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FULL PAPER

Simultaneous analysis of patin fish oil (*Pangasius micronemus*) and bandeng (*Chanos chanos*) fish oil using FTIR spectroscopy and chemometrics

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Abstract

Patin fish (*Pangasius micronemus*) and bandeng fish (*Chanos chanos*) has high levels of oils containing essential fatty acids which are necessary for the human body. Therefore, quantitative analysis of fish oils extracted from patin fish and bandeng fish was very important. This research aimed to combine Fourier transform infrared (FTIR) spectroscopy with partial least square (PLS) and principal component regression (PCR) for simultaneous analysis of the binary mixture of patin fish oil (PFO) and bandeng fish oil (BFO). The method has consisted of extraction of both oils, preparation of calibration and validation samples, scanning of samples using FTIR spectrophotometer, and developing the calibration and validation models using PLS and PCR. The simultaneous analysis of PFO in a binary mixture with BFO was performed based on first derivative spectra at combined wavenumbers of 3050-2800, 1800-1700, and 1500-650 cm^{-1} assisted with PLS. In addition, BFO in binary mixtures with PFO was determined using the first derivative spectra using the same wavenumbers. The coefficient of determination for calibration (R_{cal}^2) and validation (R_{val}^2) were 0.9998 and 0.9994, with low errors (RMSEC = 0.0072 and RMSEP = 0.0121). The combination of FTIR spectroscopy with PLS using suitable wavenumbers can be potential tools for the simultaneous analysis of PFO and BFO.

1. Introduction

Patin fish (*Pangasius micronemus*) and bandeng fish (*Chanos chanos*) are cultivated in Indonesia. Besides their meat, fish oils also have a huge potency to be developed as a dietary supplement (Siscovick *et al.*, 2017). Patin fish oil (PFO) and bandeng fish oil (BFO) were composed mainly of polyunsaturated fatty acids (PUFA). PFO contained a high level of omega-6 fatty acid while BFO contains a high level of omega-3 fatty acid. In the future, PFO and BFO may be combined as food supplements. Fish oil supplementation provides some beneficial effects on human health such as preventing cardiovascular disease and is also essential for child brain development (Ramaswami, 2016; Gao *et al.*, 2017; Hansen *et al.*, 2017).

Verifying the quality and identity of each fish oil was needed for the following reasons: well-defined quality, legal compliance, dosage, and product safety (Danezis *et al.*, 2016; Bansal *et al.*, 2017). Conventional methods were used to establish reference materials to analyse adulteration and guarantee quality (Danezis *et al.*, 2016; Sabir, *et al*., 2017; He *et al.*, 2020; Gao *et al.*, 2021). However, the fish oil components were composed of some complex components whose reference materials were hard to find. Multivariate analysis based on specific fish oil characteristics was a more conclusive method to discriminate each component (Jha *et al.*, 2016; Avramidou *et al.*, 2018; Mohammed *et al.*, 2021). Authentication of different oils and fats has been performed using multivariate analysis between triacylglycerol and fatty acid fingerprints using gas chromatography (GC) and proton transfer reaction mass spectra (PTR-MS) (van Ruth *et al.*, 2010).

FTIR spectroscopy was a common method to

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analyse fish oil characteristics based on their fingerprint in nature (Poonia *et al.*, 2017; Valand *et al.*, 2020). This method was reported to be effective for distinguishing each oil by its spectral difference (Rohman, 2017). Combined with partial least square (PLS) and principal component regression (PCR), the FTIR spectroscopy method has been claimed effective to authenticate patin fish oil with palm oil as an adulterant (Putri *et al*., 2019), cod liver oils from other fats and oils as adulterants (Rohman and Che Man, 2009). Besides that, it was also claimed effective to authenticate virgin oils with paraffin oils as an adulterant (Amit *et al.*, 2020) and avocado oil in ternary mixtures with sunflower and soybean oils (Jiménez-Sotelo *et al.*, 2016). However, the study related to the simultaneous analysis of 2 different fish oils in a mixture has not been conducted. The novelty of this research was to perform simultaneous analysis between 2 different fish oil in a mixture, which was never conducted before.

Furthermore in this research, FTIR spectroscopy combined with PLS and PCR methods was used to conduct a simultaneous quantitative analysis of PFO and BFO. Based on the literature review process, there were no sufficient findings related to combining FTIR spectroscopy with PLS and PCR for quantitative analysis of PFO and BFO simultaneously. Hence, the main objective of this research was to quantitatively analyze and optimize PFO and BFO using FTIR spectroscopy combined with PLS and PCR simultaneously.

