Use of ATR-FTIR spectroscopy to detect the changes in extra virgin olive oil by adulteration with soybean oil and high temperature heat treatment

Abstract: The structural changes induced in extra virgin olive oil (EVOO) by adulteration with soybean oil (SBO) and heat treatment at 185°C for 4 and 8 h were investigated using Attenuated Total Reflectance – Fourier Transform Infrared (ATR-FTIR) spectroscopy. Our results revealed that the band around 3006 cm⁻¹ recorded shifts versus the percentage of adulterant. The changes in the absorbance at 3006 cm⁻¹ (A3006) and in the ratio of the maximum heights of the bands at 3006 and 2925 cm⁻¹ (A3006/A2925) were used to evaluate the EVOO adulteration. The regression analysis of A3006 and A3006/A2925 versus the percentage of adulterant was used to calculate the detection limits of adulteration. The time course of spectral changes showed that the oil heating caused notable modifications in the intensity of the absorption bands and induced no shifts in their exact position. The most relevant changes were reflected by conjugation and cis-trans isomerisation of double bonds, the formation of epoxides and widening of the band in the C=O region due to formation of secondary oxidation products. This study highlights that ATR-FTIR spectroscopy may be a promising means to differentiate among pure and adulterated oils and to study the thermo-oxidative processes in oils undergoing thermal stress.

Keywords: ATR-FTIR spectroscopy, extra-virgin olive oil, soybean oil, adulteration, thermo-oxidation.

1 Introduction

EVOO adulteration with various less expensive edible oils (i.e. sunflower oil, soybean oil, corn oil) represents a great danger not only for economic reasons but also in terms of health and safety of EVOO consumers [1,2]. For this reason, the authenticity of EVOO has currently become an important issue for producers, consumers, and quality control evaluators [3,4]. Moreover, the adulterated EVOO can be used as a cooking medium in different high temperature heat applications [5,6]. The heat treatments of vegetable oils specific to food industry involve the maintaining of oil at a high temperature, in the range 170–220°C [7-9]. On the one hand, in the course of high temperature exposure, the oil undergoes severe changes including thermo-oxidation, cis-trans isomerisation, cyclization, polymerization and hydrolysis. On the other hand, the changes are difficult to predict because of the large numbers of variables involved, depending on the oil composition and the specific process conditions [10-12].

Lipid oxidation is the main undesirable process that occurs during heating of edible oils containing lipid molecules with polyunsaturation [13,14]. Lipid degradation leads to the formation of hydroperoxides as primary oxidation products. Further, hydroperoxides are degraded to secondary oxidation products (aliphatic aldehydes, ketones, lactones, alcohols, acids and hydrocarbons) which are more stable during heat exposure and have the potential to affect the taste and produce undesirable nutritional effects on consumers [9,15-17]. The high temperature treatments of oil induce compositional changes by decomposition of polyunsaturated (PUFAs), monounsaturated (MUFAs) and saturated fatty acids (SAFAs). It has long been recognized that oil oxidation is
generally influenced by many factors such as the fatty acid composition (i.e. the degree of unsaturation), processing technique, heat, light, and the presence of antioxidants. The oxidative stability of oils is an important indicator of their performance and shelf-life and also ensures that the oils show a good resistance during exposure to high temperature [13,18]. It has been emphasized that edible oils with a high content of oleic acid are more stable towards oxidation both during storage in ambient conditions as well as during high temperature heat treatments as compared to oils with high levels of PUFAs [19,20]. According to results reported by Nikovska (2010) [21], a decrease in the oxidative stability of oils results from an increase of the degree of unsaturation, due to greater availability of PUFAs.

