FOOD RESEARCH

The employment of FTIR spectroscopy and chemometrics for authentication of pumpkin seed oil from sesame oil

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Abstract

The adulteration practice of high price oils such as pumpkin seed oils (PSO) with lower ones could be motivated by economic gains. The objective of this study was to apply FTIR spectroscopy in combination with chemometrics of multivariate calibrations and discriminant analysis for the authentication of PSO. A total of fifteen oils were scanned using FTIR spectrophotometer at mid-infrared regions (4000-650 cm^{-1}) and subjected to principal component analysis (PCA) using absorbance values at whole mid-IR regions to know oil having a close similarity to PSO in terms of FTIR spectra. Two multivariate calibrations namely principle component regression (PCR) and partial least square regression (PLSR) along with FTIR spectra modes (normal, derivative-1, and derivative-2) were optimized to get the best prediction models. In addition, discriminant analysis (DA) was used for classification of PSO and PSO adulterated with oil adulterant. The results showed that among 15 oils, sesame oil (SeO) had the closer score plot in terms of the first principle component and second principle components with that of PSO. Based on the statistical parameters selected (higher R^2 and lowest errors), FTIR spectra in derivative -1 mode at wavenumbers of 1800-663 cm^{-1} were selected for quantification of PSO in SeO with coefficient of determination (R) values of 0.9998 and 0.9994 in calibration and validation models, respectively. The values of root mean square error of calibration (RMSEC) and root mean square error of prediction obtained were 0.003% and 0.006%, respectively. DA using 10 principle components could clearly discriminate PSO and PSO adulterated with SeO with accuracy levels of 100%. FTIR spectroscopy in combination with chemometrics could be an effective means to detect the adulteration of PSO with SeO.

1. Introduction

Pumpkin (*Cucurbita maxima*) belongs to Cucurbitaceae family which widely grows in tropical regions and has a relatively high economic importance around the world. Food industry has exploited to use pumpkin for the production of juices, purees, jams and alcoholic beverages (Jiao *et al*., 2012). Meanwhile, pumpkin seed refers to the edible seed of a pumpkin, containing some bioactive compounds frequently used as herbal medicines and functional foods. Pumpkin seeds are also commonly used in culinary practices mainly in Southeast Asian countries. In Indonesia, pumpkin is one of the popular vegetables consumed as considered as a functional food due to the extraordinary rich sources of bioactive compounds having beneficial health effects (Montesano *et al*., 2018). In addition, pumpkin seed oil (PSO), a strongly dichromatic viscous oil extracted from

pumpkin seed, has gained great attention in fats and oils industry not only as edible oil but also as a potential nutraceutical (Rezig *et al*., 2012).

PSO has been reported to contain phytosterols, phenolic compounds, antioxidants, tocopherols, and small levels of carotenoids responsible to some biological activities which are beneficial to human health (Cuco *et al*., 2019) including prevention of gastric, breast, colorectal and lung cancers (Elfiky *et al*., 2012), retardation of hypertension progression antihypertension (Zuhair *et al*., 2000), prevention of prostate disease, mitigation of hypercholesterolemia and arthritis, alleviation of diabetes mellitus by enhancing hypoglycaemic activity, reduction of bladder and urethral pressure (Fruhwirth *et al*., 2003), improving bladder compliance and urinary disorder in human overactive bladder (Nishimura *et al*., 2014), and offering

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good antioxidant sources (Naziri *et al.*, 2016).

Due to the beneficial effects of PSO to human health, PSO has high price in the market, as a consequence, PSO may be adulterated with other oils. Thus, analytical methods which are reliable for adulteration analysis of PSO are highly needed. Some methods have been proposed, developed and used for authentication of PSO which include molecular spectroscopy (ultraviolet-visible, near-infrared, mid-infrared spectroscopy) (Lankmayr *et al.*, 2004), gas chromatography for analysis of Δ7-phytosterols (Mandl *et al.*, 1999), liquid chromatography (Butinar *et al.*, 2010), and carbon isotope analysis of the individual fatty acids by the use of gas chromatography-combustion-isotope ratio mass spectrometry (GC/C/IRMS) (Spangenberg, 2001).

