Rapid detection of tuna fish oil adulteration using FTIR-ATR spectroscopy and chemometrics for halal authentication

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ABSTRACT

High-quality Tuna fish oil (TFO) is vulnerable to adulteration with lower-quality oil such as pork oil (PO) by unethical players for economic reasons. PO is a nonhalal oil that is prohibited to be consumed by certain religions such as Muslim and Jewish. This research aimed to develop rapid authentication methods using Fourier transform infrared (FTIR) spectroscopy combined with chemometrics for the detection of PO in TFO. FTIR spectroscopy provided fingerprint spectra for TFO and PO. TFO demonstrated two peaks at the carbonyl vibration. The peak at 1,710 cm⁻¹ was specific to TFO whereas the peak at 1,032 cm⁻¹ was specific to PO. Partial least square–discriminant analysis (PLS-DA) and orthogonal projections to latent structures–discriminant analysis (OPLS-DA) were successfully used to detect PO adulteration in TFO by discriminating and classifying TFO and TFO adulterated PO, and PO samples with OPLS-DA showed high accuracy (R²X = 0.998, R²Y = 0.735) and good predictivity (Q² = 0.552). Meanwhile, the concentration of PO in the mixtures of TFO-PO was successfully predicted using PLS and OPLS with high accuracy (R² = 0.9928) and good precision. OPLS indicated a better model than PLS with lower error evaluated using root mean square error of estimation (RMSEE) and root mean square error of cross-validation (RMSECV). The obtained RMSEE and RMSECV were 2.81 and 2.94, respectively. This developed method could detect the lowest PO (5%) presented in TFO. Therefore, it can be concluded that there was an analytical technique based on FTIR spectra combined with principal component analysis, PLS-DA, and OPLS-DA could be used for halal authentication of TFO. Further, it can be developed for halal authentication of fish oils.

INTRODUCTION

Tuna fish has been widely consumed worldwide due to its high nutrition required for human health. The oil from tuna fish, called TFO, contains various nutritional properties that make it valuable. TFO contains monounsaturated fatty acids and polyunsaturated fatty acids which are important for human nutrition. The oil also rich in omega-3 such as docosahexaenoic acid and eicosapentaenoic acid (Suseno et al., 2014). The nutrition
Food adulteration becomes a serious issue because it decreases the quality and harms the safety of food products. PO is one of the cheapest oils widely used around the world because of its versatility (Lee et al., 2018). However, nonhalal oil such as PO is truly prohibited to be consumed according to the Islamic shariah law (Ghazali and Tukiran, 2021). Halal consumption has become a lifestyle and culture for Muslim and non-Muslim communities in the world. The phenomenon of consumers choosing halal food or transacting using shariah products is an unavoidable thing. This happens not only because it fulfills the demands of religious law but also because consumers are becoming more aware that halal food products are potentially healthier and safer, which increases halal products’ popularity (Teng et al., 2013). It can be said that the halal lifestyle is marked by the increasing awareness of halal in the community. Currently, the demand for halal foods dramatically increased not only in Muslim countries due to people’s awareness of consuming halal foods (Alzeer et al., 2020; Lubis et al., 2016). In addition, Yang and Huang (2017) stated that the buying behavior of non-Muslim consumers was significantly affected by the awareness of halal food products. If we talk about the data, according to the Business Research Company (2022), the size of the global halal food market is expected to grow from $1,134.14 billion in 2021 to $2,290.35 billion in 2022. The compound annual growth rate (CAGR) was reported to be 13.8%. In 2026, the growth of the halal food market is forecasted to be $2,228.63 billion at a CAGR of 14.6%. One of the things that can support this growth is technology; advanced technology in halal food products is a key trend gaining popularity in the halal market. Therefore, halal laboratories are actively engaged in the development of analytical techniques for ensuring food safety and maintaining quality standards with the increasing demand for halal foods.

