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ORIGINAL ARTICLE

Evaluation of Extra Virgin Olive Oil Adulteration with Edible Oils using ATR-FTIR Spectroscopy

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Abstract

Extra virgin olive oil (EVOO) is categorized as expensive oil due to high-quality nutritional value. Unfortunately, EVOO is easily adulterated with other low-quality edible oils. Therefore, this study was done to differentiate and analyze the adulteration of EVOO with other edible oils using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy. The study was used several edible oils included canola oil, corn oil, sunflower oil, and soybean oil as an adulterant for EVOO. The adulterant EVOO samples were prepared by mixing with dissimilar concentrations of the solely edible oils (20 %, 40 %, 60 % and 80 % (v/v)). The main functional groups of EVOO and other edible oils are O-H, C-H, C=C and C=O groups were assigned around 3500 cm^{-1}, 2925 cm^{-1}, 3006 cm^{-1} and 1745 cm^{-1} wavenumbers, respectively. From the comparison of EVOO and other adulterant edibles oil spectra, it showed that the EVOO has the lowest absorbance intensity at around 3006 cm^{-1} represented double bond which is closely related to the composition of oil sample. The adulteration of EVOO was evaluated by analysing the changes in the absorbance based on the linear regression analysis graph of the bands at 3006 and 2925 cm^{-1} and the limit of detection (LOD) was measured. The graph of A3008/A2925 with good relative coefficients (R^2) and lower LOD is more favourable than the linear regression graph of A3006 versus percentage of edible oils added in EVOO. This study showed that ATR-FTIR spectroscopy is a convenient tool for analysing the adulteration of EVOO.

Keywords: Extra virgin olive oil, Edible oils, ATR-FTIR spectroscopy, Adulteration.

Introduction

Extra Virgin Olive Oil (EVOO) is extracted from olive fruits, *Olea europaea L*. commonly used as a cooking medium, and commercially used in cosmetic and medical applications. The production

of EVOO is solvents free using cold pressing technique with temperature below the boiling point of oil (Boskou, 2015). EVOO is a higher quality oil and contains higher nutritional value which is good for human health. The composition of fatty acids in EVOO included 55-83 % oleic acid, 3.5-21 % linoleic acid, 7.5-20 % palmitic acid and 0.5-5 % stearic acid (Quintero-Flórez et al., 2015). The advantage of EVOO consumption is believed can prevent chronic diseases such as heart attack or colon cancer (Gorzynik-Debicka et al., 2018).

Furthermore, the consumption of EVOO can help to maintain body weight due to the low content of saturated fats (Galvão Cândido et al., 2017). Thus, the price market for olive oil products becomes expensive. Since that, some irresponsible market players are trying to add low-priced edible oils into EVOO so-called "EVOO adulteration". Adulteration of EVOO is known as substitutions or addition of other less expensive edible oils in the production of EVOO to lower the cost and gain a large quantity of oil. As consequence, it can cause bad impact on the consumer health and safety due to the shelf life of the final product and the fake nutritional values (Boskou, 2015; Guillen & Cabo, 1997).

Commonly, chromatographic techniques are used to analyse the adulteration of EVOO (Brkljaca et al., 2013; Carranco et al., 2018), but these techniques are time-consuming, labour demanding and producing toxic waste (Rohman & Man, 2011). Therefore, Fourier Transform Infrared (FTIR) spectroscopy is an alternative technique that is simple and easy to analyse the adulteration of EVOO that inessential of sample preparation as common practices in chromatographic technique (Alhanash et al., 2018; Azadmard-Damirchi & Torbati, 2015). Moreover, FTIR spectroscopy provides other advantages such as non-destructive, non-contact measurement technique, economic, rapid and good reproducibility (Bacsik et al., 2004).

The enthusiasm for the application of FTIR spectroscopy in food analysis has been comprehensively used and substantial potential technique for determining the authenticity of EVOO up today. The FTIR technique provides quantitative and qualitative insight of the functional group structure and composition of chemical compounds contains in food substances including edibles oil. The qualitative is referring to the identification of the organic compounds, for instance, via a vibrational mode of the molecular group caused by the existence of certain functional groups in the infrared spectrum at an exact frequency. Meanwhile, the quantitative determination is based on the intensities of the bands in the spectrum related to the concentration following Beer's Law (Vlachos et al., 2006).