FTIR spectroscopy coupled with chemometrics has been used for authentication of high priced oils including authentication extra virgin olive oil from palm oil (Rohman and Che Man, 2010), authentication virgin coconut oil from palm oil (Rohman and Che Man, 2009), sesame oil from corn oil (Nurrulhidayah *et al.*, 2014), beef fat from dog meat (Rahayu *et al.*, 2018), and authentication of fish patin oil from palm oil (Putri *et al.*, 2019). However, there is a limited study on the application of FTIR spectroscopy in combination with chemometrics for authentication of pumpkin seed oil (PSO). Therefore, this study was aimed to develop FTIR spectroscopy combined with multivariate calibrations and discriminant analysis for authentication of PSO from sesame oil.

2. Materials and methods

2.1 Materials

Pumpkin seed oil, canola oil, coconut oil, corn oil, extra virgin olive oil, garlic oil, ginger oil, grape seed oil, black seed oil, hazelnut oil, palm oil, rice bran oil, soya bean oil, sesame oil and sunflower oil were purchased from several markets in Yogyakarta, Indonesia. The fatty acids composition of pumpkin seed oil (PSO) and sesame oil (SeO) were in agreement with those in Codex Alimentarius Commission and used to confirm that both oils were not adulterated previously with other oils. The solvents and reagents used were of pro-analytical grade.

2.2 Principal component analysis of oils

Pumpkin seed oil (PSO), canola, coconut, corn, extra virgin olive, garlic, ginger, grape seed, black seed, hazelnut, palm, rice bran, soya bean, sesame and sunflower oils were subjected to FTIR spectral measurements. Oils having close score plot value using absorbance values at whole mid-infrared regions with

PSO was used as oil adulterants. Based on this, sesame oil was selected as oil adulterant.

2.3 Preparation of calibration and validation samples

For quantitative analysis purposes, a series of calibration and validation (prediction) samples with known concentrations of PSO were prepared. For making calibration samples, 20 samples consisting of PSO in binary mixtures with SeO at concentration ranges of 0– 100.0% v/v were prepared. The oil concentrations were selected randomly with the aid of Excel software (Microsoft Inc., USA). For preparing validation samples, a series of different samples was preparing in the concentration ranges covered by calibration samples. All samples were analyzed using FTIR spectrophotometer.

2.4 Discriminant analysis

For discriminant analysis (DA), pure PSO and PSO adulterated with SeO in the concentration range of 0.5–50.0% (v/v) was prepared. Pure PSO was assigned as authentic, meanwhile, PSO mixed with SeO was called as adulterated. Both classes (unadulterated and adulterated samples) were classified and discriminated using DA on the basis of their FTIR spectra.

2.5 FTIR spectra acquisition

The acquisition of FTIR spectra of studied samples was performed using FTIR spectrometer (Thermo Scientific Nicolet iS10, Madison, WI), controlled with the operating Omnic software. The measurements were done in mid-infrared region of 4000-650 cm^{-1} with 32 scanning and the resolution was 8 cm^{-1} using horizontal attenuated total reflectance (HATR) composed of ZnSe crystal. All FTIR spectra were corrected against FTIR spectrum of air as background. After every scan, a new reference air background spectrum was taken. These spectra were recorded as absorbance values at each data point in triplicate.

2.6 Chemometrics analysis

Chemometrics analysis including multivariate calibration and discriminant analysis was performed using TQ Analyst software version 9 (Thermo Fisher Scientific, Inc.). For quantification, multivariate calibrations used were PLS and PCR. Principal component analysis was carried out using software Minitab version 17 (Minitab Inc., USA).