Therefore, ensuring and warranting the halal status of food products was important. In some cases, however, advanced analytical techniques are required for the authentication of halal products because visual observation is not able to judge the authenticity of halal food products due to the similarity in characteristics and appearance between halal and nonhalal products (Hassan et al., 2018; Hosain et al., 2022). In this study, authentic TFO is slightly different from PO; however, in the adulterated form of TFO and PO, there is no significant difference observed in their visual appearance. Therefore, a rapid, effective analytical method capable of detecting PO in TFO is highly required.

Various methods using some instruments have been developed for the authentication of fish oils. Methods based on gas chromatography such as gas chromatography using a flame ionization detector and mass spectrometry detector (GC-MS) have been developed by most researchers for fish oils authentication by focusing on fatty acids analysis (Putri et al., 2019; Rohman et al., 2021; Yi et al., 2014). However, several disadvantages such as time consumption, complex sample preparation steps including derivatization, and laborious effort due to the use of some chemical reagents have become a serious concern. Vibrational spectroscopy methods such as FTIR spectroscopy offered rapid analysis without complex sample preparation steps and did not require much amount of solvent (Rohman et al., 2020; Windarsi et al., 2021). Moreover, FTIR spectroscopy has the capability for fingerprint analysis which provides specific spectra for each sample. FTIR spectroscopy has been developed and widely used for the adulteration detection of edible fats and oils due to its high effectiveness and efficiency (Arslan and Çağlar, 2019). However, the huge dataset obtained from FTIR spectra requires advanced statistical analysis to manage and process the data. Chemometrics, a powerful multivariate analysis, has been combined with FTIR analysis to process the extracted FTIR dataset (Balan et al., 2020). Bunaciu et al. (2022) have successfully used FTIR spectroscopy to analyze sunflower oil adulteration by the addition of other edible oils. In addition, FTIR-combined chemometrics has been successfully used to analyze various fats and oils including beef fat, chicken fat, milk fat, avocado oil, virgin coconut oil, and virgin olive oil. Results showed that FTIR spectroscopy and chemometrics were successfully used to detect and classify the adulterants present (Li et al., 2019; Neves and Poppi, 2020; Rahayu et al., 2018; Vasconcelos et al., 2015). Moreover, the employment of FTIR spectroscopy combined with chemometrics has been applied for halal testing of food products, including oils and fats (Che Man and Rohman, 2011; Guntarti et al., 2019; Witjaksono et al., 2017). The adulteration detection of Gabus (Chaunna striata) fish oil with different edible oils (palm and corn oils) has been successfully performed using FTIR spectroscopy combined chemometrics (Irnavati et al., 2021). However, studies on the analysis of nonhalal components for fish oils authentication using FTIR spectroscopy and chemometrics remain limited. There is a lack of reports on the detection of PO in high-quality fish oils such as TFO using FTIR-spectroscopy-combined chemometrics. Therefore, the aim of this study was to develop rapid detection of PO in TFO using FTIR spectroscopy combined with chemometrics techniques for halal authentication.

MATERIAL AND METHODS

Sample collection and preparation

TFO was extracted from the meat of yellowfin tuna fish. The oil was obtained using a direct press extraction method employing a room temperature of 25°C (Stanisavljevic et al., 2007). We were using the temperature of 25°C for the direct press extraction method because it is the room temperature in the laboratory. Besides being simple and efficient to operate the press equipment at this temperature, 25°C also did not deteriorate the nutrition value and metabolites constituents in the sample. The sample of PO was obtained from the supermarket in Yogyakarta, Indonesia. The adulterated samples of TFO with PO were prepared by mixing TFO and PO using several concentration levels of PO (%v/v) ranging from 0% to 100% v/v with a total volume of 1 ml. A total of 21 series of samples were prepared as shown in Table 1. Each series was prepared in three replicates.

