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# ORIGINAL: Efficient Detection of Extra Virgin Olive Oil Adulteration via UV and FTIR Spectra in Combination with Heat-Mediated Oxidation Method

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**ABSTRACT**

The main aim of this study is simple and fast authentication of extra virgin olive oil by different spectroscopic techniques individually and also in combination with minimal chemical waste. UV spectra of the EVOO and mixed olive oil samples were recorded before the heating test and then along the thermal degradation experiments at the 45- and 90-mins intervals set for the analysis. The EVOO and mixed oils samples showed high absorption values around 240-300 nm band. The results showed that the characteristics of FTIR spectra including peak number, peak position and peak shape in mixed samples were significantly different from EVOO samples. According to the studies, the frequencies of around 2920 $cm^{-1}$ and 2856 $cm^{-1}$ could be related with C–H stretching (e.g. cis-double bonds) and with –C–H asymmetrical and symmetrical stretching in methylene groups. The frequency at 2925 $cm^{-1}$ is associated with aliphatic CH2 groups. Around 1366 $cm^{-1}$ and 1451 $cm^{-1}$, these frequencies could be associated with the bending vibrations of C–H groups. The results reveal that the UV–VIS and FT-IR analytical tools are the most suitable and reliable tools to detect and quantify high levels (over 10%) of adulteration in mixes of EVO with other vegetable oils.

## Introduction

Olive oil as a natural fruit juice have been obtained from olive fruits of Olive tree (Olea europea L.), which is grow and culture in Mediterranean climates using mechanical and physical procedures (1). The unique chemo-physical characteristics of olive oil including a balanced unsaturated fatty acid content, phenolic, tocopherols and pigments compounds make it the most valuable oil with human health benefits (2). In contrast with other vegetable oils from sunflower, soybean, maize, palm and peanut fruits, olive oil has the unique characteristics on human nutritional and health qualities. According to the reports, consumption of olive oil leads to reduce blood cholesterol levels (LDL) and prevention of the heart artery occlusion risk due to its high level

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unsaturated fatty acid content (3, 4). Furthermore, with recognizing the sensory qualities such as its interesting taste and health benefits on the prevention of the occurrences of chronic diseases like diabetes and obesity, the consumption of olive oil has been highly increased worldwide so far (5-7). With increasing of demand in olive oil consumption, various types of inauthentic products such as fake olive oils that mixed with other vegetable oils have been supplied to the market to obtain unfair gain (8). The European Union (EU) stated that olive oil is in the top of the list of commodities susceptible of fraud (e.g. mislabeling, substitution, or even true counterfeit) (9-14). Nevertheless, the blending of olive oil with other vegetable oils to obtain different health and nutritional properties as well as economic value for consumers with wide variety of choices is common practice in some countries. The major added adulteration oils are: oilseed oil, refined olive oil, olive pomace oil, synthetic products made from olive oil fatty acids, which are by-products of the refining process, and high oleic acid oils. Other common adulteration is also including the low-quality olive oil that the free fatty acids are eliminated by the methods such as rinsing with alkaline water and oils that have been mildly deodorized (Mildly deodorizing virgin olive oil is not allowed) and olive pomace oil treated with dichromate (9-15). Recently, Europol has reported that large quantity of fake olive oils prepared with addition of pigments (chlorophyll and β-carotene) and soya oil into sunflower oil were detected in Germany (16). However, These kind of adulterations in olive oil production causes threats and challenges both the suppliers and the health of the ultimate consumers (15, 17). Therefore, it is essential to develop viable techniques for detecting adulterated olive oils or fraud such as the substitution or mix of olive oils with other vegetable oil samples (e.g. corn, safflower, sunflower, soy and canola) requires a wide range of analytical and chemometric tools (9-15).

In last two decades, to detect addition of lower price seed and/or vegetable oils such as hazelnut (18), palm (5), almond (19), corn, sunflower and soybean oils (11, 17, 20, 21) and lower quality olive oils (refined or pomace olive oil) (22) in extra virgin olive oils have already been investigated by different non-targeted fingerprinting techniques ranging from Fourier Transform (FT) infrared (IR) (5, 15) and dielectric spectroscopy (17) to UV-Vis spectroscopy (12, 23, 24), MIR spectroscopy (9) and fluorescent spectroscopy (12). Furthermore, both high- (25-27) and low-field NMR techniques (22, 24, 28, 29) have been utilized. Moreover, chemo-metrical approaches such as principal component analysis (PCA) either in combination or independent from the described methods were used to detect EVOO olive oil adulteration (4, 12, 15, 17, 19, 21, 30, 31).

