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ORIGINAL PAPER

Analysis of Cod-Liver Oil Adulteration Using Fourier Transform Infrared (FTIR) Spectroscopy

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Abstract Analysis of the adulteration of cod-liver oil with much cheaper oil-like animal fats has become attractive in recent years. This study highlights an application of Fourier transform infrared (FTIR) spectroscopy as a nondestructive and fast technique for the determination of adulterants in cod-liver oil. Attenuated total reflectance measurements were made on pure cod-liver oil and cod-liver oil adulterated with different concentrations of lard (0.5–50% v/v in cod-liver oil). A chemometrics partial least squares (PLS) calibration model was developed for quantitative measurement of the adulterant. Discriminant analysis method was used to classify cod-liver oil samples from common animal fats (beef, chicken, mutton, and lard) based on their infrared spectra. Discriminant analysis carried out using seven principal components was able to classify the samples as pure or adulterated cod-liver oil based on their FTIR spectra at the selected fingerprint regions (1,500–1,030 cm^{-1}).

Keywords Adulteration · Cod-liver oil · Lard · PLS · Discriminant analysis · FTIR · Spectroscopy · Lipid chemistry · Lipid analysis

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Introduction

Oils are complex mixtures containing a wide range of compounds, mainly triacylglycerols (TAGs), diacylglycerols (DGs), free fatty acids (FFAs), phospholipids, and other minor components. The most important group of compounds is represented by TAGs, which are, in chemical terms, glycerol esterified with fatty acids (FAs) [1].

Recently, cod-liver oil has been the focus of a growing interest due to its nutritional advantages. It contains high levels of the long-chain n-3 fatty acids *cis*-5,8,11,14,17-eicosapentaenoic (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic (DHA), which are believed to play a preventive role in cardiovascular disease and in the alleviation of other health problems [2]. Epidemiological studies also revealed that there is an inverse relationship between high levels of fish-oil consumption and low mortality due to coronary heart disease (CHD) or breast cancer [3]. Animals fed cod-liver oil also demonstrated reduced body weights [4].

Cod-liver oil (annual production ~10,000 tons) has long been marketed, first as a source of vitamins A and D and now also as a source of the long-chain n-3 fatty acids EPA and DHA [5]. Fish-oil products based on natural fish oil or their derivatives are frequently being introduced to the market as functional foods (capsules) or medicines. Microencapsulated fish oil has been introduced for enrichment of foodstuffs including bread, infant formulas, baby food, soups, and prepared food, such as pizza [6].

Adulteration has been a serious problem in the trade of fats and oils for a long time because there is a great difference in price for different oil products. Adulterants were sometimes added deliberately and occasionally accidentally [7]. Adulteration involves addition of cheaper oils. The potential adulterants of fish oil are animal fats. Fish

oils are the most expensive oils compared to animal fats such as mutton, beef, chicken, and lard. Therefore, fish oil is subjected to adulteration to increase economic profits. Usually, complex, time-consuming, and tedious chemical treatment of test samples is required for analysis of adulteration, typically using chromatographic methods [8].

Because of cod-liver oil's therapeutic value, it is important to have a reliable method either for its identification or for the detection of potential adulterants. FTIR is one of the most popular methods used for authentication analysis, especially in fats and oils. FTIR spectroscopy has been applied for authentication of virgin olive oil [9, 10], extra virgin olive oil [11], virgin coconut oil [12], and animal fats [13]. FTIR has also been used for characterization of commercial cod-liver oil, and it provides information about the molar percentage of polyunsaturated acyl groups in cod-liver oil samples [14]. However, a literature search suggests that there is no information available related to the use of FTIR spectroscopy in cod-liver oil, especially related to adulteration.

In this study, Fourier transform infrared using attenuated total reflectance (FTIR–ATR) spectroscopy combined with chemometrics was used as a rapid, nondestructive, and chemical-free preparation approach for detection of cod-liver oil adulterated with lard, which greatly resembles cod-liver oil in chemical composition. Further analysis was carried out to distinguish cod-liver oil from other animal fats on the basis of their FTIR spectra using the discriminant analysis method.

