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The Application of FTIR Spectroscopy Combined Chemometrics for Analysis of Keting Fish Oil in Binary Mixture with Patin Fish Oil and Palm Oil

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Abstract: Keting fish (Mystus gulio) is a local fish from Indonesia. The quality of keting fish oil needs to be maintained to avoid counterfeiting. The use of Fourier transform infrared (FTIR) spectroscopy can be developed for quantitative analysis of Keting fish oil (KFO) in binary mixtures with Patin fish oil (PFO) and palm oil (PO). In this present research, PLS and PCR models were used to construct a multivariate calibration for the KFO content in the binary mixtures analysis at fingerprint region frequencies of 1,500-1,000 cm^{-1}. The results showed that PLS with first derivative FTIR spectra existed for quantitative analysis of KFO-PFO with a value of the R^2 = 0.997, the RMSEC = 0.0204%, and the RMSEP = 0.0028%. Meanwhile, R^2, RMSEC, and RMSEP values acquired for KFO in binary mixture with PO were 0.999, 0.0133%, and 0,0059%, respectively. In most cases, FTIR spectroscopy work as a suitable technique for the determination of KFO in mixtures with other oils.

Keywords: FTIR, keting fish oil, binary mixture, chemometrics

1. INTRODUCTION

Fish oils contain bioactive compounds especially polyunsaturated fatty acids (PUFAs). Docosahexaenoic acid or DHA, and eicosapentaenoic acid or EPA are the omega-3 PUFAs, established functional food, nutritional supplements, and pharmaceutical ingredients [1]. The beneficial of omega-3 fatty acids for human health such as preventing diabetes, atherosclerosis, and playing an important role in the development of brain function in kids [2]. Keting fish oils (Mystus gulio) are usually characterized by a high content of omega-6 and omega-3 PUFAs such as linoleic acid (27.55%) and docosahexaenoic acid (3.26%). Omega-3 DHA a necessary role on the establishment of the structure and growth of the brain, especially in children [3]. Keting fish is one of the local freshwater fish in Indonesia. This fish is mostly consumed by the community because of its savory taste [2]. Oils extracted from Keting fish are known as Keting Fish Oil (KFO). Currently, many studies

related to fish oil have been carried out, especially catfish like Patin fish oil (Pangasius micronema) and Lele fish oil (Clarias geriepinus). In this study, Patin Fish Oil (PFO) was selected as a mixture because it has a close fatty acid profile with KFO [4]. In addition, palm oil (PO) was selected as cheap vegetable oil. This research can be additional information about the class of catfish oil.

Since a long time ago, cases of oil adulteration have been found, including in fats and oils. Some unethical players try to blend fish oil with vegetable oil to get the economical profits. The identification of adulteration practice in fats and oils its difficult to, when the oil adulterant has close similarity in terms of chemical composition and color to that of the original oil. Therefore, the development of rapid and simple analytical methods capable of detecting adulterations in KFO is very important [5].

The application of FTIR with ATR (Attenuated Total Reflectance) has been developing in food studies, especially for the characterization, classification, and authentication of the edible oils and fats [6]. ATR-FTIR spectroscopy is a rapid method, non-destructive, and does not require any sample preparation step [7] [8]. The combination of FTIR spectroscopy with chemometric analysis has been used for detection of Patin fish oil adulterated corn oil [5], detection of pork oil adulterated with snakehead fish oil [9], and detection of Tuna fish oil with pork oil [10].

FTIR spectroscopy with chemometric technique is an important analytical method to identify adulteration of fish oil, because of its capability as a fingerprint area. Chemometrics technique is concerned with the analysis of multivariate data on a measurement. It has the ability to manage the huge data from instrument measurement to be more interpretable and understandable [11]. Multivariate calibration methods are partial least squares (PLS) and principal component regression (PCR) are the most widely used for quantitative analysis. The accuracy of analytical methods was evaluated by coefficient of determination (R^2) for the relationship between the actual value and predicted values. Meanwhile, the precision was assessed by root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) [12]. Accordingly, the aim of this study was to develop a PLS and PCR calibration method for the analysis of ATR-FTIR spectroscopy specific on the fingerprint area for the quantitative analysis of KFO with binary mixture with PFO and PO.

