	Equation		R^2		RMSEC (%v/v)	RMSEP (%v/v)
		Calibration	Validation	Calibration	Validation		
PLS	Normal	$y = 0.9737x + 0.2142$	$y = 0.9852x + 0.9104$	0.9674	0.9689	5.50	3.59
	First derivative	$y = 0.9994x + 0.0268$	$y = 1.0078x - 0.7823$	0.9994	0.9985	0.805	2.22
	Second derivative	$y = 0.9964x + 0.1433$	$y = 1.0087x - 0.8251$	0.9995	0.9983	0.778	2.43
PCR	Normal	$y = 1.0034x - 0.0449$	$y = 1.0054x - 0.3167$	0.9961	0.9938	2.54	2.61
	First derivative	$y = 0.9993x + 0.0334$	$y = 1.0075x - 0.7332$	0.9993	0.9985	0.889	2.07
	Second derivative	$y = 1.0006x - 0.1186$	$y = 1.0078x - 0.7082$	0.9989	0.9980	1.14	2.18

PCR and PLS calibration using first derivative FTIR spectra have the best values in terms of high R^2 and low of RMSEC and RMSEP values (table 2). The first derivative spectra could identify the targeted spectra from the mixed samples by increasing the spectral resolution. The R^2 values obtained using PLS and PCR calibration

model were of 0.9994 and 0.9993 (fig. 3) and 0.9985 in validation model (fig. 4). The high R^2 value and low values of RMSEC and RMSEP indicated that calibration and validation models using PLS and PCR were accurate and precise to measure the levels of PFO adulterated with PO.

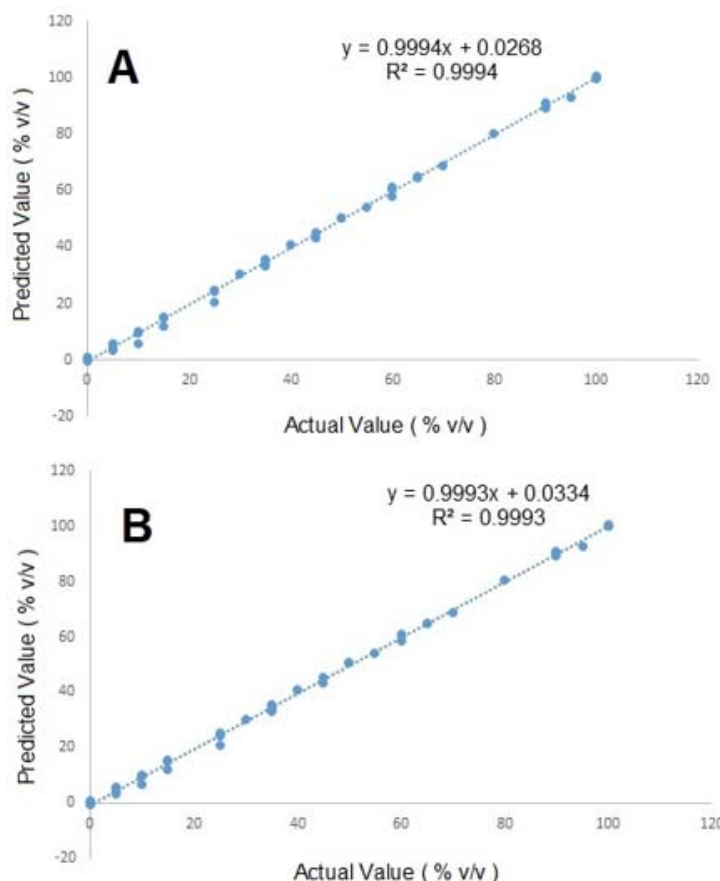


Fig. 3: Calibration model for the relationship between actual (x-axis) and FTIR predicted values of patin fish oil adulterated with palm oil using partial least square (A) and principle component regression (B) with first derivative spectra.