2. Materials and methods

Bandeng fish oils and patin fish oils were obtained from a local fish breeder in Pati, Central Java, Indonesia. Hexane and acetone pro-analytical grades for sampling cleaner were purchased from E. Merck (Darmstadt, Germany).

2.1 Sample preparation

Both fish were descaled, filleted, and cleaned. Three kilograms of clean fish flesh were placed in an aluminium tray. Samples were dried using a cabinet dryer at 50°C for 24 hrs. The extraction method was adapted from the previously reported method by Honold *et al*. (2016). Dried samples were placed into a pressing chamber covered with a filter cloth. The pressing process was performed using 100 kN force for 2 mins. The extracted oil was centrifugated at 5000×*g* for 10 mins to separate the sediment.

2.2 Preparation of calibration and validation samples

PFO and BFO were mixed into binary mixtures with accumulated levels of 100%. The total volume of binary mixtures was 2 mL, while the total amount of samples was 25. For instance, sample 1 was composed of 96.5% of PFO and 3.5% BFO. The validation samples were prepared independently using the same spanned concentration from 0-100 % as in calibration samples. The selection of its percentage in Table 1 was based on random order as suggested by Excel software (Microsoft Inc., USA).

Table 1. Sample composition between PFO and BFO percentage

Sample code	% PFO	% BFO
1	96.5	3.5
2	62.0	38.0
3	48.0	52.0
4	95.5	4.5
5	93.0	7.0
6	25.5	74.5
7	17.5	82.5
8	28.5	71.5
9	34.0	66.0
10	80.5	19.5
11	10.5	89.5
12	67.0	33.0
13	87.5	12.5
14	17.5	82.5
15	0.0	100.0
16	85.0	15.0
17	36.0	64.0
18	57.5	42.5
19	5.0	95.0
20	40.5	59.5
21	100.0	0.0
22	66.5	33.5
23	21.5	78.5
24	12.5	87.5
25	10.5	89.5

2.3 FTIR spectroscopy analysis

All samples were scanned using an FTIR spectrophotometer (Thermo Scientific Nicolet iS10, Madison, WI) according to Irnawati *et al.* (2020). Meanwhile, the obtained spectra were processed using Omnic software. The samples were measured in 11 different wavenumber regions between 4000–650 cm^{-1} with a resolution of 16 cm^{-1}, and scanning numbers of 25 replicates (2 for calibration and 1 for validation). Horizontal Attenuated Total Reflectance (HATR) composed of ZnSe crystal was used as used sampling accessory. A correcting action was carried out by scanning a new reference air as background after every sample scanning. Triplicate data points were used to make a correlation between the predicted value and FTIR spectra. The spectra data were recorded as absorbance values.

2.4 Data analysis

TQ analyst 9.7.0.27 (Thermo Fisher Scientific Inc.)

software was used for chemometrics analysis. Partial least square (PLS) and principal component regression (PCR) were used to make a correlation between actual values of PFO and BFO with predicted values and using FTIR spectra. For quantitative analysis, 25 mixtures of samples containing PFO and BFO were analysed using spectral regions between 4000 cm^{-1} to 650 cm^{-1}. The normal, 1st derivative and 2nd derivative spectra were observed to increase spectral resolution. The root mean square error of calibration (RMSEC), root mean square error of cross prediction (RMSEP), coefficient of determination for calibration (R_{cal}^2), and coefficient of determination for validation (R_{val}^2) were calculated using, TQ analyst software.

3. Results and discussion

Patin fish oil (PFO) and bandeng fish oil (BFO) were chosen based on their popularity in Indonesia and their nutritional values (Sugata *et al.*, 2019; Ilza and Sukmiwati, 2020). PFO contains high omega-3 while BFO contains high omega-6. The mixture between PFO and BFO was predicted to be developed as a dietary supplement in the future. Sample binary mixtures were scanned in wavenumber regions between 4000 cm^{-1} and 650 cm^{-1}. Figure 1 reveals FTIR spectra of patin fish oil (PFO) and bandeng fish oil (BFO) at mid infrared region (4000-650 cm^{-1}). Based on their spectra, peaks at 3007 cm^{-1} was indicating [=C-H cis (stretching)], 2953 cm^{-1} [-CH_3 (asymmetric stretching)], 2922 cm^{-1} [-CH_2 (asymmetric stretching)], 2852 cm^{-1} [-CH_2 (symmetric stretching)], 1744 cm^{-1} [-C=O (ester stretching)], 1652 cm^{-1} [-C=C cis (stretching)], 1463 cm^{-1} [-CH_2 (bending)], 1377 cm^{-1} [-CH_3 (bending)], 1238, 1117, 1163, 1098, 1031 cm^{-1} [-C-O (stretching)], 966 cm^{-1} [HC=CH- trans (out of plane)], 913 cm^{-1} [- HC=CH- cis (out of plane)], and 722 cm^{-1} [$(-CH_2)_n$ cis] (Che Man *et al.*, 2011).