According to the reports, the effectiveness of IR-FT and UV-vis spectroscopy has been proven in the field of quality control and adulteration detection of various oils, especially EVOO and mixed olive oils with different absorption rates among variety of olive oils and their different constituents (5, 12, 15, 23, 24). Therefore, further analysis is needed to test the capabilities and increase this efficiency and reduce the minimum detectable rate and facilitate the process. Per our knowledge, there is not preliminary studies in the literature about the detection of mixed olive oil using heat treatment in combination with IR-FT and UV-vis spectroscopies. Therefore, hypothesis of this research is that extra virgin olive oil could be differentiated from other seed or vegetable oils including refined canola oil, palm olein and sunflower in a mixture by using heat treatment in combination with FT-IR and UV-vis spectroscopies. Therefore, the main aim of this study is simple and fast authentication of extra virgin olive oil by different spectroscopic techniques individually and also in combination with minimal chemical waste.

# Methods

## Olive oil samples

Extra virgin olive oil (EVOO) native to Iran, Iranian commercial seed oil samples

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including sunflower oil (SO), Palm Oil (PO) and canola oil (CO) were purchased from the local supermarket and used as adulterants. Oil mixtures were prepared using the vegetable oil samples added to EVO. The adulteration levels of each type of the aforementioned vegetable oils in extra virgin olive oil were 5%, 10%, 30% (w/w). There were five samples with three replicates for each adulteration level. All samples (n = 60) were mixed in a test tube using a micropipette. Both pure oil and mixture samples were kept in a dark and cold place before analysis. Commercial label was the only source of information used to define the origin of the oil used.

### Chemical reagents

N-hexane for UV-vis analysis, sodium hydroxide, hydrochloric acid, petroleum ether, formic acid 98% (lab grade); acetonitrile and methanol, both of chromatographic grade; linoleic, linolenic and oleic acids (standard grade), were all purchased from Sigma-Aldrich (Milan, Italy).

### Heat treatment of EVOO and mixed oil samples

The oil samples incubated at 120 °C for 11 h in oven with 45-min intervals (Tefal model 1250, Paris, France). After reaching ambient temperature, the samples were stored in a freezer at -20 °C, which were tested on three replicates. From the oxidation diagrams, three points were selected for spectroscopic tests including 0, 270 and 540 min after the slope of the oxidation phase (32).

### UV analysis

The samples for UV analysis were prepared by diluting 250 mg of the oil samples in isooctane solution and diluted to a 0.8 mg/mL concentration. The absorbance of each mixture was measured at 232 to 400 nm by spectrophotometer (JenWay 6105 UV-VIS, Esses, England) and spectral shapes were plotted (33).

### FTIR spectroscopy

FTIR spectra were carried out using spectra of oil samples in a thin film as described by Gergen (34). The spectra are recorded by placing the sample on an optical crystal with high refractive index. This generates a reflected beam, which is absorbed up to a few mm in the sample and originating in turn a second beam that is recorded as a spectrum. FT-IR spectroscopy was performed by FT-IR spectrometer SHIMADZU FTIR-8300 model in the range of 500 to 4000 cm and resolution of 2 cm at an average of 20 scans at room temperature. A small amount of the oil sample (1 to 1.5 ml) was placed between two well-polished KBr plates to form a thin layer and their spectra were subtracted from those obtained from blank and pure KBr plates as a control sample.

## Results

### Spectra Interpretation

UV spectra of the EVOO and mixed olive oil samples were recorded before the heating test and then along the thermal degradation experiments at the 45- and 90-mins intervals set for the analysis. The EVOO and mixed oils samples showed high absorption values around 240-300 nm band. The broad band around 300 nm might be associated with phenolic compounds (23, 35-40). In addition, absorption bands around 320–330 nm might be associated with phenolic compounds (e.g. hydroxycinnamic acid derivatives) in the EVO samples analyzed as reported by other authors (23, 35-37, 39, 40). The absorption band at 240 nm has been also reported to be associated with elenolic acid, a significant phenolic present in EVO (23, 35-37, 39, 40). The region between 240 nm and 400 nm might be related to electron transfer (23, 36, 37, 39, 40). However, other reports indicated that the absorption at 240 nm is associated with linoleic acid, due to the occurrence of conjugated dienes and the transition of HOMO to LUMO electrons in the UV region (23, 35-40).

