

The authentication of catfish oil (*Clarias gariepinus*) from patin fish oil using FTIR spectra and chemometrics

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Abstract

Catfish is a freshwater fish widely cultivated in Indonesia. The high production of catfish creates an opportunity for the utilization of catfish as a source of raw materials for fish oil. Catfish oil is reported to contain high polyunsaturated fatty acids (PUFAs) having good benefits on human health and high price in the market, therefore CFO is subjected to adulteration practice. The purpose of this study was to authenticate catfish oil (CFO) from the oil adulterant of Patin fish oil (PFO) using FTIR spectroscopy and chemometrics. CFO was extracted by the dry rendering method combined with pressing methods. Classification of fish oils using principal component analysis (PCA) was successful for separating CFO from other fish oils. Multivariate calibration of partial least squares (PLS) gives the best quantitation model at wavenumbers 4000-600 cm⁻¹ applying the second derivative spectra model with an R² value of 0.9999; RMSEC 0.0041; and RMSEP 0.0087. FTIR spectrophotometry combined with chemometrics provides fast and accurate results for the authentication of CFO.

1. Introduction

Based on FAO, The State of World Fisheries and Aquaculture 2016, Indonesia is the largest aquaculture producer in Southeast Asia and the third largest world production after China and India. This shows that Indonesia has a big influence on increasing the competitiveness and acceptability of aquaculture products in regional and international markets. Catfish is one of the freshwater fish commodities widely cultivated in Indonesia due to its nutritional content, in which each 100 g of catfish contains 18 g protein, 2.9 g fat, minerals and vitamins (Snyder, 2023). The high production of catfish creates an opportunity for the utilization of catfish as a source of raw materials for producing catfish fish oil (CFO) (Ferazuma *et al.*, 2011). CFO is known to contain 40.32% saturated fatty acids and 59.65% unsaturated fatty acids (Pandiangan *et al.*, 2020). The presence of unsaturated fatty acids, mainly polyunsaturated fatty acids (PUFAs), has several beneficial effects on human health, including helping the process of brain development (intelligence), the development of the sense of sight, and the immune system of infants and toddlers.

In addition, PUFAs also have an important role in preventing some diseases, such as lowering cholesterol, preventing heart disease, hypertension, arthritis, other inflammatory, and autoimmune diseases (Zaloga, 2021).

Due to the high price of CFO in the fats and oils industry, the adulteration practice may take place by substituting CFO with other edible oils (fish oils or vegetable oils) to get the economic gains. Adulteration of oil is difficult to detect if the adulterated oil is similar in chemical composition and color to the original oil. As a result, the verification of edible fats and oils must be done through reliable and fast analysis methods (Ye and Meng, 2022). Several analytical methods have been successfully developed and widely used to detect counterfeiting practices, most notably molecular spectroscopy and chromatographic analysis, including fluorescence spectroscopy (Jiménez-Carvelo *et al.*, 2019), vibrational spectrometry (Rohman and Che Man, 2011; Cozzolino, 2014), nuclear magnetic spectroscopy (Triyasmono *et al.*, 2022), high-performance liquid chromatography (Carranco *et al.*, 2018), and gas chromatography (Schipilliti *et al.*, 2011).

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A popular method approach for the determination of adulteration effects is to apply physical analysis methods to determine the effects of adulteration on the physicochemical properties of fish oil, especially if the instrument is equipped with sophisticated data processing, such as multivariate or chemometric data analysis (Rohman *et al.*, 2021). One type of vibrational spectroscopy, FTIR, has succeeded in becoming a promising and advantageous method for oil and fat authentication, as the analysis is fast, reliable, and simple in sample preparation and non-destructive or considered as a green analytical chemistry (Rohman *et al.*, 2021). Furthermore, FTIR spectra can provide useful information about the molecular composition, structure, and interactions in a sample (Li *et al.*, 2018).

