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Authentication of cod liver oil from selected edible oils using FTIR spectrophotometry and chemometrics

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Abstract

Cod liver oil (CLO) is one of the expensive oils in the market. This attracted unethical players in fats and oils industry to adulterate CLO with selected oils. This study is intended to authenticate CLO from sunflower, corn, and grape seed oils using FTIR spectroscopy in combination with chemometrics. FTIR spectra of CLO, sunflower oil (SFO), corn oil (CO), grape seed oil (GSO), and their binary mixtures were measured in mid infrared region (4,000 – 650 cm-1) using 32 scanning with resolution of 4 cm^{-1}. Quantitative analysis of CLO in binary mixtures with SFO, CO, and GSO was assisted with multivariate calibration of partial least square (PLS). The selection of SFO, CO and GSO as oil adulterants was based on the close similarity of these oils to CLO in terms of FTIR spectra, as analyzed using principal component analysis. CLO in GSO is determined using normal spectra at wavenumbers region of 1250 – 950 cm^{-1}. The equation for the correlation between actual value of CLO (x-axis) and FTIR predicted value (y-axis) is $y = 0.9938x + 0.1567$ with R^2 value of 0.9962. PLS using normal spectra at wavenumbers of 1350-900 cm^{-1} is preferred for the quantification of CLO in binary mixture with CO with coefficient of determination (R^2) value of > 0.9999 and root mean square error of calibration (RMSEC) value of 0.48% (v/v). The wavenumbers of 1350-900 cm^{-1} along with FTIR normal spectra are suitable for quantification of CLO in binary mixture with GSO. Based on the high value of R^2 and low values of errors, it can be concluded that FTIR spectroscopy coupled with chemometrics can be used for analysis of CLO adulterated with sunflower, corn, and grape seed oil.

Introduction

Cod liver oil (CLO) is natural oil having some beneficial effects to human health, therefore, CLO can be taken into account as functional food oil (Rohman and Che Man, 2012). CLO is rich of omega-3 fatty acids and is frequently used as omega-3 fatty acids supplementation (Perveen *et al.,* 2013). CLO has been reported to have anti-inflammatory properties caused by the omega-3 fraction (Kehn and Fernandes 2001), antidiabetic (Hunkar *et al.,* 2002), anticancer (Dyck *et al.*, 2011), hepatoprotective (Salam*a et al.,* 2013), antidepressant and anti-anxiety effects (Perveen *et al.,* 2013), and neuroprotective qualities in epileptic conditions caused by damage in the hippocampus (Ferrari *et al.,* 2008). Besides, CLO has been reduced cardiometabolic risk factors (Abeywardena *et al.,* 2011) and ameliorated cognitive impairment induced by chronic stress (Trofimiuk and Braszko, 2011).

Currently, the authentication of functional food oils having expensive price in the industry of fats and oils such as CLO and extra virgin olive oil, is an attractive issue for consumers, producers, and regulatory agencies to the legal compliance, economic, food quality, and religious reasons (Kamm *et al.,* 2001). The adulteration of CLO usually involves the replacement of CLO with low grade ones or dilution of CLO with oils having similar physical characteristics (color, odor) (Lizhi *et al.,* 2010; Rohman and Che Man, 2012). Such adulterants for CLO are plant oils such as sunflower oil, corn oil and grape seed oil. The authentication issues have encouraged food scientist to develop and propose analytical techniques capable of detecting and quantifying oil adulterants.

Numerous techniques have been used for authentication of fish oils, including CLO, namely gas chromatography (GC) (Standal *et al*., 2008), high performance liquid chromatography (Bosque-Sendra *et al.,* 2012), 13C nuclear magnetic resonance (NMR) (Standal *et al.,* 2011), and vibrational spectroscopy (Rohman and Che Man, 2012). One of instrumental technique offering such a method is Fourier transform infrared (FTIR) spectroscopy.