Materials and Methods

Materials

Cod-liver oil was obtained from the local market in Jogjakarta, Indonesia. Animal fats were prepared by rendering adipose tissues of the corresponding animals. The rendering was done at 90–100 °C for 2 h in the oven. The melted fat was strained through triple-folded muslin cloth, dried by addition of anhydrous Na_2SO_4, and then centrifuged at 3,000 rpm for 20 min. The fat layer was decanted, shaken well, and centrifuged again before being filtered through Whatman filter paper. The filtered samples were used for further analysis.

Calibration and Validation Standard

A set of 15 standards consisting of cod-liver oil and lard was prepared by mixing of both at ratios 1–50% v/v. For validation, 25 independent samples were constructed. Pure cod-liver oil and pure lard as well as their blends were analyzed using FTIR spectrometer. The spectral regions where the variations were observed were chosen for developing the partial least squares (PLS) model.

Discriminant Analysis

Cod-liver oil and lard were mixed to obtain a series of standard or trained sets of 10 pure and 18 adulterated samples containing 1–50% of lard. The samples containing lard were assigned as adulterated, while a series of pure cod-liver oil was marked cod-liver oil and classified using FTIR spectra. In the second part of the study, cod-liver oil and four animal fat samples were compared and classified using discriminant analysis.

FTIR Instrumental Analysis

A Nicolet 6700 FTIR spectrometer (Thermo Nicolet, Madison, WI) equipped with a detector of deuterated triglycine sulphate (DTGS) and connected to software of the OMNIC operating system (Version 7.0 Thermo Nicolet) was used to obtain FTIR spectra of oils. Drops of oil samples were placed in contact with attenuated total reflectance (ATR) on a multibounce plate of ZnSe crystal at controlled ambient temperature (25 °C). FTIR spectra were collected in the frequency range of 4,000–650 cm^{-1} by co-adding 32 scans and at a resolution of 4 cm^{-1} with strong apodization. All spectra were ratioed against a background of an air spectrum. After every scan, a new reference air background spectrum was taken. The plate was carefully cleaned by wiping with hexane twice followed by acetone and dried with soft tissue before filling in with the next sample. The spectra were recorded as absorbance values at each data point in triplicate.

Statistical Analysis and Validation

The software TQ Analyst version 6 (Thermo Electron, Madison, WI) was used for chemometrics analysis, including discriminant analysis and PLS. The leave-one-out cross-validation procedure was used to verify the calibration model. The values of root mean standard error of cross validation (RMSECV) and coefficient of determination (R^2) were used as the validity criteria for the calibration. The validation was further investigated using the mean difference (MD) and standard deviation of difference (SDD) for accuracy and reproducibility.

Results and Discussion

The animal fats and cod-liver oil are essentially constituted of fatty triglyceride esters with different substitution patterns, a number of fatty acids, and degrees of saturation of

the chains and of other minor components. Due to the fingerprint capability of FTIR, the spectra of oils can be used as a potential tool which allows one to make a first differentiation. Figure 1 shows FTIR spectra of cod-liver oil and animal fats (chicken, mutton, beef, and lard) with the characteristic peaks of animal fats as described by Guillen and Cabo [15]. The entire range of spectra looks very similar for all oils evaluated, for the reasons described before.

However, a detailed investigation in the fingerprint region, especially in the wavenumber range of 1,500–1,000 cm^{-1}, revealed that there are visual differences for absorption peaks at 1,162 cm^{-1} (peak a, Fig. 1) and two adjacent peaks at 1,117 (b) and 1,097 cm^{-1} (c). The peak at 1,162 cm^{-1} was attributed to C–O stretching and CH_2 bending, whereas peaks at 1,117 and 1,097 cm^{-1} were assigned to –C–O stretching [14].

To assess calibration and validation models of cod-liver oil adulterated with lard, the peak at region 1,035–1,030 cm^{-1}, due to the stretching vibrations of the C–O group in esters that consists of two asymmetric coupled vibrations C–C(=O)–O and O–C–C, was used.

