INTRODUCTION

Known as the highest olive oils, extra-virgin olive oil (EVOO) command high-priced vegetable oil because EVOO is only obtained by means of the physical and mechanical processes of fresh and healthy fruits of olive (Olea europaea) in the appropriate maturation stage without any treatments which cause its original composition changed [1, 2]. As a consequence, EVOO is taken into account as high-quality edible oil due to its nutritional benefits; the appreciated features, pleasant taste, and enjoyable flavor [3, 4]. The evidence from epidemiological studies (especially from dietary intervention and cohort studies) trials revealed that the consumption of EVOO is associated with increased longevity [5].

Besides, EVOO is also reported in inhibiting some disease risk factors, such as diabetes, metabolic syndrome and obesity [6] and preventing certain cancers, especially breast and digestive tract cancers [7]. EVOO is rich phenolics compound which contributed to antioxidant activities and its stability [8]. In addition, pumpkin seed oil (PSO) is also considered as one of the functional edible oils [9]. PSO contained high levels of vitamin E, carotenoids and unsaturated fatty acids, contributing to beneficial effects on human health in prevention of high cholesterol levels, prostate cancer, and molecular degeneration [10, 11]. As a consequence, P SO may also be adulterated with cheaper seed oil like palm oil [12].

In fats and oils industry, both EVOO and PSO revealed advantageous effects to human health, desirable characteristics and limited production; therefore, they commanded expensive price which attracted to be adulterated intentionally with other edible oils [13]. Among the adulteration practices, the addition of EVOO with low price edible vegetable oils including palm oil and seed oils like soybean, corn, sunflower, pomace, and rapeseed oils is the most common fraud [5, 14]. As a consequence, numerous methods based on chemical or molecular biology properties have been developed and used for analysis of oil adulterants in EVOO, and among the most reported ones is FTIR spectroscopy [15, 16].

The combination of FTIR spectroscopy with multivariate analysis or chemometrics has been used for authentication studies of EVOO from palm oil [17], corn, soybean, sunflower, rapeseed [18], corn, sunflower, soybean and hazelnut oils [19], and analysis of palm with canola oil [20] and lard in binary mixtures [21]. From literature, there are limited studies reporting the combination of FTIR spectroscopy and chemometrics employed for analysis of palm oil as adulterant in EVOO and PSO in ternary mixtures; therefore, this study was intended to analyze palm oil mixed with EVOO and PSO in ternary mixture.

MATERIALS AND METHODS

Materials

Palm oil (SunCo), pumpkin seed oil (Happy Green), canola oil (Mazola), coconut oil (Kara), corn oil (Mazola), extra virgin olive oil (Bertolli), garlic oil (Double Pagoda), ginger oil (Double Pagoda), grape seed oil (Borges), black seed oil (Al-Aflah), hazelnut oil (Fora), rice bran oil (Oryza Grace), soya bean oil (Mazola), sesame oil (Lee Kum Kee) and sunflower oil (Mazola) were obtained from local markets in Yogyakarta. The other chemicals and solvents used in this study were in the level of pro-analytical grade.

Determination of fatty acids composition

The composition of fatty acids composed PO, PSO, and EVOO was determined by gas chromatography equipped with a detector of flame ionization (GC-FID). Before being analyzed using GC-FID, the studied oils were subjected to derivatization procedure to obtain derivatize methyl ester of fatty acid (FAME). The derivatization procedure and GC-FID analysis were performed according to the literature [22]. The studied oils (50 µl) were added with 1.0 ml of n-hexane and 0.2 ml of 0.2 N NaOH. The solutions were subjected to heating at 70 °C (10 min). Every 2 min, solutions were shaken vigorously, added with 1.5 ml of BF3, and heated at 70 °C (10 min). The solutions were cooled and followed by addition of 1.5 ml of saturated NaCl solution. The supernatant containing FAME was injected into GC-FID (GC-FID Agilent 7890B GC (Germany).
Separation of analytes was performed using column HP-5 (30 m × 0.32 mm × 0.25 μm). The carrier gas used was ultrapure helium with the flow rate of 2.99 ml/min. The temperatures of injector and detector were set at 260 °C. The injection was carried out in splitless mode with ratio of 15:1. The column temperature was set in gradient manner as follows: 160 °C and hold for 2 min, 160-270 °C (10 °C/min rate), and finally hold for 5 min. In addition, flow rate of the air and hydrogen ultrapure were adjusted at 400 and 40 ml/min, respectively. The retention times of FAME standards (Sigma, Aldrich, USA) were used to identify FAMEs in studied oils. The percentage of fatty acid was computed as relative percentage (%) using internal normalization technique as in [23].