FTIR spectroscopy is ideal for authentication analysis of edible fats and oils due to its simplicity,

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even in some cases without sample preparation. Besides, FTIR spectroscopy is called as fingerprint technique (Rohman *et al.,* 2014). Due to the complexity of FTIR spectra, some researchers use chemometrics to assist data treatment for qualitative and quantitative purposes. Our group has used FTIR spectroscopy for authentication of CLO from lard for halal authentication study (Rohman and Che Man, 2009), analysis of mutton fat as adulterant in CLO (Rohman *et al.,* 2012), analysis of CLO in binary mixture with corn oil (Rohman *et al.,* 2011), and authentication analysis of CLO from beef fat using fatty acid composition and FTIR spectra (Rohman and Che Man, 2011). In this study, FTIR spectroscopy using horizontal attenuated total reflectance (FTIR–HATR) as sample handling technique combined with chemometrics was used for detection and quantification of sunflower oil, corn oil, and grape seed oil for authentication study of CLO.

Materials and Methods

Materials

Cod liver oil (CLO), sunflower oil (SFO), corn oil (CO) and grape seed oil (GSO) were purchased from super market in Yogyakarta. In order to assure the authenticity of these oils, we used gas chromatography for identification of fatty acid composition. Standards of fatty acid methyl esters (FAMEs) were purchased from Sigma (Sigma Chemicals, St. Louis, MO). All reagents and solvents used during fatty acid analysis and FTIR spectra measurement were of pro analytical grade.

Analysis of fatty acid composition

Fatty acid analysis was carried out according to our previous paper using gas chromatography (Rohman *et al.,* 2015). During gas chromatography process, we use capillary column of DB-5 (0.25 mm internal diameter, 30 m length, and 0.2 μm film thickness) from Restex Corp (Bellefonte, PA, USA). The initial temperature for the column oven was 120◦C (hold for 1 min), then increased into 180◦C (8◦C/min), ramped to 240◦C (10◦C/min), and held at 240◦C for 5 min. The temperatures of the detector and injector were set at 240◦C. The flow rate of helium as the carrier gas was set at 6.8 mL/min. The peak integration was performed using a built-in data-handling program provided by GC manufacturer (Shimadzu GC-2010). Fatty acids were reported as relative percentage of the total peak area. Standard FAMEs and treated samples were run on GC under the same conditions.

Analysis of cod liver oil in binary mixture with sunflower, corn and grape seed oil

In order to seek the oils having the same characteristics to CLO, we perform principal component analysis (PCA). PCA is unsupervised pattern recognition technique which allow to look for the classification among samples with similar properties (Miller and Miller, 2005). We evaluated some oils for PCA study, namely canola oil, coconut oil, cod liver oil, corn oil, olive oil, rice bran oil, soybean oil and sunflower oil. As variables, we used fatty acid composition of these oils as determined using gas chromatography. The edible oils having similar the first principle component (PC1) and second principle component (PC2) score plots are considered to have similar profiles.

Calibration and validation

During quantitative analysis, we prepare training set samples as calibration and validation samples. For calibration, a number of training sets consisting of CLO in binary mixtures with SFO, CO and GSO at concentration ranges of 0– 50.0% v/v was prepared. For validation, a series of independent sample was also constructed. The spectra of pure CLO, SFO, CO and GSO as well as their binary mixture were analyzed using FTIR spectrophotometer.

FTIR Instrumental Analysis

FTIR analysis was performed according to Lukitaningsih *et al.,* (2012). The spectra of all oil samples were scanned using an ABB MB3000 FTIR spectrophotometer (Clairet Scientific, Northampton, UK). The FTIR spectra of all evaluated samples were acquired in mid infrared region (650-4000 cm^{-1}). The instrument was equipped with a deuterated triglycine sulfate (DTGS) detector and KBr as the beam splitter. The spectra were scanned at a resolution of 4 cm^{-1} with 32 scanning. The FTIR spectra were processed using FTIR software of Horizon MB version 3.013.1 (ABB, Canada).

Chemometrics Analysis

PCA was accomplished using Minitab software version 16 (Minitab Inc., USA), while PLS, used for quantitative analysis, was performed using Horizon MB FTIR software version 3.0.13.1 (ABB, Canada). The values of the root mean square error of calibration (RMSEC) and coefficient of determination (R^2) were used as the validity criteria for the calibration model. The predictive ability of PLS calibration model was further used to calculate the validation or prediction samples, and its data are analyzed using Excel (Microsoft Corp., USA).