PLS Calibration and Validation

For the calibration model, absorbances of lard at concentrations ranging from 0 to 50% in cod-liver oil were recorded (Fig. 2). A PLS calibration was employed to determine the relationship between actual lard value (% v/v) and FTIR-predicted lard value (% v/v) in cod-liver

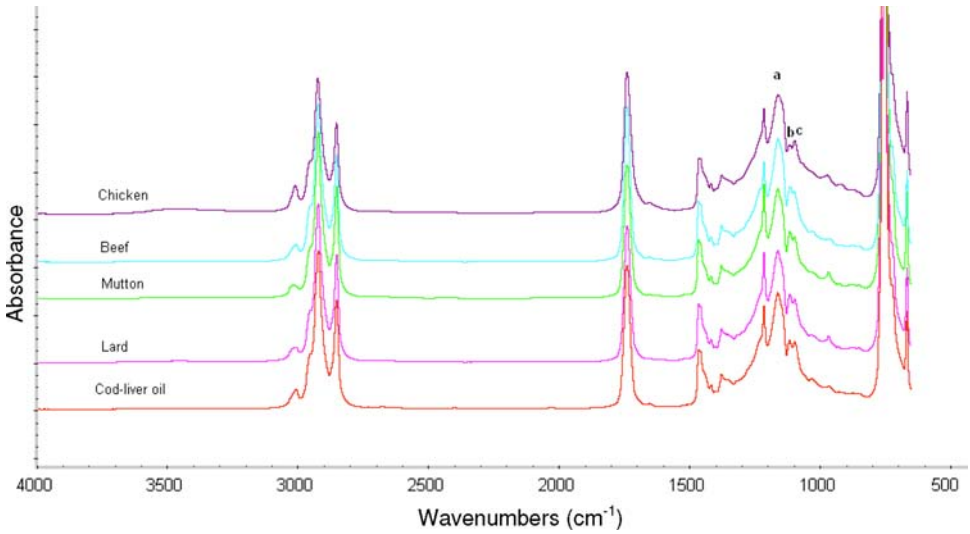

Fig. 1 FTIR spectra of cod-liver oil, lard, mutton, beef, and chicken fats at wavenumber region of 4,000–650 cm^{-1}

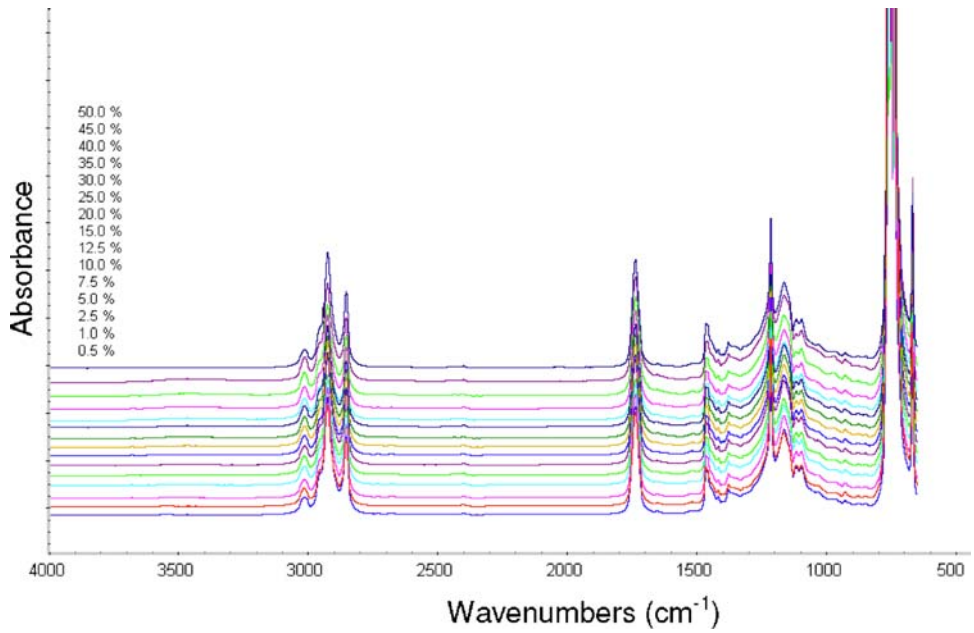

Fig. 2 FTIR spectra of cod-liver oil adulterated with lard at concentrations ranging from 0.5 to 50%