The FTIR capability, known as the "fingerprint spectral technique", is an ideal technique to distinguish between original oil samples and adulterants. Furthermore, the combination of principal component analysis (PCA), partial least-squares (PLS), principal component regression (PCR), multivariate calibration chemometrics and attenuated total reflection (ATR) FTIR spectroscopy results in a rapid authentication technique. This combination of techniques has successfully authenticated various oils, including cod liver oil (Rohman and Che Man 2009), red fruit oil (Rohman *et al.*, 2011), patin fish oil (Putri *et al.*, 2020) and edible oil (Ye and Meng, 2022). Therefore, this study aims to authenticate catfish contaminated with Patin fish oil (*Pangasius micronemus*) using FTIR spectroscopy combined with chemometrics. In addition, the PLS calibration technique is used for quantitative analysis.

2. Materials and methods

The tools used in this study were glassware (Pyrex), microtube (Effendorf, Biologix), digital analytical balance (Mettler Toledo, Ohaus), 15 mL conical (Iwaki), hotplate magnetic stirrer (Stuart CB162), magnetic bar, micro pipette (Effendorf), ultrasonicator (J.P. Selecta), vortex mixer (Thermolyne), blue tip, yellow tip, white tip (Biologix and Effendorf), pH meter (HANNA), Brookfield DV-I Prime viscometer, Konica Minolta CR-Colorimeter 400 (Minolta, Spectrophotometer, USA), Thermo Scientific iS10 FTIR spectrophotometer (Thermo Fisher Scientific, USA), Vis-Nirs SpectraStar XT-R spectrophotometer (KPM Analytics, USA).

2.1 Sample preparation

Catfish, previously identified in the Biology Laboratory of the Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia was cleaned by

removing any components which can interfere with the extraction process, such as dirt, for maximizing the extraction process. The preparation process includes cleaning the intestines of the fish, removing the fins, cutting the size of a fist, and washing all the fish samples. The fish is cut to the size of a finger to maximize the contact area of the fish sample with the heat from the cabinet dryer during fish oil extraction (Nur Ikhsan *et al.*, 2021).

2.2 Extraction of catfish oil

Extraction of catfish oil (CFO) was carried out using the dry rendering method, namely drying with a cabinet dryer and pressing with a hydraulic press. Samples that have been dried with a cabinet dryer are then wrapped in a filter cloth to remove any impurities during the pressing process. The cloth used must be clean and have pores that allow oil to escape from the pores of the filter cloth. After the sample was properly wrapped, the filter cloth containing the sample was placed in the press cylinder. The pressed cylinder was then mounted on the hydraulic press and then tightened by turning the lever on the top of the machine until it locks. The tool was then turned on, and the force used is 100 kN for 2 min. The extraction process was characterized by a hydraulic press that would press the sample down and force the oil present in the sample to come out of the cylinder side. The oil obtained was then collected in a dark container and then weighed (Nur Ikhsan *et al.*, 2021).

2.3 Catfish oil refining

Refining was carried out by mixing 10 g of catfish oil with 1 g of anhydrous sodium sulfate (Na_2SO_4) in a plastic Falcon tube. Then, after homogenization with a vortex for 5 min to ensure that the impurities and water are completely bound. Precipitation was carried out using centrifugation at 5000 rpm for 15 min. Then, the oil was separated from the sediment using a pipette, and the purified oil was transferred to a new Falcon container (Nadhiro *et al.*, 2018). Subsequently, the treatment was carried out using an absorbent, specifically bentonite, which had been activated by heating at 250°C for approximately 1 hour. Catfish oil that has been added with sodium sulfate (Na_2SO_4), then bentonite with a concentration of 2% by weight of the oil. The oil is then stirred at 500 rpm while heated to 40°C for 30 min. The oil was filtered, and the weight of the resulting oil was measured. The oil obtained was pure catfish oil (Indah *et al.*, 2022).

2.4 Authentication of catfish oil

The analysis of adulteration of catfish oil with Patin Fish oil (PFO) was carried out using FTIR spectroscopy in the region of 4000-400 cm^{-1} to obtain data related to

functional groups from the samples tested. Each oil was tested on an FTIR spectrophotometer in the MIR region of 4000-650 cm^{-1} . The absorbance results for each wave number were analyzed using principal component analysis. Oil with a close score plot to catfish oil was used as a counterfeiting agent in this study (Putri *et al.*, 2020).