The studies carried out in the last decade motivate the investigation of the potential application of FTIR spectroscopy to detect EVOO adulteration by addition of other lower cost edible oils [22,23]. Vegetable oil differentiation by this technique is possible according to their nature and composition on the base of spectral changes at specific wavenumbers, because some differences appear in the intensity and frequency at which the maximum absorbance of the bands is recorded in infrared spectra [24-27]. Also, the suitability of FTIR spectroscopy to appreciate the oxidative state of edible oils undergoing thermal stress during high temperature applications specific to the food industry, by analyzing the changes in the frequency and intensity of major absorption bands, has been explored in several studies [6,28-31]. The results of these studies highlight the potential of FTIR spectroscopy and its advantages in terms of speed and relatively low cost per analysis in the research carried out on edible oils and fats. Therefore, this technique could be an effective analytical tool to classify vegetable oils, a fast method to detect the adulteration of a vegetable oil with other lower price edible oils, or to study the thermo-oxidation processes of edible oils during high temperature heat treatments.

In agreement with these concerns, our study continues the efforts to demonstrate the ability and usefulness of FTIR spectroscopy to detect EVOO counterfeiting by soybean oil addition, as a potential adulterant agent. Particularly, we investigate changes in oil blends in response to thermal stress due to heating at high temperature (185 ± 5°C for 4 and 8 h), by considering the most relevant changes recorded in infrared spectra, by detecting and identifying the functional groups arising in the course of thermo-oxidative degradation of lipids, and by using characteristic wavenumber regions of the common oil oxidation end products.

### 2 Experimental procedure

#### 2.1 Samples

In this study we used extra-virgin olive oil (EVOO), refined soybean oil (SBO) and blends of EVOO deliberately adulterated by addition of SBO at varying levels, in the range 10–90% (v/v), at intervals of 10 units. The oil mixtures were strongly manually shaken for homogenization and stored in closed glass bottles in dark. The pure oils and blends were investigated by ATR-FTIR spectroscopy, non-oxidized, after preparation, as well as subjected to heating at a simulated frying temperature of 185 ± 5°C for 4 and 8 h. The vegetable oils were produced in Italy and purchased from local hypermarkets (Timisoara, Romania): EVOO by PIETRO CORICELLI SPA, Spoleto and SBO by VALSOIA SPA, Bologna.

#### 2.2 The heat treatment of oil samples

25.0 ± 0.5 g of oil samples were weighed in Pyrex Petri dishes (12 cm inner diameter) and placed in an electric convection oven (Esmach, Italy, 1200 W, 50 Hz) equipped with a temperature control unit and continuously heated at simulated frying temperature of 185 ± 5°C, in the dark, for 4 and 8 h. Preliminary tests were performed in order to obtain information about thermal regimes of heating. The temperature of oil samples subjected to heating was monitored by a calibrated chromel-alumel thermocouple (HI 935009, Hanna Instruments). The dishes were introduced into the oven without their lids in order to facilitate the exposure of the oil samples to the circulating air. After each heat treatment, the samples were taken out of the oven, covered, allowed to cool at room temperature, poured into glass bottles and stored in dark refrigeration conditions at 4–6°C until analysis. Heating treatments were carried out in duplicate. The oil samples were kept at room temperature (25°C) for 24 h prior to FTIR measurements.

#### 2.3 ATR-FTIR spectra acquisition

Infrared spectra were recorded on an FTIR-8400S (Shimadzu, Japan) equipped with an Attenuated Total Reflectance (ATR) accessory. The ATR-FTIR spectra were obtained against the background of air spectrum. Background scan and oil samples were sequentially measured at room temperature (25°C) from 4000 to 400 cm⁻¹ with a scanning time of 60 s and 4 cm⁻¹ resolution. After every scan of an oil sample, a background of new
reference air spectrum was performed. Also, the ATR plate was cleaned with a soft tissue soaked in acetone to remove any residues from previous oil samples. The ATR cleanliness was assessed by comparing the obtained background spectrum to the previous one. All spectra were processed with the computer software program Spectrum for Windows XP Professional (Shimadzu). The assignment of the recorded bands to the specific functional groups was done by comparison with data reported by previous studies on edible oils.

2.4 Statistical analysis

The Origin 7.0 software program was used for performing the linear regression analysis of spectral data versus percentage of adulterant agent.