In this recent study, the identification of functional groups in EVOO and edible oils (canola oil, corn oil, sunflower oil and soybean oil) were presented. The adulteration of EVOO with additional edible oils was determined by evaluating the shift of bands and calculating the limit of detection (LOD) value. The advanced Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) was used in this work. Attenuated total reflectance (ATR) in conjunction with FTIR provides more rapid, useful, automatable, and well-ease sample handling. ATR-FTIR had improved in handling the preparation of a liquid sample by directly poured onto the ATR plate. It also improved the ratio signal-to-noise, multiplexing, and higher energy and resolution (Van de Voort, 1992). The use of ATR-FTIR spectral analysis shown noticeable differences in the intensity of band characteristics of the difference of nature and composition content of edible oils (Guillen & Cabo, 1997).

Materials and Methods

Preparation and Characterization of Samples

The extra virgin olive oil (EVOO), canola oil, corn oil, sunflower oil and soybean oil were supplied from a trusted brand. In order to quantify the adulteration, the mixtures of oil were made by 20 %, 40 %, 60 % and 80 % (v/v) for each of the canola oil, corn oil, sunflower oil and soybean oil with EVOO separately (Poiana et al., 2015). The stock mixture of oil was kept in the darkroom to avoid

oxidation of the oil. The IR spectra of samples were recorded using ATR-FTIR spectroscopy (PerkinElmer 99365 Spectrophotometer, USA).

Calculation of the Detection Limit (LOD)

The limit of detection (LOD) was computed using the calibration plot of spectral data for adulteration of EVOO with adulterant edible oils. The LOD was calculated by dividing the triplicated standard deviation of the intercept with slope of the calibration curves in Figure 3. The LOD was calculated using Equation (1) and (2) (Poiana et al., 2015):

$$\text{LOD (\%)} = (Y_{LOD} - \text{intercept})/\text{slope} \quad (1)$$

where: Y_{LOD} was found using Equation (2):

$$Y_{LOD} = \text{intercept} + 3SD \quad (2)$$

where: SD = standard deviation.

Results and Discussion

Identification of the Functional Groups of EVOO and Other Edible Oils

The spectra of ATR-FTIR for EVOO and adulterant edible oils (Fig. 1) shows that there is no noticeable distinguishes between spectral features. The spectra obviously indicate the main functional groups in the studied edible oils. Table 1 tabulated the summary of functional groups corresponding to the ATR-FTIR spectra for edibles oil samples. A similar observation was revealed by Nurwahidah et al., (2019), Vanstone et al., (2018), Poiana et al., (2015), and Vlachos et al., (2006).