**Table 1:** The composition of palm oil (PO) in ternary mixtures with extra virgin olive oil (EVOO) and pumpkin seed oil (PSO)

| No | Concentration (% v/v) | PO | EVOO | PSO |
|----|----------------------|----|------|-----|
| Sample 1 | 23 | 34 | 42 |
| Sample 2 | 5 | 0 | 95 |
| Sample 3 | 18 | 2 | 80 |
| Sample 4 | 46 | 44 | 10 |
| Sample 5 | 25 | 28 | 47 |
| Sample 6 | 26 | 3 | 71 |
| Sample 7 | 36 | 41 | 23 |
| Sample 8 | 26 | 19 | 55 |
| Sample 9 | 15 | 46 | 39 |
| Sample 10 | 40 | 1 | 59 |
| Sample 11 | 31 | 24 | 45 |
| Sample 12 | 25 | 43 | 32 |
| Sample 13 | 23 | 27 | 50 |
| Sample 14 | 18 | 46 | 36 |
| Sample 15 | 12 | 8 | 80 |
| Sample 16 | 34 | 13 | 53 |
| Sample 17 | 39 | 49 | 12 |
| Sample 18 | 18 | 24 | 58 |
| Sample 19 | 23 | 26 | 51 |
| Sample 20 | 27 | 18 | 55 |

**Discriminant analysis**

Discriminant analysis (DA) for grouping samples was carried out, according to Putri et al. [29]. Discriminant analysis (DA) was used for discriminating between pure PO and PO in ternary mixture with PSO and EVOO. For training sets, pure PO and ternary system of PO–PSO-EVOO were prepared in the range of 0.5–50.0% (v/v) of PO was prepared. Both groups (pure PO and PO-PSO-EVOO) were classified and discriminated using DA on the basis of Cooman’s plot using FTIR spectral absorbance at specific wavenumbers as variables.

**FTIR spectra acquisition**

The scanning of FTIR spectra of oil samples was done using FTIR spectrophotometer (Thermo Scientific Nicolet IS10, Madison, WI), equipped with the Omnic software for spectral processing. The measurements were performed in horizontal attenuated total reflectance (HATR) at wavenumbers (1/λ) of 4000-650 cm⁻¹ using 32 scans with the resolution of 8 cm⁻¹. 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.

**Chemo-metrics analysis**

Chemometrics analysis including discriminant analysis and multivariate calibrations of principle component regression (PCR) and partial least square (PLS) was performed using TQ Analyst software version 9 (Thermo Fisher Scientific, Inc.). Statistical parameters used for evaluation of multivariate calibrations performance were coefficient of determination (R²) and root mean square error of calibration (RMSEC), as well as root, mean square error of prediction (RMSEP).

**RESULTS AND DISCUSSION**

Table 2 compiled fatty acid (FA) composition of studied oils in which palmitic and oleic acids are main FAs composed PO. In addition, oleic acid was present as main FA component in EVOO, while PSO is mainly composed from linoleic and oleic acids. All FAs in PO and EVOO were in agreement with those specified in Codex Allimentarius Commission, while FAs in PSO in this study was in accordance with those reported in the literature [24]. These FAs data indicated that studied oils (PO, EVOO and PSO) were not previously mixed with other oils. The difference of fatty acids determined the different intensities of functional groups of studied oils in which oils with higher levels of oleic acids would have higher intensities in peaks related to unsaturated fatty acids.