3 Results and discussion

3.1 Assessing EVOO adulteration by addition of SBO

Fig. 1 depicts the ATR-FTIR spectrum recorded for EVOO at room temperature, as a descriptive example of the oil samples under evaluation. The analyzed infrared spectra contain fundamental and characteristic bands whose frequencies and intensities can clearly determine the relevant functional groups in the investigated oils. The various absorption bands of the spectra were assigned on the basis of data given in the literature [13,32].

Visual examination of spectra revealed that there are no appreciable differences between their spectral features apart from slight changes in the absorbances of some bands as well as some shifts in the exact position of the bands. This finding suggests that the oil composition affects the exact position of the bands and also affects the shifts in the infrared spectra when the proportion of fatty acids is modified. All registered spectra showed absorption bands at different wavenumbers, as follows: 3471 cm\(^{-1}\) (assigned to overtone of C=O of ester) [32], 3006 cm\(^{-1}\) (C–H stretching symmetric vibration of the cis double bonds, =CH) [13,32], shoulder at 2962 cm\(^{-1}\) (asymmetric stretching vibration of C–H of aliphatic CH\(_3\) groups due to the alkyl rest of triglycerides present in large quantities in vegetable oils) [32,33], 2925 cm\(^{-1}\) (asymmetric stretching vibration of C–H of aliphatic CH\(_2\) group) [13,33], shoulder at 2872 cm\(^{-1}\) (symmetric stretching vibration of C–H of aliphatic CH\(_3\) groups due to the alkyl rest of triglycerides present in large quantities in vegetable oils) [32,33], 2854 cm\(^{-1}\) (symmetric stretching vibration of C–H of aliphatic CH\(_2\) group) [13,33], ~1746 cm\(^{-1}\) (stretching vibration of the ester carbonyl functional groups of the triglycerides, C=O) [13], weak shoulder appeared at1711 cm\(^{-1}\) (stretching vibration of free fatty acid carbonyl group, C=O) [32,33], 1654 cm\(^{-1}\) (C=C stretching vibration of cis-
disubstituted olefins, \( RHC=CHR \) \([13,30]\), 1463 and 1458 cm\(^{-1}\) (bending vibration of C–H of CH\(_3\) and CH\(_2\) aliphatic groups) \([32]\), 1418 cm\(^{-1}\) (rocking vibration of C–H bonds of cis-disubstituted olefins) \([13,32]\), 1397 cm\(^{-1}\) (bending in plane vibrations of C–H bonds of cis-olefinic groups) \([13,30,32]\), 1376 cm\(^{-1}\) (bending symmetric vibration of C–H bonds of CH\(_3\) groups) \([13]\), 1237, 1161, 1118 and 1096 cm\(^{-1}\) (stretching vibration of C–O ester groups) \([33]\), 967 cm\(^{-1}\) (out-of-plane bending vibration of trans–HC=CH–group of disubstituted olefins) \([33]\), 914 cm\(^{-1}\) (out-of-plane bending vibration of cis–HC=CH–group) \([33]\) and 722 cm\(^{-1}\) (overlapping of CH\(_2\) rocking vibration and the out-of-plane vibration of cis–HC=CH–group of disubstituted olefins) \([13,32]\).

Fig. 2 shows the region 3050–2800 cm\(^{-1}\) of ATR-FTIR spectra recorded for pure and adulterated oils. The inset shows the shift of the band recorded at 3006 cm\(^{-1}\) as a result of EVOO adulteration by addition of SBO.