With addition of canola oil and sun flower oil in EVOO a significant increase was observed in the UV-VIS absorbances around 260-280 nm (see ***Figures 1A*** and ***2A***). However, the UV absorbance related to palm olein oil

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addition of 30% to EVOO showed a significant decrease compared with EVOO with 5% and 10% palm olein oil (***Figure 3A***). These results are in agreement with the reports reveal that either the location change or the wavelengths disappearance is associated with differences in quality or origin of the oils samples (e.g. varietal or geographical origin) (23, 35-40).

The UV–VIS spectra of the pure oil and mixture samples in tree heating times were illustrated in ***Figures 1-3***. Regrading to the results illustrated in ***Figure 1***, there is not significant difference between the EVOO mixed with canola oil of 5% and 10% samples prior to the thermal treatments of 0, 45min and 90mins due to the similarity of the fatty acid structure of canola oil and olive oil. However, the increased formation of conjugated dienes in the olive oil with 30% canola oil showed UV absorption higher than that of virgin olive (***Figure 1A-1C***) (41). The UV absorbance values of virgin olive oil and sunflower oil pre-heat treatment showed no significant difference between virgin olive oil and oils with 5 and 10% sunflower oil in contrast with EVOO with 30% sunflower oil (***Figure 2A-2C***). On the other hand, the samples containing palm olein 10 and 30% showed the higher UV spectrum absorption compared with the EVOO olive oil (***Figure 3A-3C***). According to the previously reported