FTIR spectroscopy measurement

The samples both authentic and adulterated of TFO were measured using an FTIR spectrophotometer (Bruker Vertex 80, Germany). FTIR spectrophotometer was equipped with attenuated total reflectance (ATR) sampling technique.
FTIR spectra acquisition was performed in the 4,000–600 cm⁻¹ region by placing the samples directly on the ATR crystal. The resolution used was 8 cm⁻¹ with 32 scanning. Prior to the measurement of each sample, the spectra of the background were recorded using the spectrum of air. After the measurement of each sample, the ATR crystal was cleaned using ethanol analytical grade.

Chemometrics analysis

Chemometrics technique was performed using SIMCA 14.0 software (Umetrics, Sweden) and MetaboAnalyst 5.0. Two chemometrics techniques were used in this study, namely pattern recognition and multivariate calibration. The variables used for chemometrics analysis were the wavenumber extracted from the FTIR spectra. For pattern recognition analysis, unsupervised techniques such as principal component analysis (PCA) and supervised techniques such as PLS-DA and OPLS-DA were used in this study. The PCA model was evaluated using a PCA score plot, PCA loading plot, $R^2$ value, and $Q^2$ value. The performance of PLS-DA and OPLS-DA models was observed using the score plot, $R^2X$ value, $R^2Y$ value, $Q^2$ value, and permutation test. The variables important for sample differentiation were observed using variables important for projections (VIP) values. Multivariate calibration analysis was performed using PLS and OPLS models. The models were evaluated using coefficient determination, root mean square error of estimation (RMSEE), and root mean square error of cross validation (RMSECV) values. An s-line plot was carried out in OPLS analysis to identify the important variables for creating the prediction model.

RESULTS AND DISCUSSION

FTIR spectra analysis

FTIR spectroscopy provides fingerprint spectra specific for TFO, PO, and a mixture of TFO-PO samples. The spectra of TFO and PO measured at the wavenumber ranging from 4,000 to 600 cm⁻¹ are depicted in Figure 1A. Triacylglycerols, triglycerides, and fatty acids are the main compositions of fats in oils. Therefore, the resulting FTIR vibrations from the oil samples were mainly from those compounds. The stretching vibration of –CH, CH₂, and CH₃ from aromatic and alkene could be observed at a peak of 3,011 cm⁻¹ whereas the stretching vibration of –CH₂, –CH₃, and –CH₃ from aliphatic alkane was found at peaks of 2,922 and 2,853 cm⁻¹. It could be observed that TFO had two peaks at the carbonyl (C=O) region (1,744 and 1,710 cm⁻¹). The absorption band at 1,710 cm⁻¹ was specific for TFO because it was absent in the spectra of PO. The absorption band at 1,459 cm⁻¹ was correlated to the stretching vibration of C=O. Meanwhile, absorption bands at 1,151, 1,116, and 1,097 cm⁻¹ arise from the vibration of C–O (stretching). In addition, vibrations at 1,377, 1,234, 1,032, 929, and 720 cm⁻¹ were associated with bending vibration of –CH, CH₂, CH₃, and CH₄ (Rohman et al., 2020). The absorption band at 1,032 cm⁻¹ was unique for PO because it was absent in the spectra of TFO. In the presence of adulterant (PO) with several concentration levels, both absorption bands at 1,710 and 1,032 cm⁻¹ changed as the adulterant concentration increased. The absorbance at 1,710 cm⁻¹ gradually decreased as the concentration of PO increased whereas the absorbance at 1,032 cm⁻¹ increased with the increasing concentration of PO as depicted in Figure 1B. Therefore, the vibrations at 1,710 and 1,302 cm⁻¹ could be used as a potential marker for adulteration analysis of TFO.

A study reported by AL-Kahtani et al. (2017) on the application of FTIR spectroscopy for the identification of lard added in binary mixtures of vegetable oils such as palm oil, corn oil, sunflower oil, and olive oil showed that the FTIR spectra at wavenumber region of 1,405–1,365, 1,260–1,198, 935–910, 877–857, and 857–833 cm⁻¹ could be used for identification of lard in the binary mixtures of those vegetable oils.