A first finding revealed by Fig. 2 is linked to the band around 3006 cm\(^{-1}\) assigned to the C–H stretching vibration of the cis-olefinic double bonds (=CH). Upon closer examination, it can be observed that there are notable differences in the position of this band for pure and adulterated oil samples. Vlachos \textit{et al.} (2006) \([13]\) found a close relationship between the degree of unsaturation of edible oils and the change in the exact position of the maximum of the 3006 cm\(^{-1}\) band. The maximum absorbance of this band was registered for SBO and adulterated oil samples at higher frequencies than for EVOO. As illustrated in the inset of Fig. 2, SBO has maximum absorbance at 3009 cm\(^{-1}\), whereas EVOO reaches a maximum at 3005 cm\(^{-1}\). This finding is due to the oil samples composition in fatty acids which affects the exact position of this band. SBO contains a higher proportion of linolenic or linoleic acyl groups whereas EVOO contains higher proportion of oleic acyl groups \([13,29]\). Therefore, EVOO has the lowest degree of unsaturation among the oil samples under investigation. The increase in the percentage of SBO in EVOO causes a substantial increase in the unsaturation degree, as a direct result of higher content of linoleic and linolenic acids in the triglyceride composition. The specific frequency of the methyl linoleate (3011 cm\(^{-1}\)) is greater than that of methyl oleate (3006 cm\(^{-1}\)) which explains the shift of the band maximum to higher wavenumbers \([35]\). The results of our study revealed a clear shift of this band from 3005 cm\(^{-1}\) to 3009 cm\(^{-1}\) versus the percentage of adulteration, Fig. 2, and are in agreement with those previously reported by Vlachos \textit{et al.} (2006) \([13]\). Consequently, EVOO adulteration by addition of SBO induced an obvious shift of this band to higher frequencies.

Another finding that emerges from a careful examination of the inset in Fig. 2 is that the height of
the band recorded at 3006 cm$^{-1}$ in the infrared spectrum of EVOO is obviously smaller than those registered for adulterated samples, as well as for SBO. The height of this band increased according to the extent of adulteration. Thus, in our study, the change in the absorbance at 3006 cm$^{-1}$ was taken as a measure of the changes in the degree of unsaturation in response to EVOO adulteration by addition of SBO. By linear regression analysis of the values recorded for absorbance at 3006 cm$^{-1}$ versus the percentage of SBO added to EVOO, a high correlation coefficient ($R = 0.9979$) was found, as shown in Fig. 3A.

Further, the ratio of the maximum heights of the two bands recorded at 3006 and 2925 cm$^{-1}$ was investigated as an index for the change in the degree of unsaturation of EVOO in response to adulteration by addition of SBO. The ratio A3006/A2925 is recommended to differentiate among various vegetable oils, as it is closely related to the degree of unsaturation [13,32,34].

The height of the band registered at 2925 cm$^{-1}$ undergoes small changes and not in a specific way, compared to the 3006 cm$^{-1}$ band, so it is better to use the ratio A3006/A2925 in order to monitor the EVOO falsification by addition of SBO in response to changes in the unsaturation. The 2925 cm$^{-1}$ band is assigned to the asymmetric stretching vibration of C–H from aliphatic CH$_2$ groups. The calculated ratio of the two peak heights expresses the percentage of the C–H bonds which are linked by cis double bonds (=CH) present in the oil samples. It is noteworthy that the ratio of the peak heights A3006/A2925 for non-oxidized oil samples was strongly correlated with the percentage of adulterant agent added to EVOO ($R=0.9985$), Fig. 3B.

These results support the view that the increase in the unsaturation degree of investigated oil samples is closely related to the extent of EVOO adulteration by addition of SBO.

By plotting the band intensity registered at 3006 cm$^{-1}$ and the ratio A3006/A2925 versus corresponding percentage of adulterant agent added to EVOO, the calibration curves for non-oxidized oil samples were obtained, as shown in Fig. 3. Linear regression analysis of spectral data was used to calculate the limits of detection (LOD) for EVOO adulteration by addition of SBO. The detection limits were calculated as three times the standard deviation of the intercept divided by the slope of the calibration curves, according to equation (1). The data from equations of calibration curves $Y = AX + B$ presented in Fig. 3 for non-oxidized oil samples (initial) were used.

$$\text{LOD} (%) = \frac{(Y_{LOD} \text{- intercept})}{\text{slope}}$$  \hspace{1cm} (1)

where: intercept = $B$; slope = $A$ and $Y_{LOD}$ was obtained using Eq. (2):

$$Y_{LOD} = \text{intercept} + 3 \cdot \text{SD}$$  \hspace{1cm} (2)

where: SD - standard deviation.