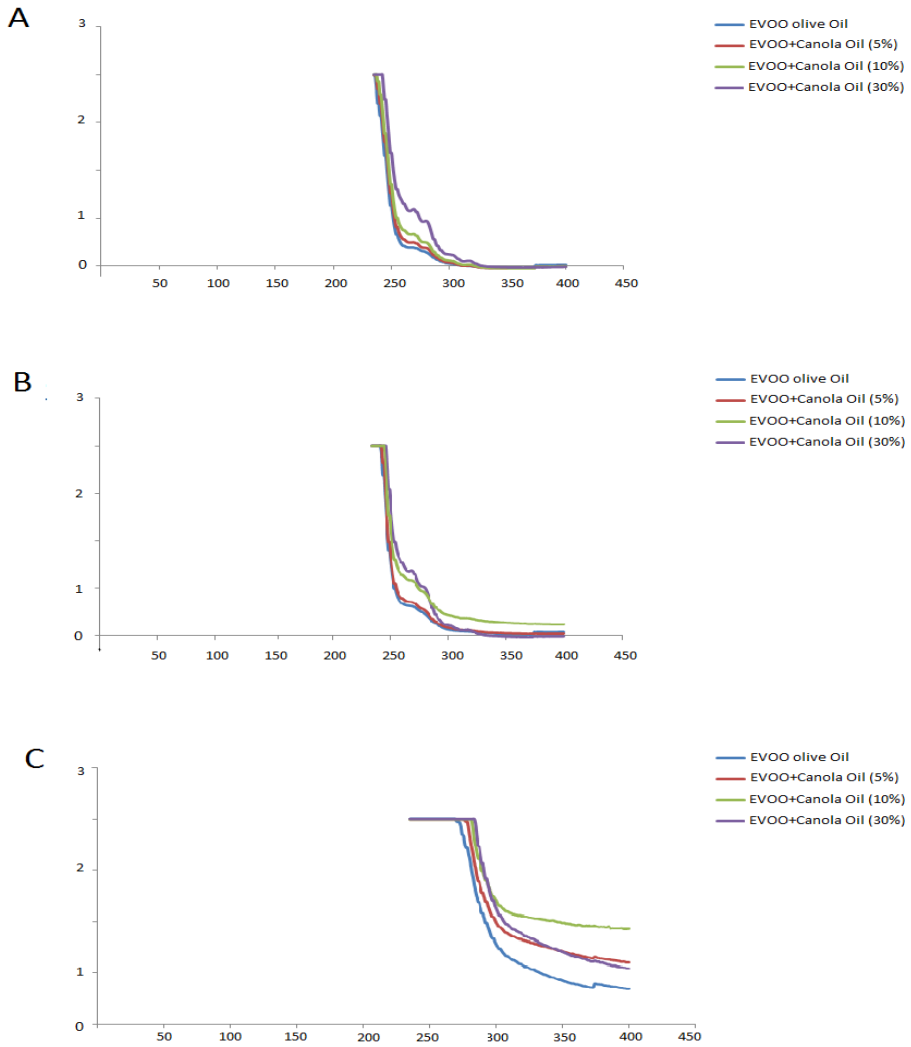


**Figure 1. UV-VIS spectra (240-400nm) of the extra virgin olive oil mixed with the different percentages of canola oil. The UV absorbance of the EVOO (blue) and its canola-mixed samples containing canola oils 5% (red), 10% (light green) and 30% (violet) were analyzed in the thermal conditions with three different durations including 0 (A), 45mins (B) and 90 mins (C).**

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studies, the most UV absorption causes the high level of oil oxidation, which form double bonds. Therefore, the number of conjugated dienes is a suitable characteristic to determine lipid oxidation level, which varies by oxygen uptake and lipid peroxides level (42).

The FT-IR spectra of extra virgin olive oil, EVOO samples containing mixed oils including canola, sun flower and palm olein oils were shown in ***Figure 4***. The results showed that the characteristics of FTIR spectra including peak number, peak position and peak shape in mixed samples were significantly different from EVOO samples. According to the studies, the frequencies of around 2920 $cm^{-1}$ and 2856 $cm^{-1}$ could be related with C–H stretching (e.g. cis-double bonds) and with –C–H asymmetrical and symmetrical stretching in methylene groups. The frequency at 2925 $cm^{-1}$ is associated with aliphatic CH2 groups. Around 1366 $cm^{-1}$ and 1451 $cm^{-1}$, these frequencies could be associated with the bending vibrations of C–H groups. Changes were also observed around 1600-1750 $cm^{-1}$ (C–O stretching) and in the fingerprint range from 1200 $cm^{-1}$ onwards with CH2 and CH3 aliphatic groups (39, 43-47). As the amount of added