Optimization of partial least square regression (PLS) and principal component regression (PCR) can be observed in Table 2 and Table 3. The selected method for (PFO) simultaneous analysis was based on the highest values of coefficient of determination for calibration (R_{cal}^2) and coefficient of determination for validation (R_{val}^2), followed by the lowest values of root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) in Table 2. The first derivative FTIR spectra at wavenumbers of 3050-2800 and 1800-1700 and 1500-650 cm^{-1} were the highest R_{cal}^2 values (0.9991); R_{val}^2 (0.9990); followed by the lowest RMSEC (0.0135); and the lowest RMSEP (0.0146) using PCR. Moreover, using PLS as the selection method resulted in the first derivative FTIR spectra at wavenumbers of 3050-2800 and 1800-1700 and 1500-650. The R_{cal}^2; R_{val}^2; RMSEC; and RMSEP value was 0.9998; 0.9994; 0.0072; and 0.0121, respectively. Meanwhile, the selected method for (BFO) simultaneous analysis was based on the highest values of coefficient of determination for calibration (R_{cal}^2) and coefficient of determination for validation (R_{val}^2), followed by the lowest values of root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) in Table 3. Similar to PFO above, the first derivative FTIR spectra at wavenumbers of 3050 -2800 and 1800-1700 and 1500-650 cm^{-1} were the highest R_{cal}^2 values (0.9991); R_{val}^2 (0.9990); followed by the lowest RMSEC (0.0135); and the lowest RMSEP (0.0146) using PCR. Furthermore, using PLS as the selection method resulted in the first derivative FTIR spectra at wavenumbers of 3050-2800 and 1800-1700 and 1500-650. The R_{cal}^2; R_{val}^2; RMSEC; and RMSEP value was 0.9998; 0.9994; 0.0072; and 0.0121, respectively.

Putri *et al.* (2019) conducted an authentication of patin fish oil from palm oil using FTIR spectroscopy combined with chemometrics. The method provided perfect discrimination (100%) of patin fish oil from palm oil with low errors (RMSEC = 0.805 and RMSEP = 2.22) and high accuracy ($R^2 > 0.999$). This research has confirmed that FTIR spectroscopy combined with chemometrics (PLS and PCR) can be used as a

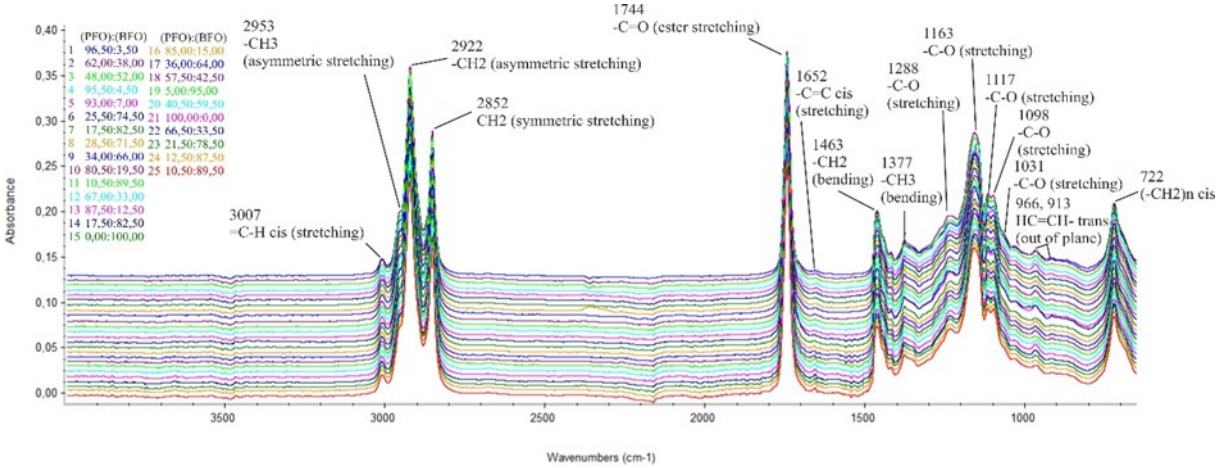

Figure 1. FTIR Spectra from binary mixtures (PFO) and (BFO)

Table 2. Optimization of principal component regression (PCR) dan partial least regression (PLS) methods to determine (PFO) component in different wavelength areas for simultaneous analysis.