**Table 2:** Fatty acid composition of fatty acids in palm oil, extra virgin olive oil and pumpkin seed oil

| Fatty acids | Palm oil | Extra virgin olive oil | Pumpkin seed oil |
|------------|----------|------------------------|------------------|
|             | Exp. | Codex | Exp. | Codex | Exp. | According to literature [24] |
| Palmitic acid (C16:0) | 38.4±0.17 | 39.3-47.5 | 11.25±0.58 | 7.5-20 | 11.05±0.12 | 9.39-12.48 |
| Palmitoleic acid (C16:1) | 0.15±0.02 | nd-6 | 0.68±0.02 | 0.3-3.5 | 0.12±0.01 | 0.10-0.14 |
| Stearic acid (C18:0) | 6.67±0.32 | 3.5-6.0 | 3.12±0.09 | 0.5-5.0 | 4.62±0.04 | 4.15-5.67 |
| Oleic acid (C18:1) | 40.79±0.45 | 36.0-44.0 | 75.12±1.05 | 55.0-83.0 | 30.56±1.15 | 29.20-36.44 |
| Linoleic acid (C18:2) | 9.35±0.05 | 9.0-12.0 | 6.97±0.07 | 3.5-21.0 | 45.4±2.45 | 42.73-55.33 |
| Arachidic acid (C20:0) | 0.31±0.01 | nd-0.5 | 0.29±0.02 | <1.5 | 0.22±0.01 | 0.14-0.30 |
| Linolenic acid (C18:3) | 0.20±0.01 | nd-1.0 | 0.43±0.01 | <0.8 | 0.36±0.01 | 0.29-0.39 |

Exp: results obtained from the experiment in this study.
Due to its nature as fingerprint analytical technique, Fourier transform infrared (FTIR) spectroscopy is an ideal method for analysis of complex mixtures such as edible fats and oils and other secondary metabolites extracted from specific parts of plants [25]. Fig. 1 showed FTIR-ATR spectra of three edible oils, palm oil (PO), extra virgin coconut oil (EVOO), and pumpkin seed oil (PSO) scanned at mid-infrared regions corresponding to 1/λ of 4000–650 cm$^{-1}$. Each peaks and shoulders in FTIR spectra come from the IR absorption by functional groups present in these oils. The interpretation of each peaks and shoulders in relation to functional groups in FTIR spectra was compiled in table 3. All Peaks in FTIR-ATR among PO, EVOO and PSO spectra are very similar. This is not surprising because the main components composing edible fats and oils were triglyceride (95-98%), glycerol esterified with three fatty acids [27]. However, upon detailed investigation, FTIR-ATR spectra of PO, EVOO and PSO reveal slight differences in terms of band intensities (absorbance's) and the exact wavenumbers in each peak and shoulders [28].

Table 3: Functional groups responsible for absorption of peaks and shoulders in palm oil, extra virgin olive oil, and pumpkin seed oil [26]

| Wavenumbers region (cm$^{-1}$) | Functional groups and vibration modes |
|-------------------------------|-------------------------------------|
| 3007                          | cis C=CH, stretching vibration      |
| 2953                          | methyl (-CH$_3$), asymmetric stretching vibration |
| 2922 and 2853                 | methylene (-CH$_2$), asymmetric and symmetric stretching vibrations |
| 1744                          | carbonyl (C=O), stretching vibration |
| 1654                          | C=C, stretching vibration           |
| 1460                          | methylene (-CH$_2$), bending vibration |
| 1376                          | methyl (-CH$_3$), bending vibration |
| 1237, 1160, 1118, 1098        | C-O, stretching vibrations          |
| 996                           | –HC=CH–(trans), bending out of a plane |
| 850                           | –HC=CH–(cis), bending out of plane   |

Table 4: The performance of principle component regression (PCR) and partial least square (PLS) for prediction of palm oil in ternary mixtures with extra virgin olive oil and pumpkin seed oil along with wavenumbers regions and FTIR spectral modes used (normal and derivatives)*