The detection limit was 7% when using data from calibration curve of A3006 versus percentage of adulterant, and a lower value of 6% when using the data from calibration curve of the ratio A3006/A2925 versus adulteration percentage. In agreement with data reported by Vlachos et al. (2006) [13], our results recommend the use of the ratio of the maximum heights of the bands recorded at 3006 and 2925 cm$^{-1}$, instead of A3006, to assess EVOO adulteration.

![Figure 3](image-url)  \hspace{1cm} Figure 3: Linear regression analysis of spectral data versus percentage of SBO added to EVOO (A: A3006; B: A3006/A2925).
3.2 Assessing the oxidative changes of oil samples during heating

In the oxidation experiment EVOO, SBO and their blends were heated for 4 and 8 h at a simulated frying temperature of 185°C and changes in their ATR-FTIR spectra in response to thermal stress were evaluated. Under thermo-oxidative conditions both major and minor components resulting from lipid degradation can evolve to give new compounds, and this is reflected by changes in the intensity of the infrared spectra absorption bands. Also, no shifts in the exact position of the bands were induced by heat treatment. Thermo-oxidative stress caused substantial changes throughout the entire infrared spectra of investigated oil samples, but the most obvious were recorded at 3006 cm\(^{-1}\), assigned to the C–H stretching vibration of cis double bonds, in the C=O region (1720–1750 cm\(^{-1}\)), in the fingerprint region (1500–900 cm\(^{-1}\)), including the portions of nonconjugated trans double bonds (967 cm\(^{-1}\)) and conjugated trans double bonds (987 cm\(^{-1}\)), as well as at 722 cm\(^{-1}\), specific for cis double bonds of disubstituted olefins.

By overlapping of infrared spectra on certain spectral regions, changes in the intensity of bands recorded at specific frequencies can be seen as a consequence of thermo-oxidative degradation of the lipid fraction of the analyzed oils.

The modifications recorded in the infrared spectra of thermo-oxidized oil samples in response to high temperature heat treatment can be explained by cis-trans isomerisation and conjugation of double bonds of PUFAs, the formation of secondary oxidation products and changes in the ratio of CH\(_2\) and CH\(_3\) terminal groups [18,36].

Also, the oxidation led to some increases in the saturation of the substrate because the cis-olefinic double bonds of the different acyl groups disappeared during the thermo-oxidation process [36].

In Fig. 4 we have grouped the most relevant changes detected in the infrared spectra of EVOO and SBO in the course of heating. These changes are closely connected with changes in the degree of unsaturation of oil samples in response to thermal stress.

Throughout the oxidation process, changes are observed in the stretching vibration of cis double bonds in the band near 3006 cm\(^{-1}\), in close connection to the composition of oil samples. The height registered for this band decreases at a rate depending on the nature of the oil sample. It is evident in the ATR-FTIR spectra of heated oil samples that a clear and pronounced decrease in the absorption intensity of the cis double bonds stretching band at 3006 cm\(^{-1}\) arises in response to heating, as a consequence of the diminution of cis double bonds, Figs. 4A1, A2. It becomes more pronounced with increased exposure time to the thermal stress, in agreement with data previously reported [32].

The loss of the cis double bonds, due to their isomerisation to trans groups and/or their breakdown to produce secondary oxidation products, is specific to lipids undergoing oxidation processes as a result of heat stress [28]. The decrease recorded in the absorbance at 3006 cm\(^{-1}\) during heating may be explained by the formation of primary oxidation products at a rate lower than that of their decomposition. The degradation of hydroperoxides of PUFAs, as primary oxidation products, results in a complex mixture of secondary products (aldehydes, ketones, acids and esters) and consequently, leads to a decrease in the degree of unsaturation due to disappearance of double bonds in the cis conformation [37]. Also, the decrease in the degree of unsaturation can be associated with a decrease in the level of free radicals that contain cis double bonds as a result of the scavenging effect of antioxidant compounds such as tocopherols, found in oil samples [32].