Results and Discussion

Fatty acid analysis

The purpose of fatty acid determination is to assure the authenticity of the used oil during this study (cod liver oil, CLO). Table 1 compiled fatty acid composition of CLO along with that found in some references. Based on Table 1, it is known that the CLO is authentic meaning that CLO are not adulterated or mixed with other oils. The fatty acid composition of sunflower oil (SFO), corn oil (CO) and grape seed oil (GSO) used in this work can be seen in our previous papers (Rohman and Ariani, 2013; Rohman *et al.,* 2015).

Fatty acid composition is a tool used for fats and oils scientist to assure the authenticity of fats and oils. This is based on the fact that fatty acid composition is unique for each fats and oils. There is no fats and oils having the same fatty acid profiles in terms of type and concentration (Che Man *et al.,* 2011), therefore, fatty acid composition is widely used for the characterization of edible fats and oils. CLO can be differentiated from vegetable oils by investigating the specific fatty acids present in marine oils like CLO. CLO can be characterized by eicosapentanoic acid (C-20:5) accounted to 16.745% and docosahexanoic acid (C-22:6) accounted to 8.882%.

Tabel 1. Fatty acid composition of cod liver oil using gas chromatography with flame ionization detector

Fatty acid composition	Concentration (%)	
	From study	From References
Lauric acid (C-12:0)	0.031	0.01 – 0.08[b]
Tridecanoic acid (C-13:0)	0.012	-
Myristic acid (C-14:0)	2.800	2.0 – 5.14[b]
Pentadecanoic acid (C-15:0)	0.199	0.1 – 0.44[b]
Palmitoleic acid (C-16:1)	3.946	3.5 - 5.5[b]
Palmitic acid (C-16:0)	10.064	5 – 12.01[b]
Heptadecanoic acid (C-17: 0)	0.149	-
Oleic acid (C-18:1)	26.167	25 – 48.8[b]
Stearic acid (C-18:0)	2.720	2.90[a]
Elaidic acid (t-C18:1)	0.015	-
Linoleic acid (C-18:2)	0.041	0.23[a]
Arachidic acid (C20 : 0)	2.940	-
Gama Linolenic acid (C18:3)	0.725	-
Cis -8,11,14Eicosenoic acid (C-20:2)	0.011	0.13[a]
Linolenic acid (C-18:3)	0.451	-
Behenic acid (C22: 0)	0.220	0.26[a]
Erucic acid (C22: 1, n-9)	1.380	-
Cetoleic acid (C22: 1, n-11)	10.213	-
Arachidonic acid (C-20:4)	0.148	0.05[a]
Tricosanoic acid (C23: 0)	0.011	-
Eicosapentanoic acid (C-20:5)	16.745	0.23[b]
Nervonic (C24:1)	0.551	-
Docosahexanoic acid (C-22:6)	8.882	-

ataken from Rohman and Che Man (2010); btaken from Medina *et al.* (1999).

FTIR spectral analysis and principal component analysis

Figure 1 shows FTIR spectra of cod-liver oil (CLO), canola oil, coconut oil, corn oil, olive oil, rice bran oil, soybean oil and sunflower oil which reveal the characteristic bands of fats and oils. The main peaks along with its wavenumbers and functional groups responsible for absorption of infrared radiation are: 3007 cm^{-1} (cis C=CH stretching), 2956 cm^{-1} (stretching vibration of -CH3), 2929 and 2859 cm^{-1} (asymmetric and symmetric stretching vibration of –CH2), 1746 cm^{-1} (C=O stretching), 1654 cm^{-1} (cis C=CH), 1463 cm^{-1} (bending vibration of –CH2), 1377 cm^{-1} (bending vibration of –CH3), 1237, 1161 and 1097 cm^{-1} (C-O ester), 963 cm^{-1} (isolated trans CH) (Lerma-Garcia *et al.,* 2010).