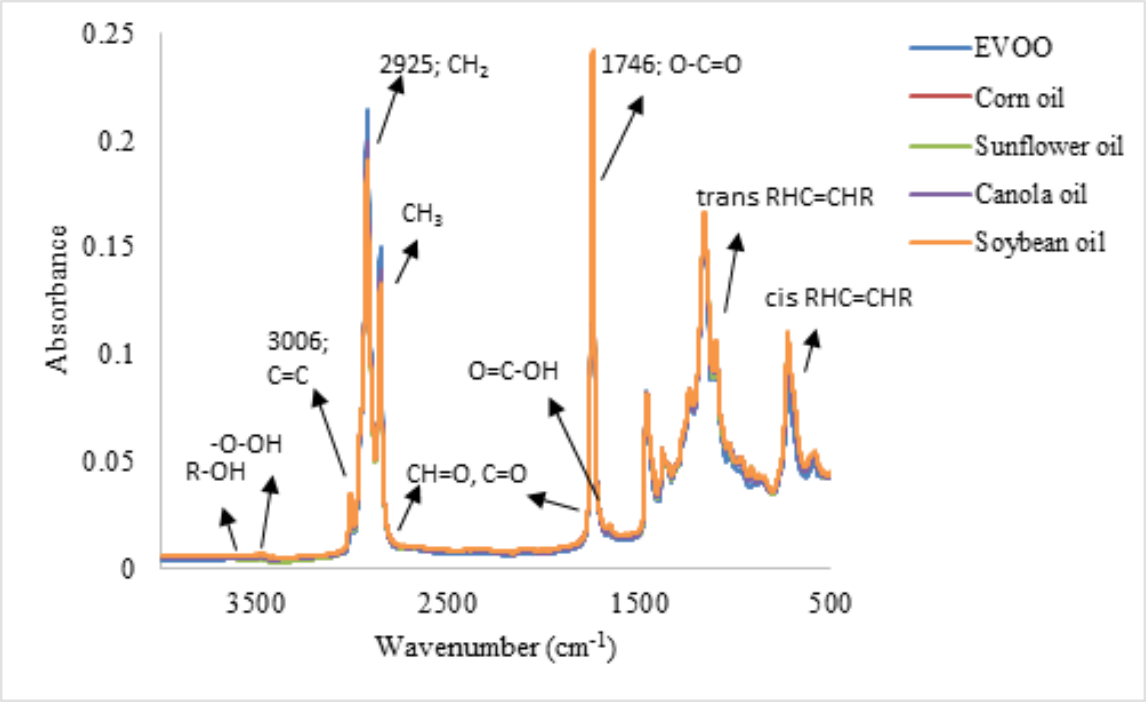


Figure 1: Spectra of edible oils.

Table 1: Summary of functional groups in edibles oil samples

Wavenumber (cm^{-1})	Functional groups
3471	overtone of C=O ester
3006	C–H stretching (*cis* double bonds, =CH)
2925	C-H stretching (CH_2 group)
~1746	C=O stretching (ester carbonyl functional groups of the triglycerides)
1654	C=C stretching (*cis*- disubstituted olefins, RHC=CHR)
1463, 1458	C–H of CH_2 and CH_3 aliphatic groups
967	*trans* –HC=CH− group of disubstituted olefins
722	CH_2 rocking and *cis* –HC=CH− group of disubstituted olefins

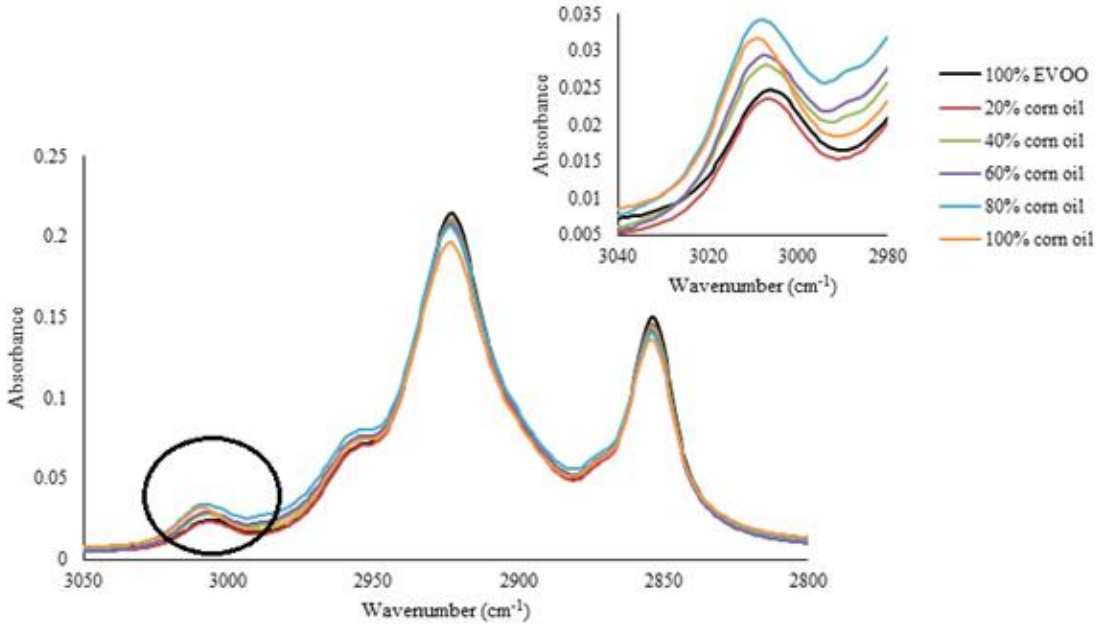
Analysis ATR-FTIR Spectra of EVOO Adulteration