Detection of PO in TFO using pattern recognition chemometrics

Three types of pattern recognition analysis were used in this study such as PCA, PLS-DA, and OPLS-DA. PCA could clearly differentiate between TFO and PO samples as depicted in the PCA score plot (Fig. 2). The PCA model had $R^2$ of 0.998 and $Q^2$ of 0.990. The $R^2$ value was used to evaluate model fitting. A model with $R^2$ closer to 1 had the goodness of fit whereas the $Q^2$ was used to evaluate model predictivity. Good model predictivity is obtained when the $Q^2$ value is more than 0.5. In this study, PCA demonstrated a good ability to differentiate TFO from PO. However, analysis using PCA could not clearly differentiate authentic TFO samples from TFO samples adulterated with several concentrations of PO (data not shown). Another study reported that PCA could detect pork adulteration in used cooking oils such as samples of frying oils from fried chicken, fried pork, and fried fish. However, PCA could not differentiate frying oil samples based on their sources (Ali and Tukiran, 2021). Therefore, in our study, PLS-DA was further used for the discrimination of

<table>
<thead>
<tr>
<th>Sample</th>
<th>Snakehead fish oil (% v/v)</th>
<th>PO (% v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>10</td>
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<tr>
<td>4</td>
<td>85</td>
<td>15</td>
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<tr>
<td>5</td>
<td>80</td>
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<td>6</td>
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<td>7</td>
<td>70</td>
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<td>8</td>
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<td>15</td>
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<tr>
<td>21</td>
<td>0</td>
<td>100</td>
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</table>
authentic TFO, adulterated TFO with PO at some concentration levels, and pure PO samples. The PLS-DA model could only partially discriminate samples as shown in the PLS-DA score plot (Fig. 3A). The score plots of TFO and adulterated TFO with a lower concentration of PO showed some overlapping with each other. In addition, samples with a high concentration of PO also overlapped with pure PO. Therefore, OPLS-DA was chosen for further analysis to obtain better discrimination results.

OPLS-DA model using three predictive components and seven x-orthogonal components perfectly classified authentic TFO, adulterated TFO with PO, and pure PO with clear separation as demonstrated in the OPLS-DA score plot (Fig. 3B). OPLS-DA could detect and discriminate PO from TFO even at the lowest concentration of PO present (5%v/v). All the mixture samples of TFO-PO could be clearly discriminated from authentic TFO. The OPLS-DA model had an $R^2_X$ value of 0.998 and an $R^2_Y$ value of 0.735 indicating good fitness which is associated with the high accuracy model. Meanwhile, the $Q^2$ was 0.552 indicating good model predictivity (Lalaleo et al., 2020). Validation using the permutation test resulted in a $Q^2$ intercept value of zero and below zero ($0, -0.581$) indicating model validity. In addition, validation using receiver operating characteristics (ROC) analysis also ensures the validity of the OPLS-DA model demonstrated by its area under the curve (AUC) value (1) in all classes. It was associated with the correct classification of samples without misclassification. Analysis of the VIP values showed that variables 2,952, 1,770, 1,743, 1,035, and 1,240 cm$^{-1}$ were found to be important variables for sample classification. These variables were associated with the vibration of functional groups from fatty acids and triglyceride contents in the oils. It can be summarized that pattern recognition chemometrics such as PCA, PLS-DA, and OPLS-DA are promising to be used for authentication of TFO based on FTIR spectra data with OPLS-DA showing better differentiation results among others.

**Multivariate calibration for halal analysis of TFO from PO**

Chemometrics multivariate calibrations were applied to detect and predict the concentration of PO added in TFO. PLS
Figure 2. PCA score plot of TFO and PO.