3. Results and discussion

In this study, FTIR spectroscopy in combination with several chemometrics techniques namely principal component analysis (PCA), multivariate calibrations of principle component regression (PCR), partial least

square regression (PLSR) was used for authentication of pumpkin seed oil (PSO). PCA was used to look for oils having similar characteristics to PSO, as depicted as score plot of first principle component analysis (PC1) and second principle component (PC2) using absorbances at whole mid-infrared region (4000-650 cm^{-1}) as variables. As shown in Figure 1, FTIR spectrum of sesame is similar to that of sesame oil (SeO), therefore SeO was selected as oil adulterant. This indicated that PSO and SeO had a similar FTIR spectrum. Besides, both oils physically also have similar color.

Figure 2 reveals FTIR spectra of sesame oil (SeO) and pumpkin seed oil (PSO) scanned at mid-infrared regions corresponding to wavenumbers of 4000-650 cm^{-1} using attenuated total reflectance as a sampling technique. Both FTIR spectra showed the typical infrared spectra of tryglycerides (TG) and this is not surprising because edible oils are mainly composed from TGs (Jimenez-Carvelo *et al.*, 2017). Each shoulders and bands

(peaks) related to absorption of infrared radiation by functional groups present in SeO and PSO. Peak at 3007 was due to stretching vibration of *cis* C=CH, while the peak at 2953 cm^{-1} originated from asymmetric stretching vibration of methyl (-CH$_3$) group. The peaks at 2922 and 2853 cm^{-1} were coming from asymmetric and symmetric stretching vibrations of methylene (-CH$_2$). The carbonyl (C=O) stretching vibration was observed at 1744 cm^{-1}, while the peak at 1654 is from C=C stretching vibration. The bending vibrations of methylene and methyl were observed at wavenumbers of 1460 and 1376 cm^{-1}, respectively. The peaks at regions of 1237, 1160, 1118, 1098 were from C-O vibrations. While, peaks at 996 and 850 were due to bending out of plane vibrations of –HC=CH– (*trans*) and –HC=CH– (*cis*), respectively (Ozulku *et al.*, 2017; Arslan *et al.*, 2019).

Due to the nature of FTIR spectra as fingerprint analytical tools, the variation in FTIR spectra of PSO and SeO existed, and this difference was exploited for
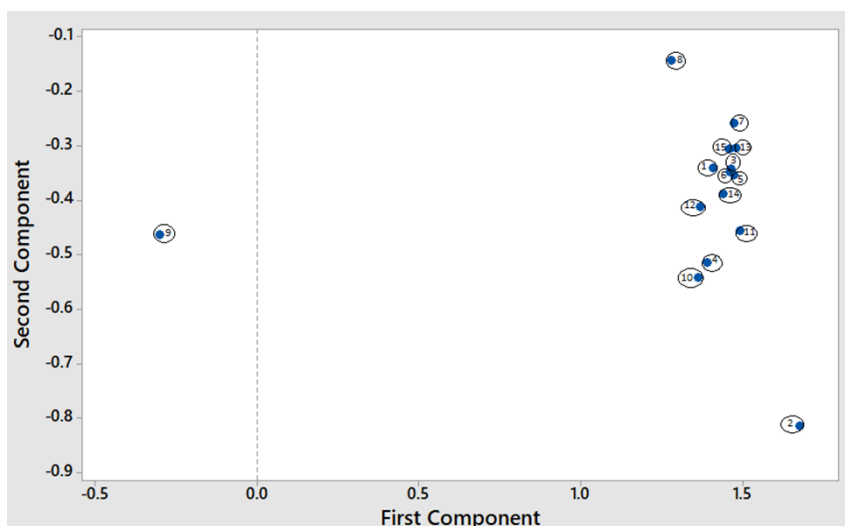