Fig. 2 shows the region of 3050-2800 cm^{-1} wavenumber recorded for EVOO and adulterated EVOO. The band of EVOO is shifted at range around 3006 cm^{-1} resulting from adulteration mixture of EVOO with adulterant edible oils. The band at 3006 cm^{-1} is consigned to the C–H stretching (symmetric vibration of the cis double bonds, =CH). Similar findings were found by Vlaschos et al., (2006) and Poiana et al., (2015). The spectra in Figs. 2(a), 2(b), 2(c) and 2(d) shows the maximum absorbance value for 100 % of corn oil, sunflower oil, canola oil and soybean oil were at 3008 cm^{-1}, 3009 cm^{-1}, 3007 cm^{-1} and 3009 $cm^{-1,}$ respectively. Meanwhile, the maximum absorbance of 100 % EVOO is at lower wavenumber 3005 cm^{-1}. It also shows that the studied adulterant edible oils have higher absorbance compared to EVOO.

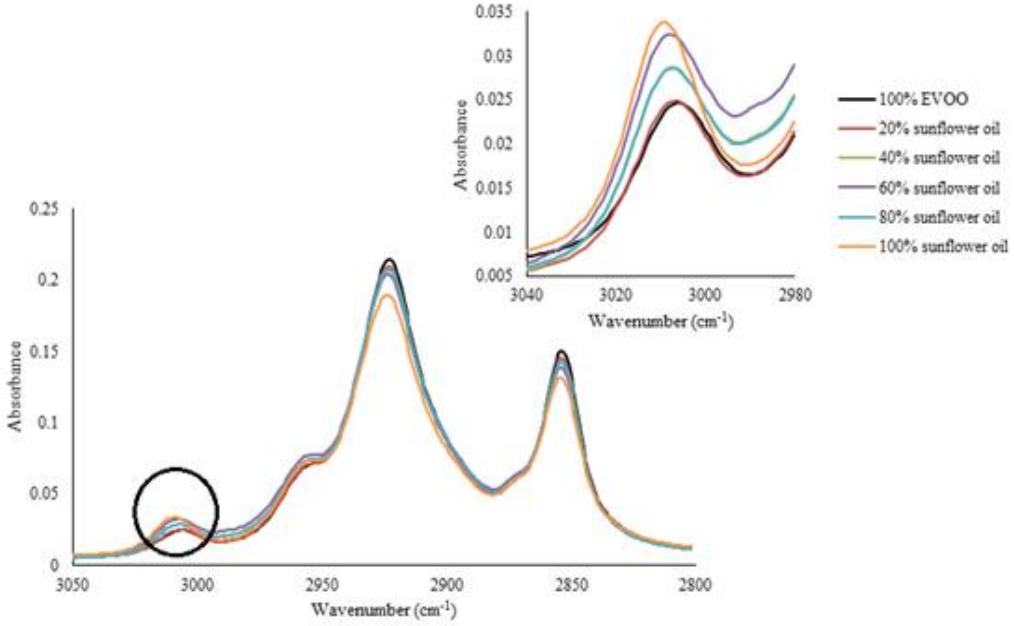
In addition, these results showed a shifted bands of each adulterated EVOO from 3005 cm^{-1} to 3008 cm^{-1} (Fig. 2(a)), 3005 cm^{-1} to 3009 cm^{-1} (Fig. 2(b)), 3005 cm^{-1} to 3007 cm^{-1} (Fig. 2(c)), and 3005 cm^{-1} to 3009 cm^{-1} (Fig. 2(d)) according to the increasingly additional of adulterant edible oils percentage into EVOO. The shift of this band is due to the oil nature and the composition of fatty acids. It is agreed with several previous studies by Poiana et al. (2015) and Vlachos et al. (2006).

Briefly, the nature and the composition of edible oils could be explained; corn oil and sunflower oil comprise a higher amount of linoleic acid which are 34.0-62.0 % and 48.0-74.0 %, respectively compared to EVOO which is comprised only 3.5-21.0 %. Meanwhile, the canola oil comprises a higher proportion of linoleic acid (21.7 %) and has linolenic acid contain rather than EVOO. The soybean oil also comprises a higher proportion of linoleic acid which is 50.0-60.0 % and has linolenic acid (5.0-9.0 %) whereas EVOO comprises a higher amount of oleic acyl group (Vlachos et al. 2006, Guillen & Cabo, 2009).