Figure 3. PLS-DA and OPLS-DA for discrimination and classification of authentic and adulterated TFO with PO.
was initially applied and the concentration of PO added in TFO was successfully predicted using PLS with the equation of \( y = x + 4.122 \times 10^{-6}. \) The PLS model demonstrated a high \( R^2 \) value of 0.9928 indicating goodness of fit that is associated with good accuracy of the PLS model. Meanwhile, low values of RMSEE (2.81) and RMSECV (2.96) showed low model error, which is correlated to good model precision. The plot of the PLS model is depicted in Figure 4A. PLS is one of the multivariate calibration methods which is widely applied to predict the concentration of target analytes. PLS searches the latent variables to know the relationships between \( X \)-matrix and \( Y \)-matrix in the prediction model. Previous studies reported the success of the use of PLS for predicting lard added in palm oil (Rohman et al., 2012) as well as lard added in crude palm oil (Ahda and Safitri, 2016) with high precision (\( R^2 > 0.99 \)) and lower error (RMSEC and RMSEE < 5%).

Apart from PLS, OPLS offers different algorithms to create the prediction model due to the application of orthogonal components. Results showed that OPLS was successfully used to detect and predict the concentration of PO in TFO with high accuracy and good precision (Fig. 4B). The unknown sample of TFO adulterated PO could be predicted using the equation of \( y = x + 7.474 \times 10^{-6} \) with the \( R^2 \) value of 0.9928. The model error was low correlated to the high precision as demonstrated in the values RMSEE (2.81) and RMSECV (2.94). From OPLS analysis, the variables having an important role in predicting the concentration of PO could be observed using an S-line plot as shown in Figure 5. Variables from 11 wavenumbers were investigated. These variables came from the vibrations of \(-\text{CH}, -\text{CH}_2, \) and \(-\text{CH}_3\) stretching, \(\text{C=O} \) stretching, \(\text{C–O} \) stretching, \(\text{C=C} \) stretching, and bending vibration of \(-\text{CH}, -\text{CH}_2, \) and \(-\text{CH}_3\) which were associated with the vibration of fatty acids, triacylglycerols, and triglycerides.

It can be summarized that multivariate calibration of PLS and OPLS could be used as rapid and effective methods for authentication of TFO by detecting and predicting the concentration of PO in TFO with high accuracy, good precision, and without time consumption.

Compared to the previous studies on tuna fish and TFO authentication, our study offered several advantages such as being simpler in sample preparation, more economical reagent

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**Figure 4.** (A) PLS model and (B) OPLS model to predict the concentration of PO in TFO.
Table 2. Comparison of a previous study and this study about tuna fish/TFO adulteration detection.