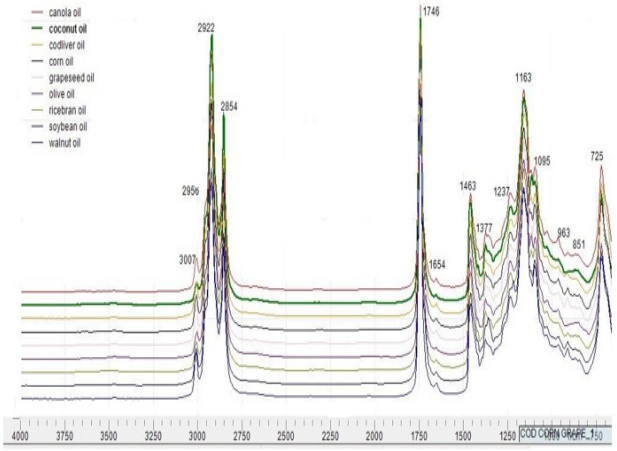

Figure 1. FTIR spectra of canola oil, coconut oil, cod liver oil, corn oil, grape seed oil, olive oil, rice bran oil, soybean oil, and walnut oil, scanned at mid infrared region (4000 – 650 cm^{-1}) using resolution of 4 cm^{-1} and 32 scanning

FTIR spectra of the studied oils are very similar with slight differences in term of peak absorbance and the exact frequencies at which the maximum absorbance due to the different nature and composition of fatty acids in CLO and others, especially at 3007 cm^{-1} and at fingerprint regions. By exploiting slight differences among FTIR spectra of CLO and others, principal component analysis (PCA) was performed in order to seek the oils having the closer similarity with CLO. Figure 2 is PCA score plot of CLO and other oils using the absorbance values as variables. PCA represents the projection of samples defined by the first principal component (PC 1) and the second principal component (PC 2). PC 1 accounts for the most variation in aborbance differences, while PC 2 accounts for the next largest variation. Based on PCA score plot, CLO, sunflower oil (SFO), corn oil (CO) and grape seed oil (GSO) are the same right quadrant with closer distance than other oils. The closer the distance between two oils, the more similar they are. It means that SFO, CO and GSO have the close similarity with CLO so that they are potential adulterants to CLO.

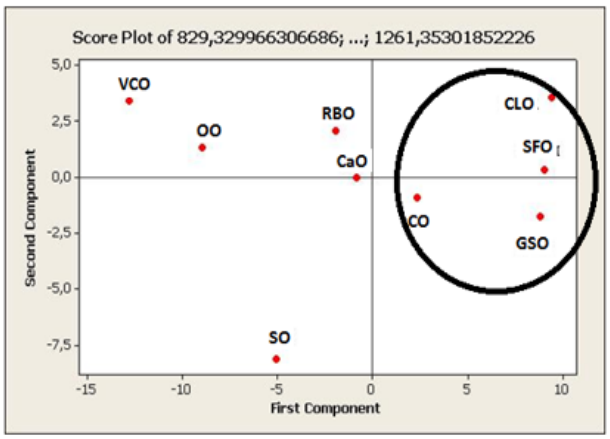

Figure 2. PCA score plot of cod liver oil (CLO), sunflower oil (SFO), grape seed oil (GSO), corn oil (CO), canola oil (CaO), soybean oil (SO), rice bran oil (RBO), olive oil (OO) and coconut oil (VCO) based on the first principle component (PC1) and second principle components (PC2)

Quantification of cod liver oil in binary mixture with SFO, CO and GSO

Quantitative analysis of CLO in binary mixtures with SFO, CO and GSO was assisted with multivariate calibration of PLS. For calibration model, the absorbance values of CLO with concentration ranged from 0 – 50% in SFO, CO and GSO were recorded. PLS calibration model was used for making the correlation between actual and FTIR predicted value of CLO (% v/v) in SFO, CO and GSO. The first step for quantification process is selection of frequency region capable of providing the highest value of coefficient of determination ($R^{2)}$ and the lowest value of root mean square error in calibration (RMSEC) and prediction (RMSEP).