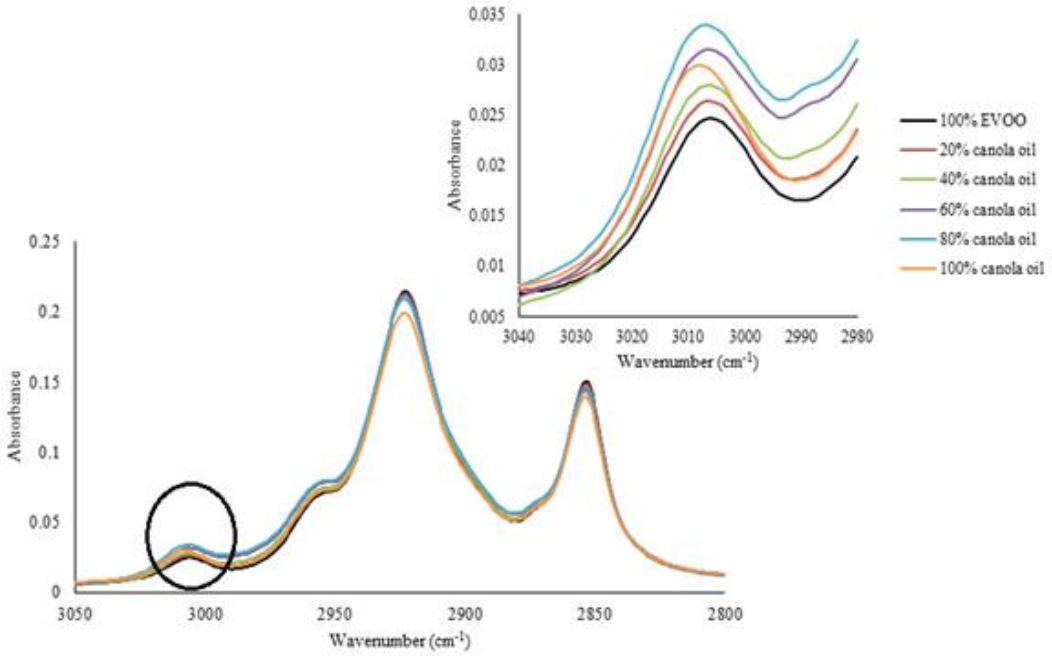
From this observation, it showed that the EVOO has the lowest degree of unsaturation fatty acids than the adulterant edible oils samples. Therefore, the obtained FTIR result with higher absorbance for edible oils is reasonable relating to the increasing degree of unsaturation fatty acids due to the higher content of linoleic acid in the triglyceride composition (Poiana et al., 2015). Table 2 summarizes the percentage of the composition of fatty acids in each edible oil.


Absorbance
Wavenumber (cm-1)
100% EVOO
20% corn oil
40% corn oil
60% corn oil
80% corn oil
100% corn oil