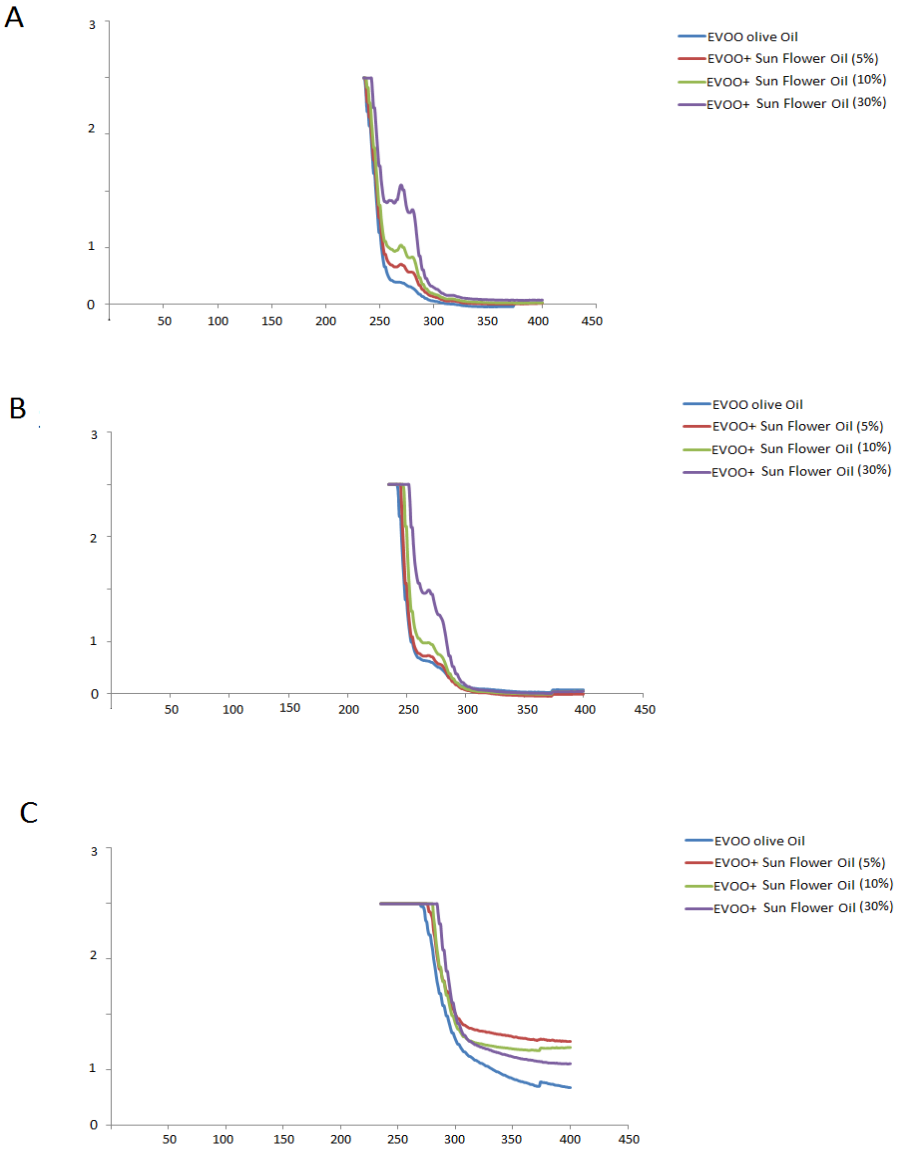


**Figure 2. UV-VIS spectra (240-400nm) of the extra virgin olive oil mixed with the different percentages of Sun flower oil. The UV absorbance of the EVOO (blue) and its sun flower-mixed samples containing sun flower oils 5% (red), 10% (light green) and 30% (violet) were analyzed in the thermal conditions with three different durations including 0 (A), 45mins (B) and 90 mins (C).**

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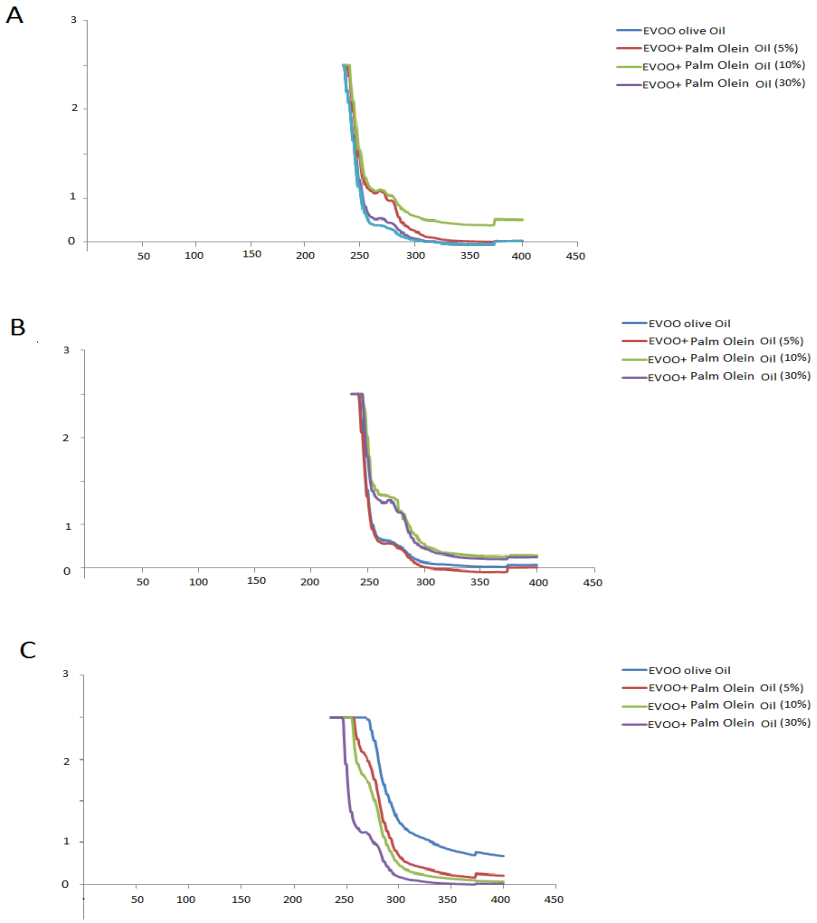