| Multivariate calibrations | Wavenumber (cm$^{-1}$) | Spectra | Calibration | Validation |
|---------------------------|------------------------|---------|-------------|------------|
|                           |                        |         | R           | RMSEC      | R          | RMSEP      |
| PCR                       | 3100-663               | normal  | 0.9785      | 0.0086     | 0.9641     | 0.0082     |
|                           |                        | derivative 1 | 0.9778     | 0.0206     | 0.9883     | 0.0230     |
|                           |                        | derivative 2 | 0.9486     | 0.0311     | 0.9582     | 0.0293     |
|                           | 1800-663               | normal  | 0.9855      | 0.0016     | 0.9505     | 0.0037     |
|                           |                        | derivative 1 | 0.9605     | 0.0273     | 0.9683     | 0.0300     |
|                           |                        | derivative 2 | 0.9040     | 0.0420     | 0.9136     | 0.0413     |
|                           | 3100-2750              | normal  | 0.9836      | 0.0177     | 0.9720     | 0.0246     |
|                           |                        | derivative 1 | 0.9806     | 0.0192     | 0.9760     | 0.0241     |
|                           |                        | derivative 2 | 0.9620     | 0.0268     | 0.9589     | 0.0298     |
|                           | 1500-663               | normal  | 0.9548      | 0.0292     | 0.9028     | 0.0433     |
|                           |                        | derivative 1 | 0.9365     | 0.0344     | 0.9271     | 0.0381     |
|                           |                        | derivative 2 | 0.8480     | 0.0519     | 0.8491     | 0.0525     |
|                           | 3100-2750 and 1800-663 | normal  | 0.9824      | 0.0183     | 0.9693     | 0.0270     |
|                           |                        | derivative 1 | 0.9787     | 0.0202     | 0.9887     | 0.0255     |
|                           |                        | derivative 2 | 0.9227     | 0.0379     | 0.9484     | 0.0321     |
|                           | 3100-2750 and 1500-663 | normal  | 0.9740      | 0.0223     | 0.9486     | 0.0316     |
|                           |                        | derivative 1 | 0.9762     | 0.0213     | 0.9875     | 0.0158     |
|                           |                        | derivative 2 | 0.8661     | 0.0491     | 0.8827     | 0.0465     |
|                           | 3100-663               | normal  | 0.9927      | 0.0119     | 0.9713     | 0.0269     |
|                           |                        | derivative 1 | 0.9959     | 0.0088     | 0.9861     | 0.0256     |
|                           |                        | derivative 2 | 0.9861     | 0.0163     | 0.9811     | 0.0210     |
|                           | 1800-663               | normal  | 0.9933      | 0.0114     | 0.9623     | 0.0326     |
|                           |                        | derivative 1 | 0.9795     | 0.0198     | 0.9836     | 0.0262     |
|                           |                        | derivative 2 | 0.9613     | 0.0271     | 0.9587     | 0.0297     |
|                           | 3100-2750              | normal  | 0.9844      | 0.0173     | 0.9723     | 0.0246     |
|                           |                        | derivative 1 | 0.9799     | 0.0196     | 0.9782     | 0.0230     |
|                           |                        | derivative 2 | 0.9911     | 0.0130     | 0.9732     | 0.0251     |
|                           | 1500-663               | normal  | 0.9718      | 0.0232     | 0.9428     | 0.0334     |
|                           |                        | derivative 1 | 0.9776     | 0.0207     | 0.9762     | 0.0225     |
|                           |                        | derivative 2 | 0.8450     | 0.0525     | 0.8544     | 0.0515     |
|                           | 3100-2750 and 1800-663 | normal  | 0.9935      | 0.0112     | 0.9670     | 0.0305     |
|                           |                        | derivative 1 | 0.9843     | 0.0173     | 0.9868     | 0.0255     |
|                           |                        | derivative 2 | 0.9978     | 0.0064     | 0.9806     | 0.0264     |
|                           | 3100-2750 and 1500-663 | normal  | 0.9962      | 0.0085     | 0.9798     | 0.0207     |
|                           |                        | derivative 1 | 0.9967     | 0.0080     | 0.9906     | 0.0152     |
|                           |                        | derivative 2 | 0.8538     | 0.0542     | 0.8450     | 0.0527     |