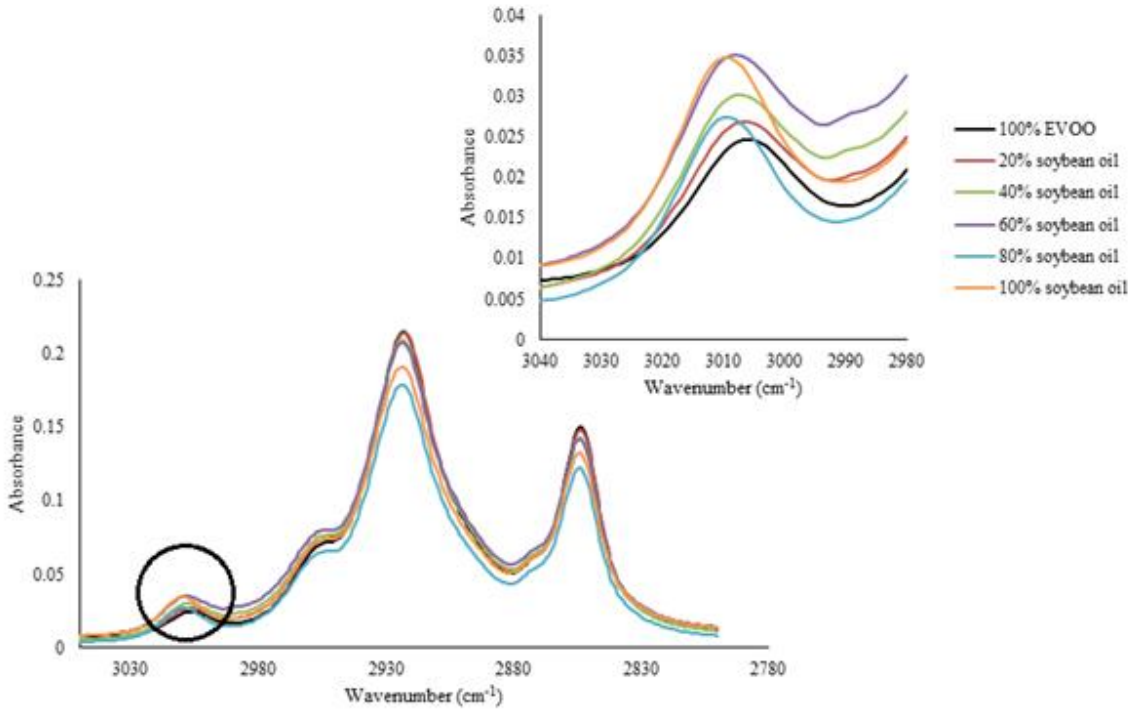
(a)


Absorbance
Wavenumber (cm-1)
100% EVOO
20% sunflower oil
40% sunflower oil
60% sunflower oil
80% sunflower oil
100% sunflower oil

(b)



(c)



(d)

Figure 2: Spectra of adulteration EVOO with (a) corn oil, (b) sunflower, (c) canola oil and (d) soybean oil at region 3050-2800 and inset at the region around 3006 cm^{-1}. In the inset shows the band shift at 3006 cm^{-1} due to adulteration response (range circled).

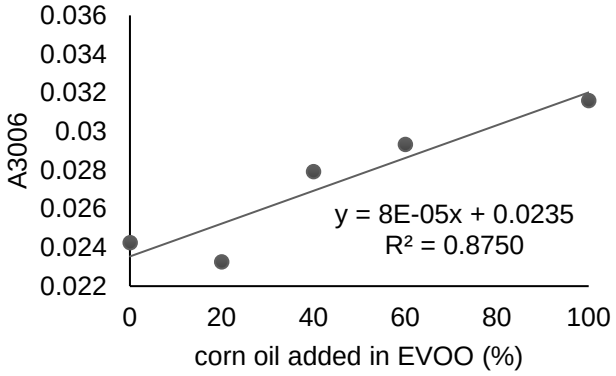
Table 2: Composition of fatty acids in edible oils (Vlachos et al., 2006; Guillen & Cabo, 2009; Kostik et al., 2013, Orsavova et al., 2015, Mirzaee Ghazani & Marangoni, 2016)

Edible oils	Linoleic acid (%) C18:2 (%)	Linolenic acid (%) C18:3 (%)	Oleic acid (%) C18:1 (%)
EVOO	3.5-21.0	0.2-1.8	64.0-83.0
Corn oil	34.0-62.0	1.0	24.0-42.0
Sunflower oil	55.0-75.0	Not detected	14.0-35.0
Canola oil	21.7	12.0	23.0-60.0
Soybean oil	50.0-60.0	5.0-10.0	18.0-26.0

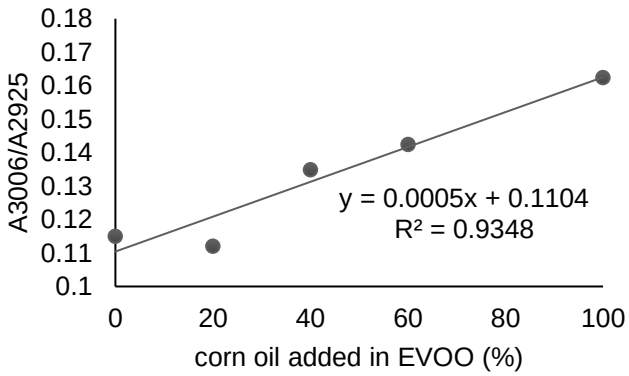
Quantification of EVOO Adulteration

Referring to the obtained result from ATR-FTIR spectra, the absorbance band at 3006 cm^{-1} was selected to express the changes in the degree of unsaturation fatty acid in response to EVOO adulteration by addition of adulterant edible oils. The intensity of the band for 100 % EVOO is lower than the intensity of the adulterated EVOO samples band. The changes of the absorbance were plotted as in Figs. 3(a), 3(b), 3(c) and 3(d).