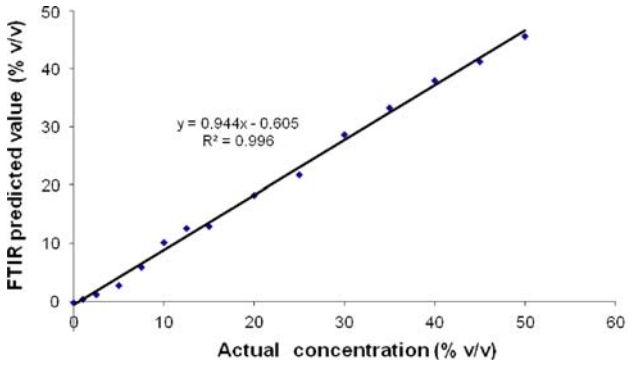

Fig. 3 Partial least square (PLS) calibration model of actual value of lard in (% v/v) versus FTIR predicted value (%)

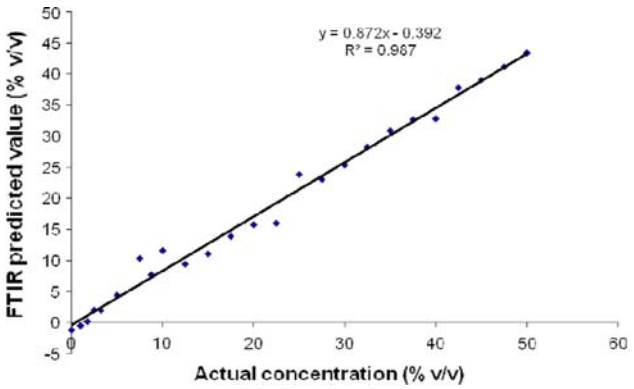

Fig. 4 Relationship between actual value of adulterant (lard) and FTIR predicted value using cross-validation by removing one standard at a time

oil for the region 1,035–1,030 cm^{-1} (Fig. 3). A good relationship was obtained with an R^2 higher than 0.99 and RMSECV of 1.04 ($y = 0.944x - 0.605$). Therefore the calibration model can be used as a tool to predict the concentration of lard adulterant in cod-liver oil.

The calibration model was further cross-validated by removing one standard at a time, and an R^2 value of 0.987 ($y = 0.872x - 0.392$) and an RMSECV value of 1.605 were obtained (Fig. 4). Confirmation and validation of the analysis region used for developing the PLS model were performed by computing the predicted residual error sum of squares (PRESS) values for different principal component (PC) factors. Eigen analysis and PRESS value suggested that the optimum number of factors is two, which demonstrates that RMSEC reaches a stable minimum after two factors. This confirms that the spectral region used for developing the PLS model for quantification of lard exhibits significant correlation with its concentration [9].

Accuracy and reproducibility of the method were further evaluated by calculating mean difference (MD) and standard deviation of difference (SDDr), respectively. Accuracy is a measure of the closeness between actual value and FTIR-predicted value. The low values of MDa (3.137) and SDDa (2.607) for cod-liver oil in mixture with lard showed that FTIR is well suited for determining whether cod-liver oil has been adulterated with lard. Meanwhile, low MDr (3.328) and SDDr (2.762) indicate that the FTIR method has high reproducibility.

Discriminant Analysis

Discriminant analysis can be used to determine the class of cod-liver oil that is similar to lard by computing the distance from each class center in Mahalanobis distance units. Once a classification model has been obtained, the membership of unknown objects in one of the defined classes can be predicted. In other words, classification methods find the relationships between a set of descriptive variables (FTIR spectra) and a qualitative variable (class of sample) [16].