<table>
<thead>
<tr>
<th>No.</th>
<th>Samples</th>
<th>Methods</th>
<th>Literature sources</th>
<th>Advantage of this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Four tunas species: <em>Katsuwonus pelamis</em>, <em>Thunnus alalunga</em>, <em>Thunnus albacares</em>, and <em>Thunnus obesus</em></td>
<td>Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)</td>
<td>(Mata et al., 2020)</td>
<td>The sample preparation is more simple and more economical and reagent is needed</td>
</tr>
<tr>
<td>2</td>
<td>Five tuna species: bigeye, skipjack, Atlantic bluefin, albacore, and yellowfin tunas</td>
<td>A multiplex PCR assay</td>
<td>(Lee et al., 2022)</td>
<td>There is no need in the current study for primer analysis. It is simpler and more economical</td>
</tr>
<tr>
<td>3</td>
<td>Four tuna species: yellowfin tuna (<em>Thunnus albacares</em>), southern bluefin tuna (<em>Thunnus maccocyii</em>), bigeye tuna (<em>Thunnus obesus</em>), and Atlantic bluefin tuna (<em>Thunnus thynnus</em>)</td>
<td>PCR-RFLP to amplify the cytochrome b gene (Cyth)</td>
<td>(Yao et al., 2020)</td>
<td>There is no need in the current study for primer analysis. It is simpler and more economical</td>
</tr>
<tr>
<td>4</td>
<td>Three tuna species: <em>Thunnus albacares</em>, <em>Thunnus obesus</em>, and <em>Katsuwonus pelamis</em></td>
<td>A one-step triplex- PCR-based assay</td>
<td>(Michelini et al., 2007)</td>
<td>Lower in cost, more efficient</td>
</tr>
<tr>
<td>5</td>
<td>Yellowfin (<em>Thunnus albacares</em>), bluefin (<em>Thunnus thynnus</em>), and albacore (<em>Thunnus alalunga</em>) tunas</td>
<td>PCR-RFLP, minor groove binder probe assays, and sequence analysis</td>
<td>(Terio et al., 2010)</td>
<td>Lower in cost, faster, and more efficient</td>
</tr>
<tr>
<td>6</td>
<td>Bigeye tuna (<em>Thunnus obesus</em>) and yellowfin tuna (<em>Thunnus albacares</em>)</td>
<td>qPCR-based methods</td>
<td>(Bojolly et al., 2017)</td>
<td>Lower in cost, faster, and more efficient</td>
</tr>
<tr>
<td>7</td>
<td>25 commercial fish oil supplements</td>
<td>GC/MS with a hierarchical clustering algorithm</td>
<td>(Khoomrung et al., 2014)</td>
<td>No need for esterification method for the sample preparation, and it is low in cost and practical</td>
</tr>
<tr>
<td>8</td>
<td>Adulterated yellowfin tuna (<em>Thunnus albacares</em>) using nitrate and nitrite</td>
<td>A colorimetric analysis using a spectrophotometer (SpectraScan PR655, Jadak) and a digital smartphone camera</td>
<td>(Sáez-Hernández et al., 2022)</td>
<td>No need for analysis using high performance liquid chromatography (HPLC) for further quantification analysis</td>
</tr>
<tr>
<td>9</td>
<td>Mixed tuna samples</td>
<td>Next-generation sequencing of PCR assay</td>
<td>(Kappel et al., 2017)</td>
<td>Lower in cost, faster, and more efficient</td>
</tr>
<tr>
<td>10</td>
<td>Skipjack tuna (<em>Katsuwonus pelamis</em>)</td>
<td>Molecular beacons in loop-mediated isothermal amplification (MB-LAMP) assay</td>
<td>(Xu et al., 2022)</td>
<td>Lower in cost, faster, and more efficient</td>
</tr>
<tr>
<td>11</td>
<td>Skipjack tuna (<em>Katsuwonus pelamis</em>) in processed fish products</td>
<td>LAMP assay</td>
<td>(Xiong et al., 2021)</td>
<td>Lower in cost, faster, and more efficient</td>
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</tbody>
</table>
needed, lower in cost, and faster. Table 2 shows the comparison between our study and the previous studies.

CONCLUSION

The authentication of TFO from PO adulteration is very important to warrant the quality and halal status of TFO to protect consumers. FTIR spectroscopy could be used as a rapid analytical method for the authentication of TFO due to its fingerprint properties. Combination with chemometrics PCA, PLS-DA, and OPLS-DA could be used to differentiate PO and TFO. Moreover, OPLS-DA successfully classified authentic TFO and adulterated TFO with 100% accuracy. Meanwhile, chemometrics of PLS and OPLS was effective to predict the concentration of PO added in TFO with high accuracy and high precision. It can be concluded that the combination of FTIR spectroscopy and chemometrics could be a promising analytical technique for the authentication of TFO. In the future, it can be developed as a standard analytical method for the halal authentication of fish oil. Therefore, further research on the application of larger samples of fish oils is required.

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AUTHORS’ CONTRIBUTIONS

Irnawati conceived and designed the research; Anjar Windarsih prepared the manuscript and made critical thinking on the manuscript; Anastasia Wheni Indrianingsih, Wuri Apriyana, and Yuli Ary Ratnawati performed research activities, data acquisition, and data interpretation; La Ode Muhamad Hazairin Nadia performed statistical analysis; Abdul Rohman conceived and designed research and critically analyzed the manuscript. All authors read and agreed on the published version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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REFERENCES


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