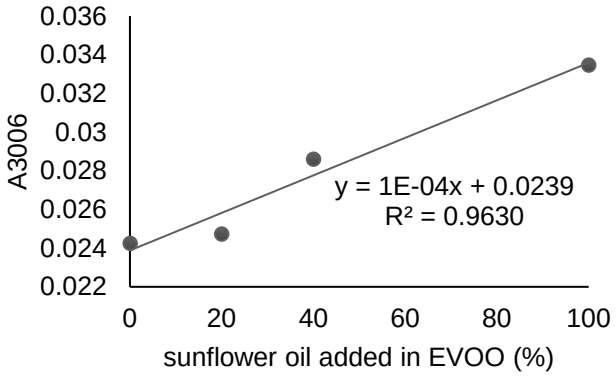
Furthermore, Figs. 3(e), 3(f), 3(g) and 3(h) shows the calibration plot of the ratio between two bands at 3006 cm^{-1} and 2925 cm^{-1} to quantify the adulteration of EVOO. The ratio between the bands (A3006/A2925) was studied as an index for the change in the degree of unsaturation of EVOO in response to adulteration by the addition of edible oils. The band at 2925 cm^{-1} is allocated to the asymmetric stretching vibration of C-H from aliphatic CH_2 group. The ratio of the peaks intensities at 3006 cm^{-1} and 2925 cm^{-1} represents the percentage of the C-H bonds which are attached by cis doubled bonds (=CH) in the edible oil. This method was referred from Vlachos et al. (2006), Guillen & Cabo (1998) and Poiana et al. (2015) with slight modification.



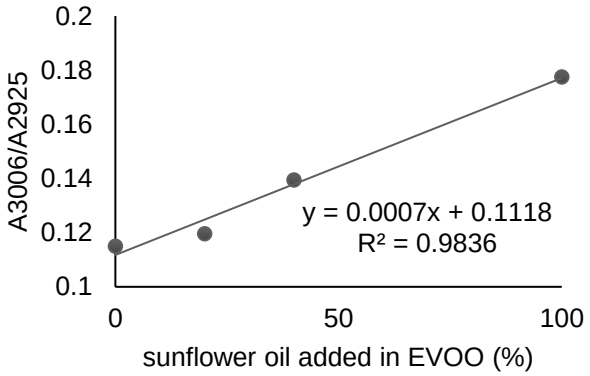
(a)



(e)



(b)



(f)