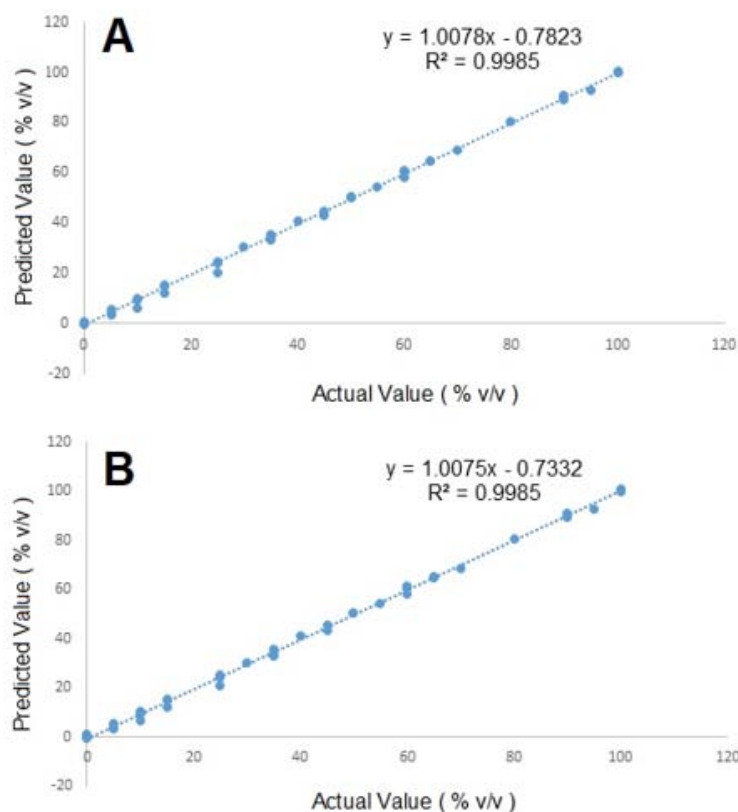


Fig. 3: The validation model for the relationship between actual (x-axis) and FTIR predicted values of of patin fish oil adulterated with palm oil using partial least square (A) and principle component regression (B) with first derivative spectra

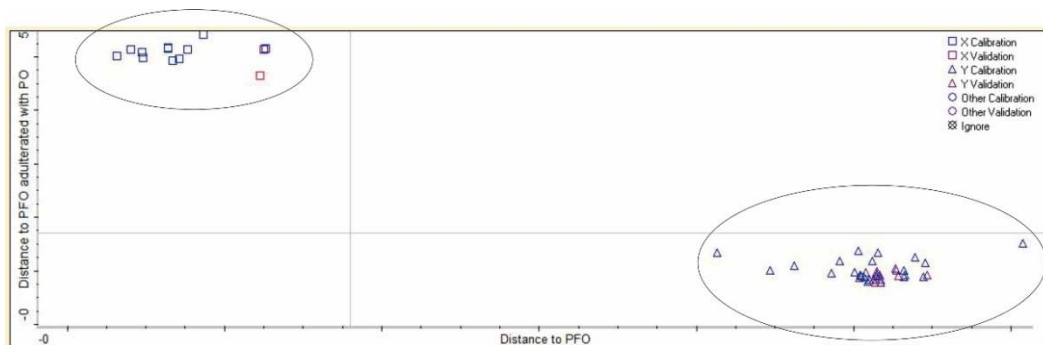


Fig. 5: The coomzans plot of patin fish oil (PFO) and palm oil (PO): (Δ) PFO adulterated with PO and (\square) pure PFO

Discriminant analysis

Discriminant analysis is a method commonly used to decide the group between pure patin fish oil (PFO) and PFO adulterated with palm oil (PO) by computing the distance from each group center based on Mahalanobis distance units. If the grouping model have been obtained, the component of unknown samples can be predicted [15]. Discriminant analysis using the combined wavenumber regions of 1001-1501, 1776-1712, and 2819-2981 cm^{-1} were used for classification.

The Coomans plot is the method to show the group of pure PFO and PFO adulterated with PO. This plot was obtained from calculating two principal components (PC) and then plotting the residual distances of samples from each two models [19]. Fig. 5 exhibited the Coomans Plot for pure PFO and PFO adulterated with PO. The x-axis described the Mahalanobis distance for pure PFO and the y-axis exhibited the distance of PFO adulterated with PO. The Coomans plot in fig. 5 exhibited the group division between pure PFO and PFO adulterated with PO. The DA model is capable of grouping the oils with an accuracy

level of 100%. Therefore, this method is a suitable method for grouping the samples and no samples were mistakenly grouped.

CONCLUSION

FTIR spectroscopy combined with chemometrics is suitable method for authentication of patin fish oil (PFO) and PFO adulterated with PO. Chemometrics method using PLS and PCR can give an accepted model for the calibration and validation with high R^2 value and low RMSEC and RMSEP values using first derivative FTIR spectra in the regions at 1001-1501, 1776-1712, and 2819-2981 cm^{-1} . Discriminant analysis could also successfully used for grouping the pure PFO and PFO adulterated with PO with accuracy level of 100%.

ACKNOWLEDGMENT

Author thanks to The Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for its scholarship so that this research can be done.

AUTHORS CONTRIBUTIONS

ARP compiled data dan prepared manuscript. AR and SR designed research, analysed data, and made critical thinking on manuscript

CONFLICT OF INTERESTS

The author declares that there is no conflict of interest

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