2.5 Sample preparation for validation and calibration

The sample used was a mixture of CFO and PFO with a concentration range of 0-100%. The mixing was carried out using a calibrated micropipette and shaken to ensure homogeneity. Then the samples were tested on FTIR spectrophotometer in the MIR region of 4000-650 cm^{-1} . Replication was carried out 3 times and recorded in the form of absorbance.

2.6 Data analysis

The results of authentication with oil adulterants were processed using the Minitab software. The quantitative analysis was carried out using the TQ Analyst software included in the FTIR spectrophotometer.

3. Results and discussion

The purpose of the authentication of catfish oil is to ensure the authenticity of catfish oil and prevent adulteration of catfish oil with other oils. In order to detect the existence of adulterating practices, this research created several models for authentication. Authentication of catfish oil was carried out by preparing a mixture of catfish oil and oil that had similarities with the FTIR spectra. Figure 1 FTIR spectra of various types of fish oil. Spectral peaks were identified at various wave numbers to determine the types of vibrations and functional groups in the oil. The determination of wave number is based on the peak wave number in the functional group (Rohman and Che Man, 2009). This study took the wave number adapted from the research by Putri *et al.* (2020) and adjusted for the peak wave number in the sample, which can be seen in Table 1.

Table 1. CFO spectral wavenumber and functional groups adapted from Putri *et al.* (2020).

Wavenumber (cm^{-1})	Functional Groups
3007	Cis- Stretching C = H
2921	Asymmetric Stretching -CH ₂
2852	Symmetric Stretchin-CH ₂
1743	Stretching -C = O
1461	Bending -CH ₂
1376	Bending -CH ₃
1234, 1158, 1116, 1097	Stretchin -C-O
965	Bending HC=CH Trans
721	Bending -CH ₂

The PCA method is a technique that can be used to classify samples based on the CFO variation that occur between samples (Miller and Miller, 2010). The variable used in PCA is the absorbance at a certain wavelength. PCA analysis aims to group correlated variables and replace them with new groups called main components (Rohman *et al.*, 2011). Based on PCA, an oil that is most similar to catfish oil will be obtained, which will then be used as a counterfeiting oil. FTIR spectral readings were carried out on all types of fish oil, consisting of catfish oil, patin fish oil, keting fish oil and pomfret oil. The absorbance value in the wave number resulting from the FTIR reading will be a variable entered in the Minitab software for PCA analysis. Grouping of samples was carried out based on Principle Component (PC) 1 and PC 2. PC 1 is the component that represents the greatest variation in the original variable, while PC 2 is the component that represents the second largest variation after PC 1. The X axis is PC 1, while the Y axis is PC 2. PCA images can be seen in Figure 2.

From the results of the PCA analysis, it was found that catfish oil had a plot score value that was closest to Patin Fish Oil (PFO). The profile similarity between catfish oil and catfish oil is shown in Figure 2, with plot score values based on their position. The difference in the distance between the plots of each oil shows the degree of similarity between the oils. The closer the distance between the oil plots, the greater the similarity. It is possible for plots that are at a close distance to be