Figure 1. The PCA score plot of pumpkin seed oil (11) and other oils. 1 = Canola Oil; 2 = Coconut Oil; 3 = Corn Oil; 4 = Extra Virgin Olive Oil; 5 = Garlic Oil; 6 = Ginger Oil; 7 = Grape Seed Oil; 8 = Black seed Oil; 9 = Hazelnut Oil; 10 = Palm Oil; 12 = Rice Bran Oil; 13 =Soya Bean Oil; 14 = Sesame Oil; and 15 = Sun Flower Oil.
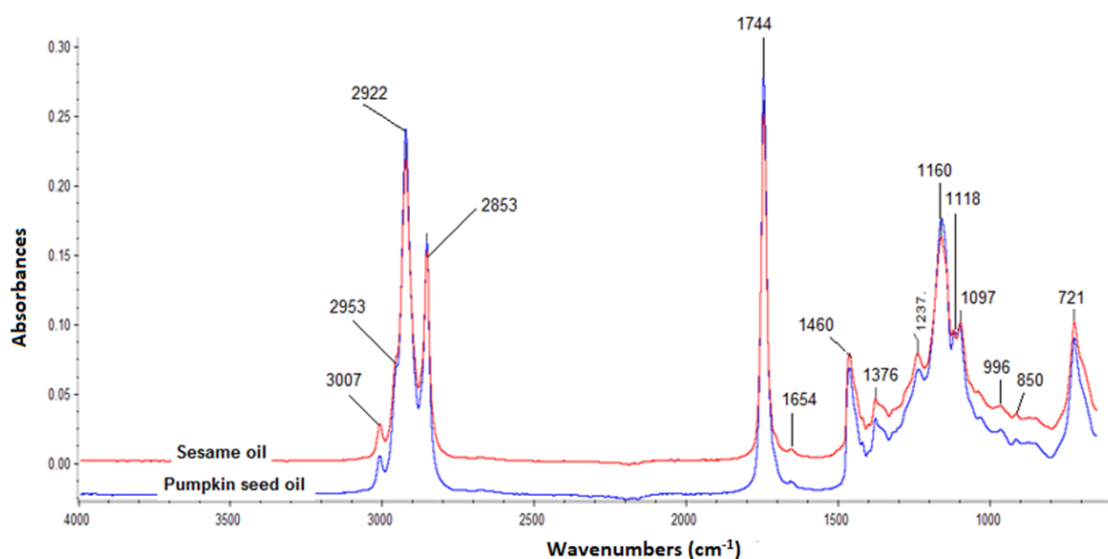

Figure 2. FTIR spectra of pumpkin seed oil and sesame oil scanned at mid infrared region (4000-650 cm^{-1}) using attenuated total reflectance mode.

Table 1. The results of FTIR spectroscopy using different wavenumbers and spectral treatments coupled with multivariate calibration intended for prediction of pumpkin seed oil (PSO) levels adulterated with sesame oil.

Multivariate calibrations	Wavenumber (cm^{-1})	Spectra	Calibration		Validation	
			R^2	RMSEC	R^2	RMSEP
PCR	3100-663	normal	0.9978	0.0096	0.9773	0.0134
		derivative 1	0.9982	0.0086	0.9975	0.0111
		derivative 2	0.9918	0.0188	0.9935	0.0052
	1800-663	normal	0.9984	0.0082	0.9983	0.0131
		derivative 1	0.9989	0.0066	0.9988	0.0084
		derivative 2	0.9971	0.011	0.9964	0.015
	3100-2750	normal	0.9955	0.0136	0.9894	0.0213
		derivative 1	0.9866	0.0235	0.9824	0.0288
		derivative 2	0.9459	0.0468	0.9265	0.0558
	1500-663	normal	0.9989	0.0066	0.9993	0.0056
		derivative 1	0.999	0.0063	0.9991	0.0063
		derivative 2	0.9972	0.0109	0.9981	0.0098
	3100-2750 and 1800-663	normal	0.9985	0.0078	0.9984	0.0116
		derivative 1	0.9992	0.0059	0.9987	0.0081
		derivative 2	0.9972	0.0107	0.9966	0.0151
	3100-2750 and 1500-663	normal	0.9975	0.0102	0.9976	0.0101
		derivative 1	0.999	0.0065	0.9989	0.0069
		derivative 2	0.9981	0.0089	0.9989	0.0081
PLS	3100-663	normal	0.9873	0.0229	0.9873	0.0236
		derivative 1	0.9999	0.002	0.9989	0.0078
		derivative 2	0.9979	0.0092	0.9947	0.0213
	1800-663	normal	0.992	0.0182	0.9914	0.0236
		derivative 1	**0.9998**	**0.003**	**0.9994**	**0.0061**
		derivative 2	0.9996	0.0041	0.9975	0.0122
	3100-2750	normal	0.9952	0.0141	0.9898	0.0209
		derivative 1	0.993	0.017	0.9846	0.0267
		derivative 2	0.9926	0.0175	0.967	0.0386
	1500-663	normal	0.9981	0.0089	0.9971	0.0113
		derivative 1	1	0.001	0.9992	0.0066
		derivative 2	0.9996	0.0039	0.9992	0.0081
	3100-2750 and 1800-663	normal	0.9981	0.0098	0.9971	0.0113
		derivative 1	0.9999	0.0015	0.9992	0.0065
		derivative 2	0.9979	0.0092	0.997	0.0148
	3100-2750 and 1500-663	normal	0.9858	0.0242	0.9848	0.0252
		derivative 1	0.9999	0.0018	0.9992	0.006
		derivative 2	0.9997	0.0035	0.9989	0.0088