Multivariate Calibration	Wavenumber (cm^{-1})	Spectra	Calibration		Validation	
			RMSEC	R_{cal}^2	RMSEP	R_{val}^2
PCR	3050-650	Normal	0.0227	0.9976	0.0195	0.9984
		1st derivative	0.0148	0.9990	0.0190	0.9984
		2nd derivative	0.0301	0.9957	0.0434	0.9921
	3050-2800 and 1800-1700	Normal	0.0203	0.9981	0.0265	0.9970
		1st derivative	0.0248	0.9971	0.0354	0.9943
		2nd derivative	0.0400	0.9924	0.0636	0.9808
	3050-2800 and 1500-650	Normal	0.0186	0.9984	0.0164	0.9988
		1st derivative	0.0130	0.9992	0.0177	0.9987
		2nd derivative	0.0215	0.9978	0.0268	0.9969
	3050-2800 and 1800-1700 and 1500-650	Normal	0.0215	0.9978	0.0240	0.9974
		1st derivative	0.0135	0.9991	0.0146	0.9990
		2nd derivative	0.0188	0.9983	0.0219	0.9978
PLS	3050-650	Normal	0.0269	0.9966	0.0285	0.9965
		1st derivative	0.0075	0.9997	0.0142	0.9991
		2nd derivative	0.0073	0.9997	0.0303	0.9962
	3050-2800 and 1800-1700	Normal	0.0644	0.9802	0.0871	0.9640
		1st derivative	0.0184	0.9984	0.0340	0.9946
		2nd derivative	0.1810	0.8308	0.193	0.8043
	3050-2800 and 1500-650	Normal	0.0235	0.9974	0.0243	0.9974
		1st derivative	0.0104	0.9995	0.0156	0.9991
		2nd derivative	0.0142	0.999	0.0241	0.9975
	3050-2800 and 1800-1700 and 1500-650	Normal	0.0236	0.9974	0.0200	0.9982
		1st derivative	***0.0072***	***0.9998***	***0.0121***	***0.9994***
		2nd derivative	0.0061	0.9998	0.0180	0.9985

The selection condition was marked with italicized bold

promising method to analyse two different oil samples simultaneously with high accuracy. Errors in Putri *et al.* (2019) research were slightly higher than in this research. It may be caused by the differences in fatty acid composition between patin fish oil and palm oil. The patin fish oil was composed mainly of unsaturated fatty acid, however, palm oil is mainly composed of saturated fatty acid (Putri *et al*., 2019). Compared with this research, both PFO and BFO are mainly composed of unsaturated fatty acids, so PFO and BFO have similar spectra to patin fish oil and palm oil.

Figure 2 presents the actual and calculated values correlation of PFO and BFO binary mixtures. Based on the chart, the errors that occurred in modelling were distributed randomly along the correlation line. Moreover, the errors obtained were not systematic. The highest values R_{cal}^2 and R_{val}^2, but the lowest values of RMSEC and RMSEP indicate the selected method performed accurate results for the analysis of PFO and BFO binary mixture simultaneously (Miller and Miller, 2018).