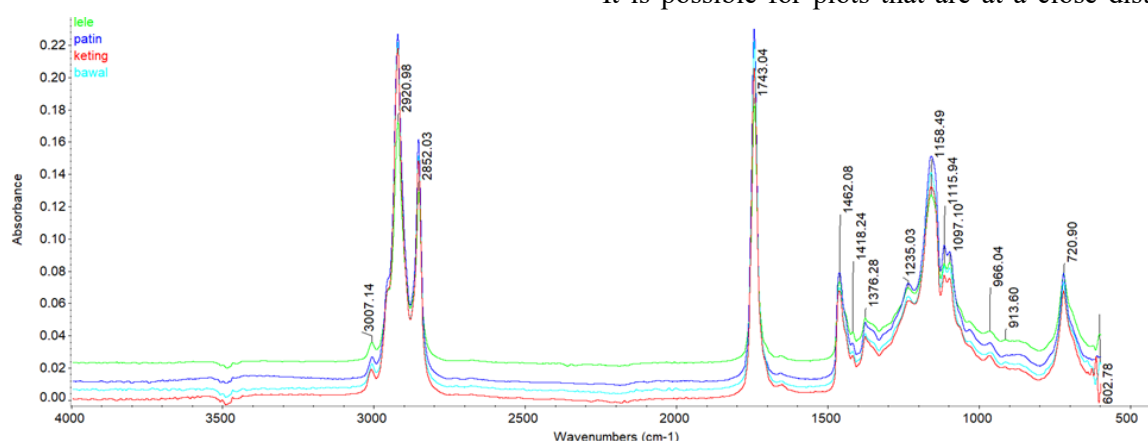


Figure 1. FTIR spectra of various types of fish oil.

joined in the same group. It can be seen in Figure 2 that catfish oil and patin fish oil are in the same quadrant, so patin fish oil was chosen as the oil adulterant.

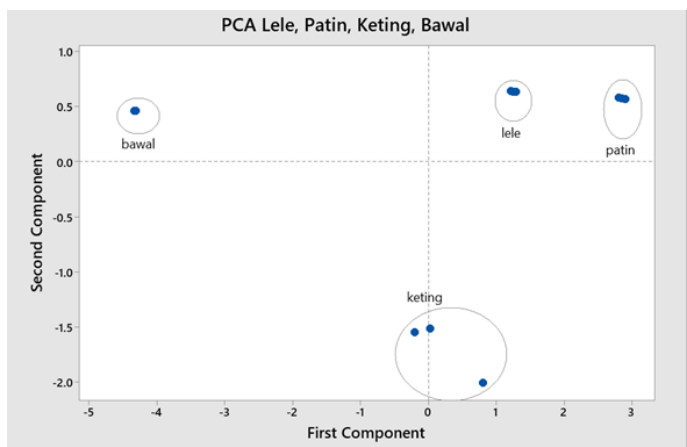


Figure 2. PCA results of various types of fish oil.

3.1 Quantitative analysis

The calibration curve was prepared by preparing a binary mixture of catfish oil and catfish oil samples with different concentrations ranging from 0-100%. Each sample was then measured for its intensity using FTIR spectroscopy. The spectra obtained were then processed using the TQ AnalystTM software to create PLS and PCR calibration models and to create a validation model with the same sample. The PLS and PCR models were constructed using the normal spectra and the first and second derivative spectra. The aim of the derivatization is to increase the sensitivity of the reading by eliminating the spectral interference. The first derivative aims to increase the spectral resolution and simplify the selection of the baseline, while the second derivative eliminates the wide-band absorption. The spectral area is optimized by selecting a specific spectral region that has an important wave number on the absorbance of functional group bonds related to the characteristics of snakehead fish oil and palm oil. In the PLS and PCR calibration techniques, linearity is used as an evaluation to see the relationship between absorbance and sample concentration. The statistical parameters observed were the value of the coefficient of determination (R^2), root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP). A high R^2 value with low RMSEC and RMSEP values indicates

success in the regression model (Putri *et al.*, 2020). The results of the analysis of the PLS and PCR calibration model of a mixture of catfish oil in catfish oil at various wave numbers can be seen in Table 2.

PLS calibration at wave number 4000-600 cm^{-1} with the second derivative spectra model shows the most reliable results for quantification of catfish oil with a high R^2 value of 0.9999. In addition, this wave number gives low RMSEC and RMSEP values, namely 0.0041 and 0.0087. A high R^2 value indicates a good correlation between the actual concentration and the predicted concentration. So that this method can be used to accurately quantify the actual levels in samples, both catfish oil and catfish oil samples. Figure 3 shows the relationship between the actual content and the predicted level of catfish oil expressed in the equation $y = 0.9721x + 0.0071$.

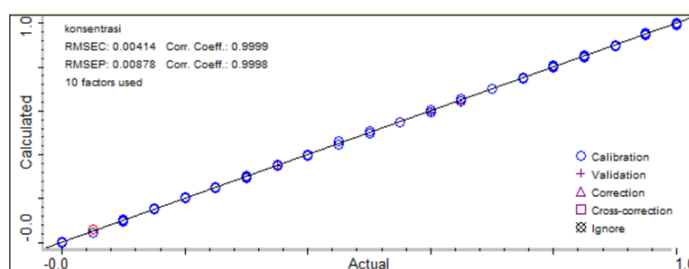


Figure 3. PLS CFO calibration and validation results.