Based on the optimization process in terms of frequency region and spectral treatment, CLO in GSO is determined using normal spectra at frequency region of 1250 – 950 cm^{-1}. The equation for correlation between actual value of CLO (x-axis) and FTIR predicted value (y-axis) is y = 0.9938x + 0.1567 with R^2 value of 0.9962, as shown in Figure 3. PLS using normal spectra at wavenumbers of 1350-900 $cm^{-1}$is preferred for quantification of CLO in binary mixture with corn oil (CO). These wavenumbers offer the best correlation between actual value of CLO and COwith R^2value >0.9999and RMSEC value of0.48% (v/v). The equation obtained is y = 0.9982x + 0.0750 (Figure 4). The calibration model was further used for prediction of validation samples. The values of R^2 for the relationship between actual value of CLO (x-axis) and FTIR predicted value (y-axis), and root mean square value of prediction obtained is 0.9999and 0.28% (v/v), respectivelywith equation of y = 1.003x + 0.0069.

The wavenumbers of 1350-900 cm^{-1}along

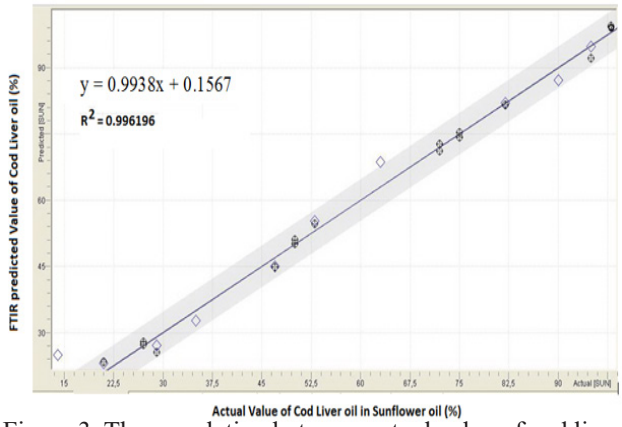

Figure 3. The correlation between actual value of cod liver oil (CLO, x-axis) and FTIR predicted value (y-axis) of CLO in binary mixture with sunflower oil (SFO)

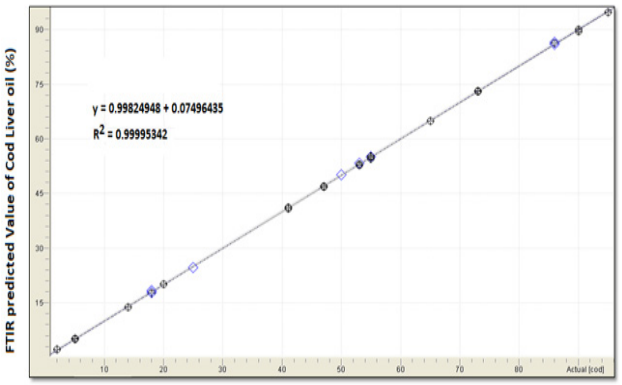

Figure 4. The relationship between actual value of cod liver oil (CLO, x-axis) and FTIR predicted value (y-axis) of CLO in binary mixture with corn oil (CO)

with FTIR normal spectra is also preferred for quantification of CLO in binary mixture with grape seed oil (GSO). The R^2 and RMSEC values obtained are 0,9999 and 0.52% (v/v) with equation of y = 0.9995x + 0.0321. The PLS validation model yielded R^2 value of 0.9998 and RMSEP value of 0.44 % (v/v) with equation of y = 0.9952x + 0.4259 (y axis = predicted value of CLO, x-axis = actual value of CLO). The high value of R^2 and low values of errors (RMSEC and RMSEP) indicated that FTIR spectroscopy coupled with multivariate calibration of PLS can be used for analysis of CLO adulterated with sunflower, corn, and grape seed oil.

Conclusion

FTIR spectroscopy coupled with chemometrics has been successfully used for analysis of CLO in binary mixture with SFO, CO and GSO. In binary mixture with SFO, CLO is determined using normal spectra at frequency region of 1250 – 950 cm^{-1}. Meanwhile, CLO in the binary mixture with CO and GSO is analyzed using wavenumbers of 1350-900 cm^{-1}. The developed method is suitable for routine

analysis of CLO authentication from SFO, CO, and GSO.

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