2. MATERIALS AND METHODS

2.1. Materials

Keting fish oil (KFO) is obtained from Keting fish purchased from the local fish market in Central Java (in Gandrungmangu - Cilacap), Indonesia. The keting fish used to have a short body of about 6-8 cm and the characterized by a shorts adipose fin base. Patin Fish Oil (PFO) is obtained from Patin Fish were available from farms in Yogyakarta, Indonesia. Fish oil extraction was carried out by dry rendering (55°C, 24 hours) combined press hydraulic (150 kN, 5 minutes) [4]. Then Palm oil (PO) was purchased from a local market in DIY, Indonesia. For quantitative analysis, the levels of binary mixtures were analyzed with the aid of PLS and PCR calibration. A set of 25 binary mixture samples containing KFO-PFO or KFO-PO was mixed in accurately measured proportions of 0–100% v/v, and shaken vigorously to ensure the total homogenization. For validation or prediction purposes, 25 independent samples were also prepared.

2.2. Method

2.2.1. ATR-FTIR spectroscopy measurement

The conditions of the FTIR spectrophotometer are as follows in Table 1. Before collecting the FTIR spectra, the ATR plate was carefully cleaned in situ using hexane and dried with a soft tissue before filling with the sample. Every scan of the sample, please take a new reference water background. Pasteur pipette can be used for a few drops of each sample placed in contact with ATR. All FTIR spectra were recorded as absorbance values at each data point in triplicate.

Table 1. The condition of the FTIR spectrophotometer A Nicolet 6700 (Thermo Nicolet Corp., Madison, WI).

Detector	DTGS
Beam splitter	KBr/Germanium
Regions	4,000-600 cm^{-1}
Sampling technique	ATR (ZnSe crystal plate)
Scanning, Resolution	32 Scan, 8 cm^{-1}
Temperature	Controlled at 25°C
Software	OMNIC V7.0 Thermo Nicolet

2.2.2. Chemometrics Analysis

PLS and PCR calibrations are models of calibration multivariate, which are performed using the software of TQ Analyst™ V6. Analysis of FTIR spectral regions in the fingerprint region 1,500-1,000 cm^{-1}. The models were evaluated by computing the values of R^2, RMSEC, RMSEP.

3. RESULTS AND DISCUSSION

3.1. Spectra Analysis

Triglycerides (TAG) are the principal component in edible fats and oils, so the FTIR spectrum of fats and oils is dominated by this. Figure 1a presents the FTIR spectra of KFO, PFO, and PO at the mid-infrared region of 4,000–600 cm^{-1}. The Results of the peaks and shoulders appearing on FTIR spectra not visible difference. Due to absorption of certain functional groups present of the sample, and the typical characteristic of absorption bands for common TAG. The analytical evaluation of the KFO, PFO, and PO spectra is given in Table 2.

Table 2. ATR-FTIR band assignments for functional groups and modes of vibration in the spectrum of Keting fish oil [13].

Frequency (cm^{-1})	**Functional group**	**Vibration modes**
3008	=C–H (cis)	Stretching
2921	–C–H (–CH2)	Asymmetric stretching
2852	–C–H (–CH2)	Symmetric stretching
1743	–C=O (ester)	Stretching
1462	–C–H (–CH2)	Bending (scissoring)
1418	=C–H (cis)	Bending (rocking)
1376	–C–H (–CH3)	Symmetrical bending
1232	–C–O	Stretching
1157	–C–O	Stretching
	–CH2–	Bending
1115, 1098	–C–O	Stretching
965	–HC=CH– (trans)	Bending out of plane
720	–(CH2) n–	Bending out of plane
	–HC=CH– (cis-olefin)	Rocking, bending out

In this paper FTIR spectra looks very similar to the three oils, shown in fig. 1a. This is due to the similar chemical composition of KFO, PFO, and PO, in terms of fatty acid compositions (Table 2). However, if one evaluates spectra closely, there is a slight difference observed. The identification of functional groups of these oil spectra is shown in Fig 1. However, two spectra of a binary mixture KFO-PFO, and KFO-PO were distinguished from peak intensities as fingerprint properties [14]. FTIR spectra of KFO-PFO was shown in Fig. 1b, both FTIR spectra can be differences in selected fingerprint regions 1,500–1,000 cm^{-1}, especially in terms of absorbance bands at a wavenumber of 1032.46 cm-1, these are the vibrations of –C–O in the stretching mode. Meanwhile, in the binary mixture of KFO with PO as shown in Fig.1c, these differences can be seen at wavenumbers of 1,418.55 cm^{-1} and 1,098.03 cm^{-1}, vibration of =C–H (cis) in the bending rocking mode and –C–O in the stretching mode, respectively [12]. These differences could be exploited as regions to optimize for chemometrics analysis [13].

Figure 1. (a) FTIR spectra of Keting fish oil at wavenumbers 4,000-600 cm^{-1}; (b) FTIR spectra of 100% Keting fish oil (KFO) combined with 100% Patin fish oil (PFO); and (c) KFO combined with 100% Palm oil (PO) at fingerprint region is 1,500-1,000 cm^{-1}.