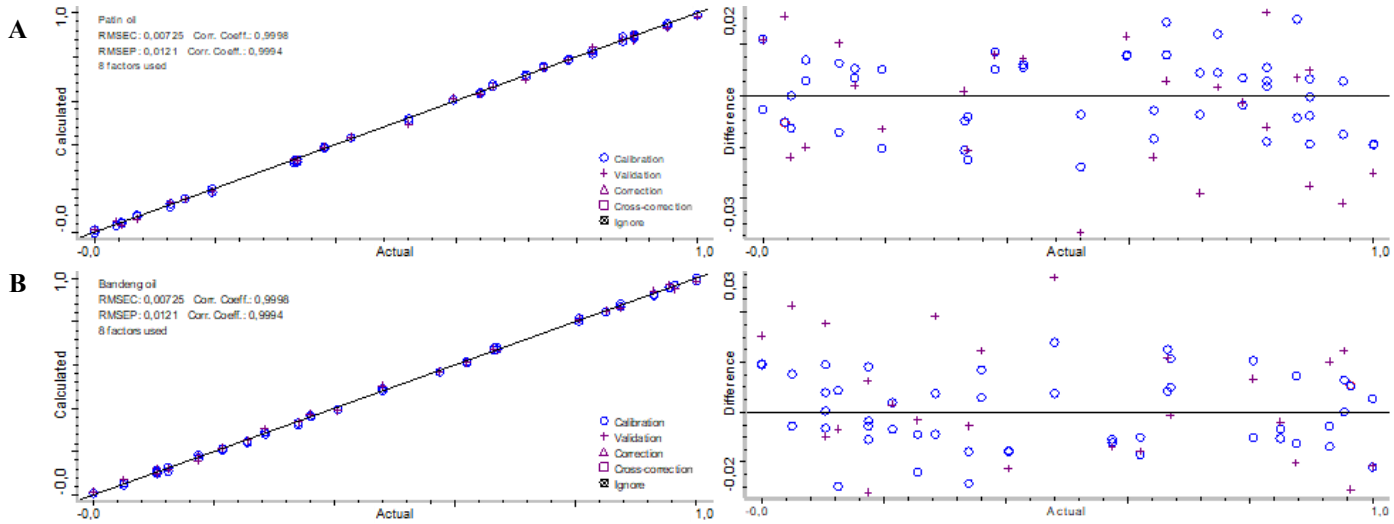

Figure 2. The correlation charts between actual and calculated of [A] (PFO), [B] (BFO) in PFO-BFO binary mixture.

Table 3. Optimization of principal component regression (PCR) dan partial least regression (PLS) methods to determine (BFO) component in different wavelength areas for simultaneous analysis.

Multivariate Calibration	Wavenumber (cm^{-1})	Spectra	Calibration		Validation	
			RMSEC	R_{cal}^2	RMSEP	R_{val}^2
PCR	3050-650	Normal	0.0227	0.9976	0.0195	0.9984
		1st derivative	0.0148	0.9990	0.0190	0.9984
		2nd derivative	0.0301	0.9957	0.0434	0.9921
	3050-2800 and 1800-1700	Normal	0.0203	0.9981	0.0265	0.9970
		1st derivative	0.0248	0.9971	0.0354	0.9943
		2nd derivative	0.0400	0.9924	0.0636	0.9808
	3050-2800 and 1500-650	Normal	0.0186	0.9984	0.0164	0.9988
		1st derivative	0.0130	0.9992	0.0177	0.9987
		2nd derivative	0.0215	0.9978	0.0268	0.9969
	3050-2800 and 1800-1700 and 1500-650	Normal	0.0215	0.9978	0.0240	0.9974
		1st derivative	0.0135	0.9991	0.0146	0.9990
		2nd derivative	0.0188	0.9983	0.0219	0.9978
PLS	3050-650	Normal	0.0269	0.9966	0.0285	0.9965
		1st derivative	0.0075	0.9997	0.0142	0.9991
		2nd derivative	0.0073	0.9997	0.0303	0.9962
	3050-2800 and 1800-1700	Normal	0.0644	0.9802	0.0871	0.9640
		1st derivative	0.0184	0.9984	0.0340	0.9946
		2nd derivative	0.1810	0.8308	0.1930	0.8043
	3050-2800 and 1500-650	Normal	0.0235	0.9974	0.0243	0.9974
		1st derivative	0.0104	0.9995	0.0156	0.9991
		2nd derivative	0.0142	0.9990	0.0241	0.9975
	3050-2800 and 1800-1700 and 1500-650	Normal	0.0236	0.9974	0.0200	0.9982
		1st derivative	***0.0072***	***0.9998***	***0.0121***	***0.9994***
		2nd derivative	0.0061	0.9998	0.0180	0.9985

The selection condition was marked with italicized bold

4. Conclusion

The combination of Fourier transform infrared spectroscopy (FTIR) with partial least square (PLS) and principal component regression (PCR) had successfully been used for simultaneous analysis of binary mixtures of patin fish oil (PFO) and bandeng fish oil (BFO). The simultaneous analysis method was based on variable absorbances values of the first derivative spectra at 3050-2800, 1800-1700, and 1500-650 cm^{-1} for PFO and BFO.

Conflict of interest

The authors declare no conflict of interest.

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