Besides the reduction in the intensity of the band at 3006 cm\(^{-1}\) band, the decrease of the absorbance ratio A3006/A2925 in response to heating provides strong evidence for the decrease in the degree of unsaturation. It should be noted that, in agreement with data reported by Van de Voort et al. (1994) [28], this finding may be attributed to a reduction in the unsaturated fatty acids content (18:2 and 18:3 fatty acids) as a result of oxidation.

Fig. 3A shows the same trend of enhanced absorbance at 3006 cm\(^{-1}\) as a result of increasing the percentage of adulterant agent in oil samples subjected to thermo-oxidative stress. Thus, even after 8 h of heating, the changes in absorbance of this band were highly correlated (R > 0.97) with the percentage of adulterant agent. Additionally, Figure 3B shows the significant positive correlation found for the ratio A3006/A2925 versus the percentage of adulterant in EVOO, even after 8 h of heating. Therefore, the changes in the absorbance at 3006 cm\(^{-1}\), as well as in the value of the ratio A3006/A2925 allow differentiating among pure and adulterated oil samples used in high temperature heat treatments.

In terms of cis-trans isomerisation of double bonds of PUFAs, this transformation can be evidenced by quantifying the changes occurring at specific frequencies assigned to unsaturation. The bands detected at 967 and 987 cm\(^{-1}\) (Figs. 4B1, B2) have been widely used to estimate the level of trans unsaturated fatty acid in oils whereas the band recorded in the region 700–725 cm\(^{-1}\), at
Figure 4: The spectral changes during heating of oil samples, related to decreases in the degree of unsaturation (A1, A2: 3006 cm\(^{-1}\); B1, B2: 967, 987 cm\(^{-1}\); C1, C2: 722 cm\(^{-1}\); D1, D2: 885 cm\(^{-1}\)).
722 cm⁻¹, (Figs. 4C1, C2) belongs to the cis double bonds of unsaturated fatty acids and has also been used for their evaluation [30]. Thus, a progressive decrease in the absorbance at 3006 cm⁻¹, as well as in the absorbance at 722 cm⁻¹ indicates the disappearance of double bonds in the cis conformation. On the other hand, in Figs. 4B1, B2 we show the changes of the absorption bands recorded at 967 and 987 cm⁻¹ in response to formation of trans isomers (nonconjugated trans and conjugated trans, respectively).

Thus, the thermal stress caused gradual changes of the cis fatty acids to trans fatty acids. The band at 987 cm⁻¹ is associated with bending vibrations of C−H trans, trans and cis, trans conjugated diene groups of hydroperoxides, as previously reported by Guillen et al. (2005) [15]. This band appeared as a result of thermal stress, in the course of heating at 185°C, and remained until the end of treatment, when it reached the maximum absorbance. Our results support the finding mentioned by Guillen et al. (2005) [15] that hydroperoxide groups are associated with conjugated double bonds. The intensity of the band attributed to conjugated trans isomers is more important in the study of oil samples oxidation compared to the band assigned to nonconjugated trans isomers [36]. The band recorded in the infrared spectra of heat treated oil samples near 967 cm⁻¹ has been assigned to secondary oxidation products such as aldehydes or ketones that contain isolated trans double bonds [15, 28]. It became more noticeable, reaching the highest value at the end of heating, after 8 h, as shown in Figs. 4B1, B2.

Therefore, the conjugation and cis-trans isomerisation of double bonds were reflected by decreases in the absorbance of cis bands at 3006 and 722 cm⁻¹ and increases in the absorbance at 987 and 967 cm⁻¹ assigned to trans conjugated and nonconjugated double bonds.