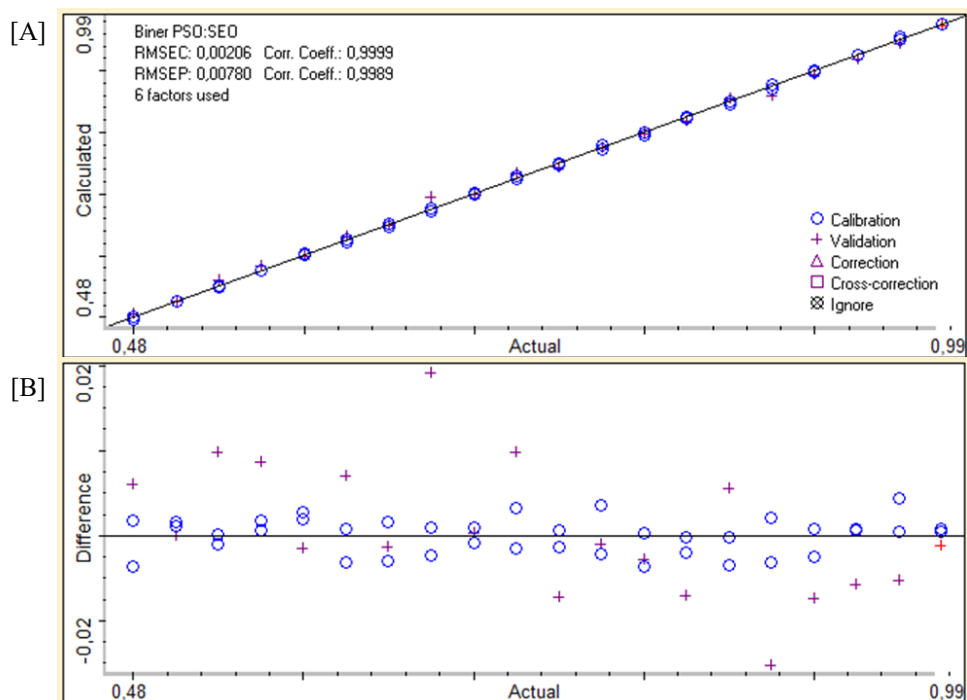**The selected variables were marked with bold.**