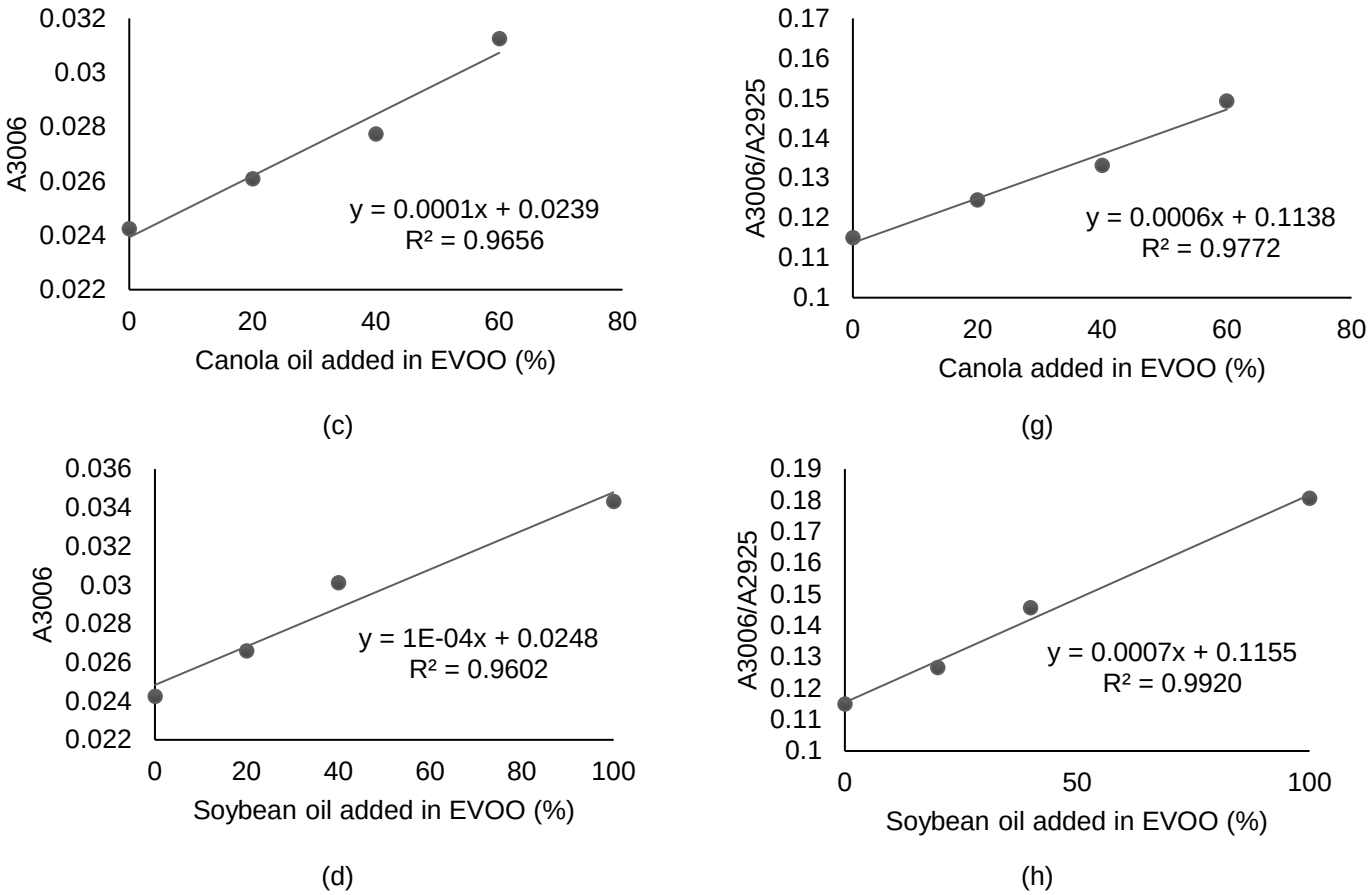


Figure 3: Calibration plots of band at 3006 cm^{-1} (a), (b), (c) and d) and the ratio A3006/A2925 (e), (f), (g) and (h) versus percentage of edible oils added in EVOO.

Table 3: Equation and R^2 value for A3006 and A3006/A2925 for each edible oil added in EVOO.

Types of adulterant	Absorbance	Equation	R^2	LOD
Corn oil	A3006	y = 8E-05x + 0.0235	0.8750	1.5875
	A3006/A2925	y = 0.0005x + 0.1104	0.9348	1.3982
Sunflower oil	A3006	y = 1E-04x + 0.0239	0.9630	1.1385
	A3006/A2925	y = 0.0007x + 0.1118	0.9836	1.0212
Canola oil	A3006	y = 0.0001x + 0.0239	0.9656	1.0521
	A3006/A2925	y = 0.0006x + 0.1138	0.9772	0.9162
Soybean oil	A3006	y = 1E-04x + 0.0248	0.9602	1.3188
	A3006/A2925	y = 0.0007x + 0.1155	0.9920	1.0784

Conclusion

This study indicated that the ATR-FTIR method was successfully analysed the adulteration of EVOO. The result demonstrated that the alterations were obtained in the band of the FTIR spectra. From the band changes, the adulteration of EVOO was assessed based on the calibration plot of band changes at 3006 cm^{-1} and at the ratio A3006/A2925. From the analysis, the ratio A3006/A2925 is suggested to examine EVOO adulteration instead of A3006 due to low LOD

values. Thus, it proved that the ATR-FTIR is a promising method with simple, economical and easy for analysing the adulteration of EVOO.

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