3.2. Chemometric Analysis

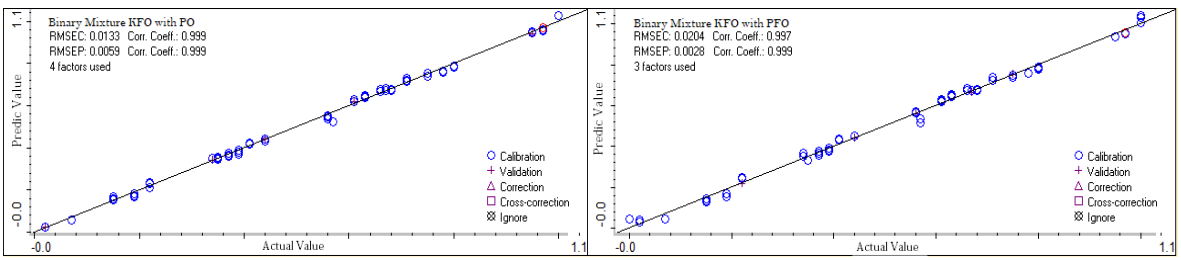
Quantification of KFO in a binary mixture with PFO and PO was performed by PLS and PCR multivariate calibration. Quantitative analysis is important to maintain the concentration of unknown analytes [16]. Quantification of KFO in a binary mixture with PFO and PO was performed using PLS and PCR algorithms. The adulterant samples were divided into calibration and prediction or validation sets. Each test consisted of 25 samples. Based on fingerprint regions, the wavenumbers used for quantification analysis are 1,500 – 1,000 cm^{-1} [5].

Table 3. Calibration for determining KFO in binary mixture with PO and PFO (1,500-1,000 cm^{-1}) using PLS and PCR techniques.

Binary Mixture	Multivariate Calibration	Spectral	R^2	RMSEC	R^2	RMSEP
KFO-PFO	**PLS**	Normal	0.866	0.1220	0.9985	0.0239
		1st Derivative*	***0.997***	***0.0204***	***0.9999***	***0.0028***
		2nd Derivative	0.969	0.0625	0.9997	0.0070
	PCR	Normal	0.898	0.1110	0.9988	0.0105
		1st Derivative	0.952	0.0744	0.9991	0.0168
		2nd Derivative	0.947	0.0788	0.9995	0.0112
KFO - PO	**PLS**	Normal	0.999	0.0134	0.9997	0.0081
		1st Derivative*	***0.999***	***0.0133***	***0.9999***	***0.0059***
		2nd Derivative	0.998	0.0190	0.9999	0.0027
	PCR	Normal	0.999	0.0150	0.9999	0.0026
		1st Derivative	0.998	0.0175	0.9997	0.0082
		2nd Derivative	0.998	0.0193	0.9999	0.0051

*Multivariate calibration selected for quantification are bolded and italicized

The correlation between x-axis (the actual values) and y-axis (the FTIR predicted values) in optimized condition for binary mixture KFO-PFO and KFO-PO can be shown in fig.2. The PLS model was subsequently validated using validation or prediction samples. The R^2, RMSEC, and RMSEP values were computed after making validation of all prediction samples. The derivatization aims to increase the sensitivity of the reading by removing the interfering spectra. The first derivative aims to increase the spectral resolution and simplifies baseline selection, while the second derivative eliminates the broadband absorption. The result KFO-PO in the *1st* derivative is reported with values of R^2, RMSEC, and RMSEP of 0.999, 0,0204%, and 0.0059% (v/v), respectively. Then for binary mixture KFO-PO in the 1st derivative is reported with values of R^2, RMSEC, and RMSEP of 0.999, 0.0133%, and 0.0028% (v/v), respectively, and we can see in fig.2(a) and fig.2(b). That's why it can be stated that FTIR spectroscopy at fingerprint region assisted with PLS calibration model can give accurate results with low errors for the determination of KFO in binary mixture.



(a) **(b)**

Figure 2. The calibration models of PLS KFO with PO (a) and KFO with PFO (b) for the relationship between actual and predicted values of KFO in binary mixture using 1st derivative spectra at 1,500–1,000 cm^{-1}.

4. CONCLUSION

FTIR spectroscopy combined multivariate calibrations, especially PLS and PCA at fingerprint regions of 1,500-1,000 cm^{-1} can be used to monitor the adulteration of KFO in a binary mixture with PFO and PO. Although the result of the PLS calibration model gives accurate results (high R^2) and low errors (low RMSEC and RMSEP), both the calibrations model succeeded in quantifying the level of KFO in a binary mixture.

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