Discriminant analysis was carried out in two steps. First, pure cod-liver oil and the mixed samples were classified into two groups: pure cod-liver oil and adulterated oils. Discriminant analysis was applied to both classes in the wavenumber range of 1,500–1,030 cm^{-1}. Figure 5 shows the Coomans plot for the classification of pure cod-liver oil spiked with 1–50% lard using seven PCs. The *x*-axis shows Mahalanobis distance to cod-liver oil, while the *y*-axis shows the distance to the adulterated oil class. The Mahalanobis distance is useful in assigning whether a set of samples with unknown values is similar to a collection set of known measured samples. The Coomans plot clearly exhibits separate groups for the pure cod-liver and adulterated oils. In this study, the discriminant analysis model classified 100% of all samples accurately according to its

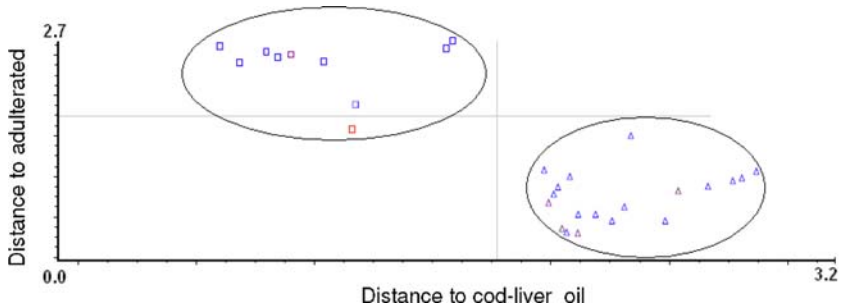

Fig. 5 Coomans plot of pure cod-liver oil (*squares*) and cod-liver oil spiked with different proportions of lard (*triangles*)

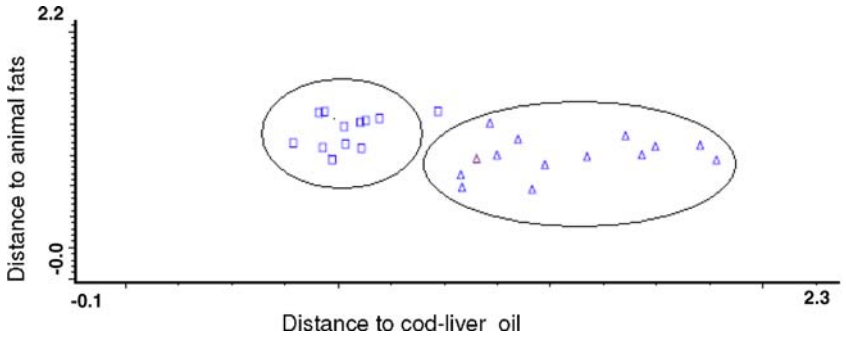

Fig. 6 Coomans plot of cod-liver oil (*squares*) and other animal fats (*triangles*)

group, meaning that no samples were misclassified into the wrong group, which could happen sometimes because of the close similarities in chemical composition between groups.

Second, the pure cod-liver oil samples were compared with animal fats such as beef, chicken, mutton, and lard. All animal oils were categorized into one group and compared with cod-liver samples. Furthermore, discriminant analysis was used to categorize the test samples into cod-liver oil and animal fats. Figure 6 exhibits the Coomans plot of cod-liver oil and other animal fats. The model successfully classified cod-liver oil samples and other animal fats. However, the Mahalanobis distances are closer to each other than those in Fig. 5. There was one misclassification reported for the discriminant analysis in this developed model.

Conclusions

It can be concluded that adulteration of cod-liver oil can be monitored using FTIR spectroscopy with the ATR sampling technique. Partial least squares can be successfully used to detect the level of adulterant, such as lard, that is most similar to cod-liver oils. Discriminant analysis allows one to make a classification of cod-liver oil and potential adulterants of animal oils.

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