**Figure 3. UV-VIS spectra (240-400nm) of the extra virgin olive oil mixed with the different percentages of Palm Olein oil. The UV absorbance of the EVOO (blue) and its palm-mixed samples containing palm olein oils 5% (red), 10% (light green) and 30% (violet) were analyzed in the thermal conditions with three different durations including 0 (A), 45mins (B) and 90 mins (C).**

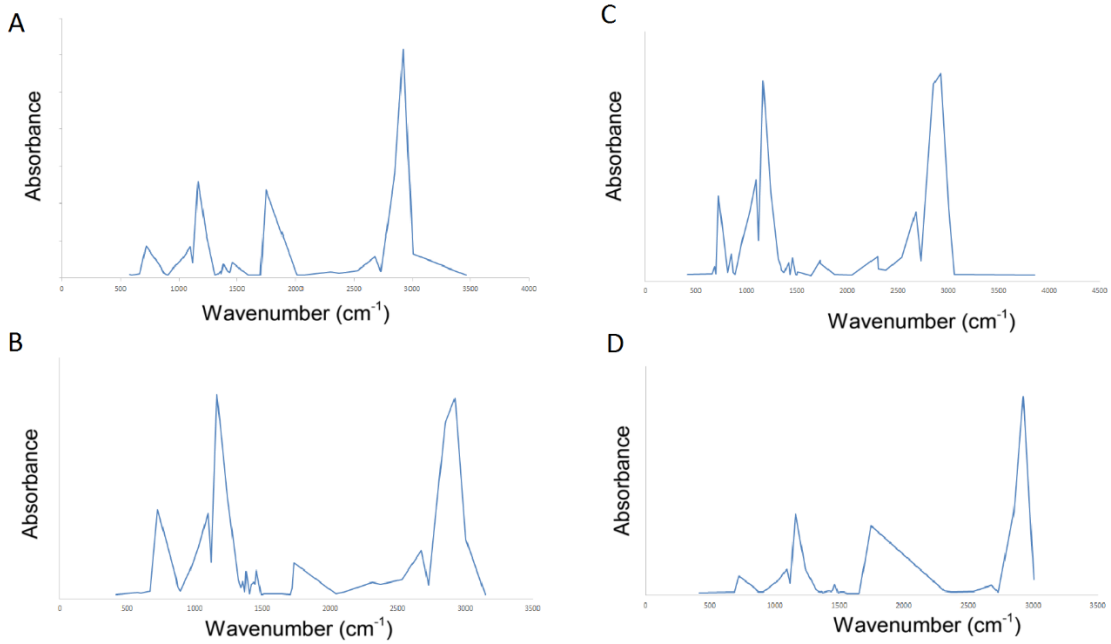


**Figure 4. FT-IR spectra (500-4000 $cm^{-1}$) of A) extra virgin olive oil, B) EVOO with canola oil, C) EVOO with sunflower oil, D) EVOO with Palm Olein oil.**