The decrease of unsaturation by disappearance of the double bonds in response to heating could be explained by the formation of epoxides. The epoxy groups, characterized by a vibration band at 885 cm⁻¹ arise in response to heating at high temperature by thermal decomposition of methyl oleate hydroperoxides and are attributed to trans epoxides [36]. The formation of epoxy groups, revealed by a weak band at 885 cm⁻¹, can be seen in the infrared spectra of thermo-oxidized oils (Figs. 4D1, D2) only after heating for 8 h at 185°C, when important concentrations of conjugated double bonds have been obtained. Thus, the changes in the conjugated trans band in the infrared spectra of oxidized samples can be associated with the formation of epoxides.

Fig. 5 illustrates the spectral changes recorded in the C=O region in the course of heat treatment applied to EVOO and SBO. The vibration of the ester carbonyl functional group of the triglycerides includes the region of 1720–1750 cm⁻¹. Actually, the carbonyl band is composed of two bands: a sharp component appearing in infrared spectra at 1743 cm⁻¹ and a broad component at about 1728 cm⁻¹ [38]. With regard to the spectral changes in the C=O region, our study shows a widening of the band recorded at 1743 cm⁻¹ for the oxidized oil samples exposed to heat for 8 h, Fig. 5.

As it has been widely reported by Moharam and Abbas (2000) [32], Guillen et al. (2005) [16], and Vlachos et al. (2006) [13], this change may be associated with the decomposition of hydroperoxides and the formation of saturated aldehydes or other secondary oxidation products such as alcohol, ketones, acids and esters, causing an absorption at 1728 cm⁻¹, which is close to, and may overlap with the stretching vibration band assigned to the ester carbonyl functional group of
the triglycerides, recorded at 1746 cm\(^{-1}\). Under thermo-oxidative conditions, the maximum absorbance in the spectral region between 1700 and 1726 cm\(^{-1}\) induces a decrease in the frequency of the band to 1743 cm\(^{-1}\), along with its broadening, when new carbonyl groups are formed from initial aldehyde and ketone compounds. The magnitude of oxidative changes recorded in infrared spectra of oil samples in response to thermal stress varied according to both the degree of EVOO adulteration and the duration of heat treatment.

**4 Conclusions**

The results of this study demonstrate that the changes occurring in the frequency and intensity of the FTIR spectra characteristic bands can provide meaningful information to detect EVOO adulteration by addition of SBO and to estimate the oxidation state of oil samples during high temperature heat treatment. The changes in the absorbance at 3006 cm\(^{-1}\) and in the ratio A3006/A2925 were used to evaluate EVOO adulteration. The detection limits of adulteration were calculated by linear regression analysis of A3006 and A3006/A2925 versus the percentage of adulterant. Our results recommend the use of the ratio A3006/A2925, instead of A3006, to assess EVOO adulteration. The thermo-oxidative stress undergone in response to heating at 185\(^\circ\)C caused significant changes throughout the entire infrared spectra of oils, but the most meaningful were interpreted by concomitant conjugation and cis-trans isomerisation of the double bonds (reflected by a decrease in the absorbance of cis bands at 3006 and 722 cm\(^{-1}\) and an increase in the absorbance of trans conjugated and nonconjugated bands, at 987 and 967 cm\(^{-1}\)), widening of the band in the C=O region at 1743 cm\(^{-1}\) (due to formation of secondary oxidation products) and the formation of epoxides, revealed by a weak vibration band at 885 cm\(^{-1}\). Our results highlight the feasibility and promising potential of ATR-FTIR spectroscopy as a fast and reagent-free method to successfully differentiate among pure and adulterated oils and in the routine evaluation of the oxidative state of edible oils subjected to heat treatments.

**Abbreviations**

ATR-FTIR spectroscopy: Attenuated Total Reflectance – Fourier Transform Infrared spectroscopy; EVOO: extra-virgin olive oil; SBO: soybean oil; SAFAs: saturated fatty acids; MUFAs: mono unsaturated fatty acids; PUFAs: poly unsaturated fatty acids; A: absorbance; R: correlation coefficient; LOD: limit of detection; SD: standard deviation.

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