4. Conclusion

CFO authentication from PFO as adulterant can be successfully done with PCA capable of separating CFO from counterfeiting agents properly. Multivariate calibration on FTIR gives the best quantitation model using the PLS technique at wave numbers 4000-600 cm^{-1} with the second derivative spectra model, with an R^2 value of 0.9999; RMSEC 0.0041; and RMSEP 0.0087. The developed method is rapid and reliable for the authentication of the CFO from the PFO.

Conflict of interest

The authors have no conflicts of interest regarding this investigation.

Table 2. Results of analysis of the PLS and PCR calibration models mixed with CFO in PFO.

Multivariate Calibration	Wavenumber	Spectrum	Calibration		Prediction	
			RMSEC	R^2	RMSEP	R^2
PLS	4000-600	Normal	0.0163	0.9986	0.0083	0.9997
		1 st derivate	0.0069	0.9997	0.0312	0.9976
		2nd derivate	0.0041	0.9999	0.0087	0.9998
PCR	4000-600	Normal	0.0868	0.9623	0.0218	0.9989
		1 st derivate	0.0882	0.9618	0.0190	0.9986
		2 nd derivate	0.0875	0.9622	0.0196	0.9988

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References

- Carranco, N., Farrés-Cebrián, M., Saurina, J. and Núñez, O. (2018). Authentication and quantitation of fraud in extra virgin olive oils based on HPLC-UV fingerprinting and multivariate calibration. *Foods*, 7 (4), 44. <https://doi.org/10.3390/foods7040044>
- Cozzolino, D. (2014). An overview of the use of infrared spectroscopy and chemometrics in authenticity and traceability of cereals. *Food Research International*, 60, 262-265. <https://doi.org/10.1016/j.foodres.2013.08.034>
- Ferazuma, H., Marliyati, S.A. and Amalia, L. (2011). Substitusi tepung kepala ikan lele dumbo (*Clarias gariepinus* sp.) untuk meningkatkan kandungan kalsium crackers. *Jurnal Gizi Dan Pangan*, 6(1), 18-27. <https://doi.org/10.25182/jgp.2011.6.1.18-27> [In Bahasa Indonesia].
- Indah, Lestari, L.A. and Rohman, A. (2022). Physicochemical characterization refined patin fish oil (*Pangasius micronema*) using bentonite and activated carbon. *Journal of Food and Pharmaceutical Sciences*, 10(2), 626–633. <https://doi.org/10.22146/jfps.4682>
- Jiménez-Carvelo, A.M., Valeria A., Lozano, V.A. and Olivieri, A.C. (2019). Comparative chemometric analysis of fluorescence and near infrared spectroscopies for authenticity confirmation and geographical origin of argentinean extra virgin olive oils. *Food Control*, 96, 22-28. <https://doi.org/10.1016/j.foodcont.2018.08.024>
- Li, Q., Chen, J., Huyan, Z., Kou, Y., Xu, L., Yu, X. and Gao, J.M. (2018). Application of fourier transform infrared spectroscopy for the quality and safety analysis of fats and oils: a review. *Critical Reviews in Food Science and Nutrition*, 59(22), 3597-3611. <https://doi.org/10.1080/10408398.2018.1500441>
- Miller, J.N. and Miller, J.C. (2010). Statistics and chemometrics for analytical chemistry. 6th ed. Harlow: Prentice Hall/Pearson. UK: Pearson Education Limited
- Nur Ikhsan, A., Rohman, A., Putri, A.R., Syifa, F., Mustafidah, M. and Martien, R. (2021). Application of FTIR spectroscopy and chemometrics for the prediction of radical scavenging activities of fish oils. *Indonesian Journal of Pharmacy*, 32(2), 166–174. <https://doi.org/10.22146/ijp.1004>