Figure 3. The relationship between actual values (x-axis) of pumpkin seed oil (PSO) in x-axis and the predicted values of PSO using FTIR spectroscopy [A] along with residual analysis [B].
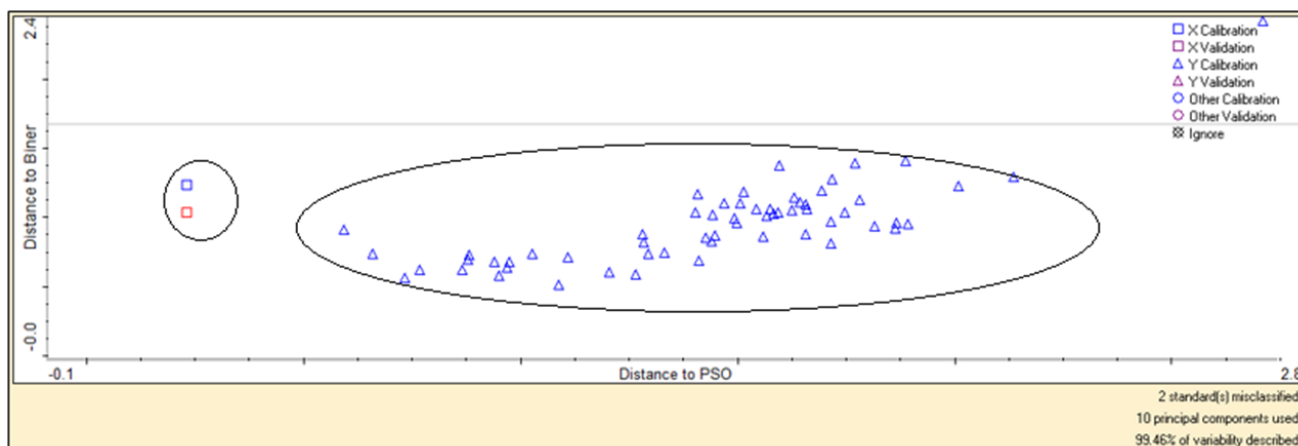
Figure 4. The Cooman's plot obtained during discriminant analysis for discrimination of pure pumpkin seed oil (PSO) and PSO mixed with sesame oil.

optimization of wavenumbers regions and spectral modes for quantitative analysis and discrimination analysis between PSO and PSO mixed with SeO. Quantitative analysis of PSO in SeO was performed with the aid of two multivariate calibrations, namely principle component regression (PCR) and Partial Least Square regression (PLS). Both PCR and PLSR are inverse regression in which concentration (*y-axis*) was modelled with predictors of principle components, a linear combination of absorbance values (*x-axis*) (Manaf *et al.*, 2007).

In order to obtain the best prediction models, multivariate calibrations (PCR and PLSR), the wavenumbers regions and FTIR spectral treatments (normal, derivative-1 and derivative-2) were optimized. Table 1 compiles the optimization results by combining the parameters and the variables selected were based on the highest coefficient of determination (R^2) and the lowest errors. PLSR at wavenumbers of 1800-663 cm^{-1} was selected for quantification of PSO in SeO with R^2 values of 0.9998 and 0.9994 in calibration and validation models, respectively. The values of root mean square error of calibration (RMSEC) and root mean square error of prediction obtained were 0.003% and 0.006%, respectively. The high values of R^2 and low values of RMSEC and RMSEP indicated that the developed models offered acceptable accuracy and precision. Figure 3A reveals the correlation between actual values (*x-axis*) of PSO and the predicted values of PSO using FTIR spectroscopy. It is clear that errors occurring during modelling were randomly occured around zero difference. This indicated that the systematic errors did not existed and the model developed are reliable to predict PSO adulterated with SeO.

The chemometrics of discriminant analysis (DA) is one of the supervised pattern recognition and is commonly used for clustering between authentic samples with adulterated ones (Rohman *et al.,* 2014). The variables used during DA were absorbances at the whole

mid-infrared region, and the Mahalanobis distances were then calculated using these absorbances. Figure 4 shows the Cooman's plot for classification purposes, and DA could classify PSO and PSO mixed with SeO successfully with the accuracy level of 100%. This result indicated that DA as an effective tool to classify high priced edible oils from potential adulterants.

4. Conclusion

Fourier transform infrared (FTIR) spectroscopy in combination with PLSR and discriminant analysis was successfully used for quantification and classification of PSO adulterated with SeO. Derivative-1 FTIR spectra at wavenumbers of 1800-663 cm^{-1} coupled with PLSR was used for quantification, while FTIR normal spectra at whole mid-infrared region were exploited for classification using DA. FTIR spectroscopy in combination with chemometrics of PLSR and DA could be effective to detect the adulteration of PSO with SeO.

Conflict of Interest

Authors declare no conflict of interest.

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