*The selected variables were marked with bold. R = correlation coefficient; RMSEC = root mean square error of prediction; RMSEP = root mean square error of prediction.
Quantitative analysis of PO in ternary mixtures with EVOO and PSO was performed by the assistance of two multivariate calibrations, namely principle component regression (PCR) and partial least square (PLS). Table 4 compiled the performance of PCR and PLS for prediction of PO in ternary mixtures with EVOO and PSO along with wavenumbers regions and FTIR spectral modes used (normal and derivatives). The wavenumbers regions were selected based on the variation existed among PO, EVOO, and PSO. In addition, derivatization was intended to resolve any extensive overlapping peaks. The selection of FTIR spectroscopy conditions (multivariate calibrations, spectral modes, and wavenumbers regions) was based on the highest coefficient of correlation (R) and the lowest errors in calibration and prediction, expressed with root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP). Finally, the first derivative FTIR spectra at the combined 1/λ regions of 3100-2750 and 1500-663 cm⁻¹ aided with PLS regression offered the best prediction models for quantitative analysis of PO in ternary mixtures with EVOO and PSO. The R values obtained for the correlation between actual values and FTIR predicted values were 0.9967 and 0.9906 in calibration and validation models, respectively (fig. 2). The low errors either in calibration and prediction models were confirmed by residual analysis (fig. 2B) in which no systematic errors were observed. The high R values and lowest values of RMSEC and RMSEP indicated that FTIR spectroscopy aided with PLS offered a reliable technique for analysis of PO as adulterants in high valuable oils such as EVOO and PSO either in binary or ternary models.

Fig. 1: FTIR spectra using attenuated total reflectance mode of palm oil, extra virgin olive oil, and pumpkin seed oil acquired at mid-infrared region (4000-650 cm⁻¹)

![FTIR spectra](image)

Fig. 2: The correlation between actual values (x-axis) and FTIR predicted values (y-axis) of palm oil in ternary mixtures with extra virgin olive oil (EVOO) and pumpkin seed oil (PSO) with a coefficient correlation of 0.9967 and 0.9906 in calibration and validation models. RMSEC = root mean square error of calibration; RMSEP = root mean square error of prediction

![Correlation graph](image)
Discriminant analysis (DA), one of the supervised pattern recognition techniques, was used for discrimination between pure PO and PO in ternary mixtures with EVOO and PSO. Both classes (pure and the mixture) were clearly separated, indicating that PO was different from EVOO and PSO (fig. 3), the accuracy levels of discrimination capacity were 100%, meaning that no training samples were misclassified in the wrong groups. Misclassification may occur if both groups are very similar. This result could be extended that DA based on Mahalanobis distance of variables used (absorbances at wavenumbers of 3100-2750 and 1500-663 cm⁻¹) was an effective means for authentication of EVOO and PSO from the possible adulteration with low-value edible oils such as palm oil.

CONCLUSION

Fourier transform infrared (FTIR) spectroscopy in combination with PLS and discriminant analysis could be successfully used for analysis of palm oil (PO) in ternary mixtures with extra virgin olive oil (EVOO) and pumpkin seed oil (PSO). The first derivative FTIR-ATR spectra at combined wavenumbers regions of 3100-2750 and 1500-663 cm⁻¹ were selected for analysis of PO in ternary mixtures with EVOO and PSO with acceptable results. FTIR spectroscopy in combination with multivariate calibration of PLS and DA could be effective means to quantify and to discriminate PO mixed into EVOO and PSO.

ACKNOWLEDGMENT

The authors thank to the Ministry of Research, Technology and Higher Education, Republic Indonesia for financial support during this study through Hibah Penelitian Dasar Unggulan Perguruan Tinggi (PUPT) 2019 with contract number 2519/UN1.DITLIT/DIT-LIT/IT/2019.

ABBREVIATION

FTIR = Fourier transform infrared; ATR = attenuated total reflectance; PCR = principle component regression; PLS = partial least square; RMSEC = root mean square error of calibration; RMSEP = root mean square error of prediction; DA = Discriminant analysis; PO = palm oil; PSO = pumpkin seed oil; EVOO = extra virgin olive oil; 1/λ = wavenumbers; R = coefficient of correlation

AUTHORS CONTRIBUTIONS

Irnawati performed research activities and analyzed data. AR, SM and SR designed research, prepared manuscript and made critical thinking on manuscript.

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Fig. 3: The discriminant analysis expressed by Cooman’s plot for the discrimination of palm oil and palm oils in ternary mixtures with extra virgin olive oil and pumpkin seed oil.
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