- Nadhiro, U., Subekti., Tjahjaningsih, W. and Patmawati. (2018). Quality characteristics of bali sardinella (*Sardinella lemuru*) oil purified with bentonite as an adsorbent. *IOP Conference Series: Earth and Environmental Science*, 137, 012012. <https://doi.org/10.1088/1755-1315/137/1/012012>
- Pandiangan, M., Kaban, J., Wirjosentono, B. and Silalahi, J. (2020). Analisis asam lemak omega 3 dan 6 pada minyak ikan lele secara GC-FID. *Jurnal Riset Teknologi Pangan dan Hasil Pertanian (Retipa)*, 1, 22–29. <https://doi.org/10.54367/retipa.v1i1.908> [In Bahasa Indonesia].
- Putri, A.R., Rohman, A., Riyanto, S. and Setyaningsih, W. (2020). Authentication of patin fish oil (*Pangasius micronemus*) using FTIR spectroscopy combined with chemometrics. *Indonesian Journal of Chemometrics and Pharmaceutical Analysis*, 1(1), 22-27. <https://doi.org/10.22146/ijcpa.487>
- Rohman, A. and Che Man, Y.B. (2009). Analysis of cod-liver oil adulteration using fourier transform infrared (FTIR) spectroscopy. *Journal of the American Oil Chemists Society*, 86(12), 1149–1153. <https://doi.org/10.1007/s11746-009-1453-9>
- Rohman, A. and Che Man, Y.B. (2011). The use of fourier transform mid infrared (FT-MIR) spectroscopy for detection and quantification of adulteration in virgin coconut oil. *Food Chemistry*, 129(2), 583–588. <https://doi.org/10.1016/j.foodchem.2011.04.070>
- Rohman, A., Che Man, Y.B. and Riyanto, S. (2011). Authentication Analysis of Red Fruit (*Pandanus Conoideus* Lam) Oil Using FTIR Spectroscopy in Combination with Chemometrics. *Phytochemical Analysis*, 22(5), 462-467. <https://doi.org/10.1002/pca.1304>
- Rohman, A., Ghazali, M.A.B., Windarsih, A., Irnawati, Riyanto, S., Yusof, F.M. and Mustafa, S. (2020). Comprehensive review on application of FTIR spectroscopy coupled with chemometrics for authentication analysis of fats and oils in the food products. *Molecules*, 25(22), 5485. <https://doi.org/10.3390/molecules25225485>
- Rohman, A., Putri, A.R., Irnawati, Windarsih, A., Nisa, K. and Lestari, L.A. (2021). The employment of analytical techniques and chemometrics for authentication of fish oils: a review. *Food Control*, 124, 107864. <https://doi.org/10.1016/j.foodcont.2021.107864>
- Schipilliti, L., Dugo, G., Santi, L., Dugo, P. and Mondello, L. (2011). Authentication of bergamot essential oil by gas chromatography-combustion-isotope ratio mass spectrometer (GC-LC-IRMS). *Journal of Essential Oil Research*, 23(2), 60-71.

- <https://doi.org/10.1080/10412905.2011.9700447>
- Snyder, C. (2023). Is catfish healthy? Nutrients, benefits, and more. Retrieved on January 20, 2024 from Healthline website: <https://www.Healthline.com/nutrition/Is-Catfish-Healthy>.
- Triyasmono, L., Schollmayer, C. and Holzgrabe, U. (2023). Chemometric analysis applied to ^1H NMR and FTIR data for a quality parameter distinction of red fruit (*Pandanus Conoideus*, Lam.) oil products. *Phytochemical Analysis*, 34(7), 788-799. <https://doi.org/10.1002/pca.3196>
- Ye, Q. and Meng, X. (2022). Highly efficient authentication of edible oils by FTIR spectroscopy coupled with chemometrics. *Food Chemistry*, 385, 132661. <https://doi.org/10.1016/j.foodchem.2022.132661>
- Zaloga, G.P. (2021). Narrative review of n-3 polyunsaturated fatty acid supplementation upon immune functions, resolution molecules and lipid peroxidation. *Nutrients*, 13(2), 662. <https://doi.org/10.3390/nu13020662>