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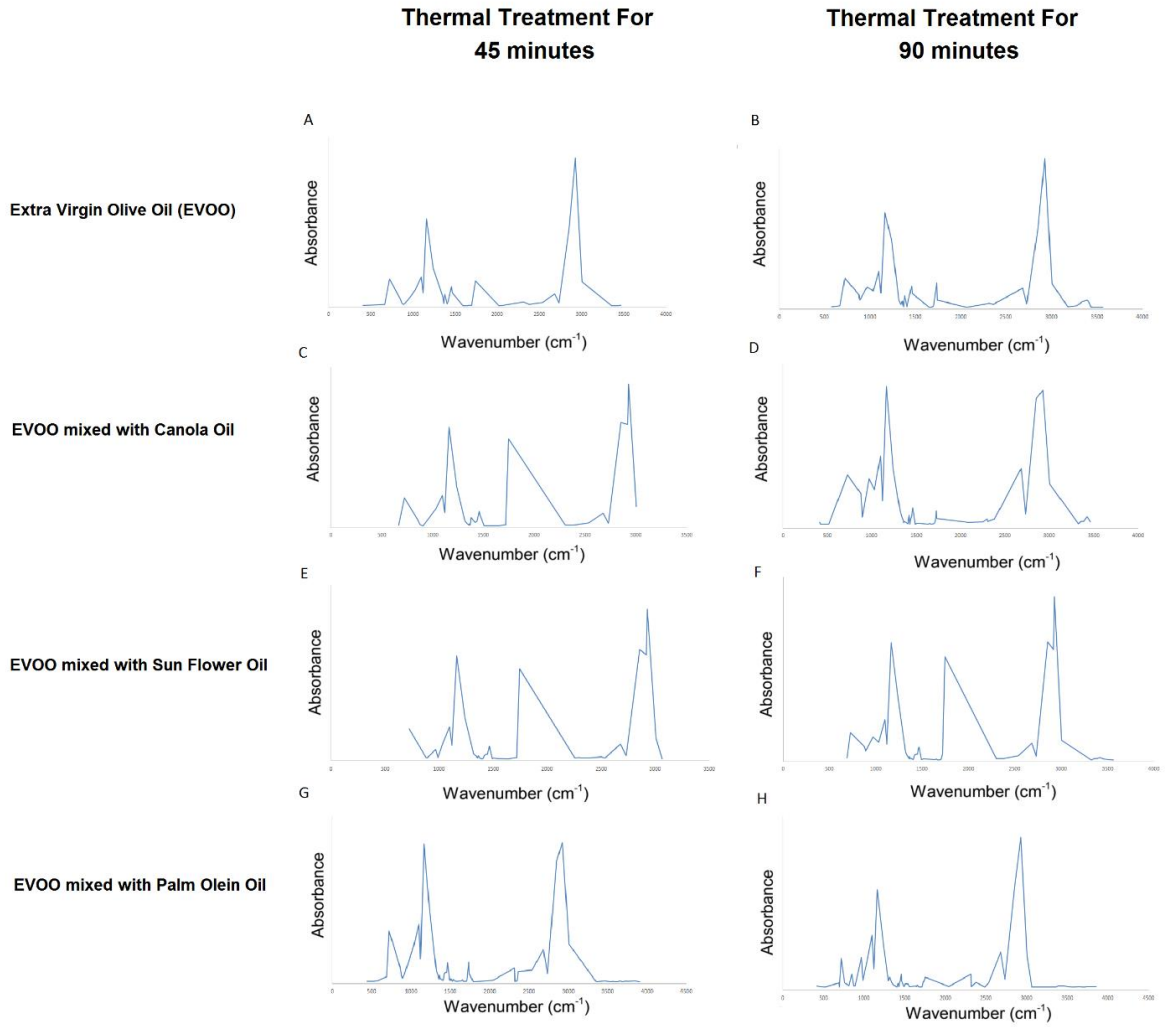


**Figure 5. FT-IR spectra (500-4000 $cm^{-1}$) of the A,B) extra virgin olive oil, C, D) EVOO with canola oil, E, F) EVOO with sunflower oil, G, H) EVOO with Palm Olein oil under thermal treatments.**

vegetable oil to the EVO increases, the values at 2922 $cm^{-1}$ and 2858 $cm^{-1}$ increases as reported by (48). Similar results were reported by other authors (39, 43-47). Furthermore, we analyzed FT-IR spectra in EVOO and mixed samples during two thermal duration treatments. The results showed that thermal treatment of EVOO samples for 45mins lead to decrease in the absorbance in the range of 1200-1400$cm^{-1}$ (***Figure 4A***, ***Figure 5A***) as well as canola and sun flower mixed EVOO samples (***Figure 5C*** and ***5E***). However, in palm olein mixed samples, thermal treatments cause different FT-IR spectra characteristics with increase at 700-800$cm^{-1}$ and decrease in the range of 1200-1400$cm^{-1}$ (***Figures 4D*** and ***5G***). With increasing the thermal duration to 90 mins, the mixed samples excepted sunflower mixed samples show the significantly decrease FTIR absorbance in the range of 1200-1400$cm^{-1}$ (***Figure 5***). By agreement with Didham, Truong (48), the results reveal that the UV–VIS and FT-IR analytical tools are the most suitable and reliable tools to detect and quantify high levels (over 10%) of adulteration in mixes of EVO with other vegetable oils (canola, sunflower and palm oils).

## Conclusion

The present results reveal that the UV–VIS and FT-IR analytical tools are the most suitable and reliable tools to detect and quantify high levels (over 10%) of adulteration in mixes of EVO with other

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vegetable oils).

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