



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

Sepideh Gholami Khesht
Elahe Kavusi
Maryam Mousavi
Yaser Sharaj Sharifi

Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran.
Department of Agricultural Biotechnology, Tabriz University, Iran.
Department of Agricultural Biotechnology, Payame Noor University, Isfahan branch, Iran.
Department of Agricultural Biotechnology, Sari Agricultural Sciences and Natural Resources University, Iran.

ARTICLE INFO

Submitted: 16 Dec 2020
Accepted: 28 Dec 2020
Published: 30 Dec 2020

Keywords:

**Adulteration;
FT-IR spectroscopy;
Heat-Mediated Oxidation Method;
Olive oil;
UV-vis spectroscopy**

Correspondence:


Yaser Sharaj Sharifi, Department of Agricultural Biotechnology, Sari Agricultural Sciences and Natural Resources University, Iran.

Email: sharifiyaser71@gmail.com

ORCID: 0000-0002-2560-2812

Citation:

Gholami Khesht S, Kavusi E, Mousavi M, Sharaj sharifi Y. Efficient Detection of Extra Virgin Olive Oil Adulteration via UV and FTIR Spectra in Combination with Heat-Mediated Oxidation Method. Tabari Biomed Stu Res J. 2020; 2(4):38-47.

 **10.18502/tbsrj.v2i4.5470**

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

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

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, Essex, 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

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*. Regarding 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

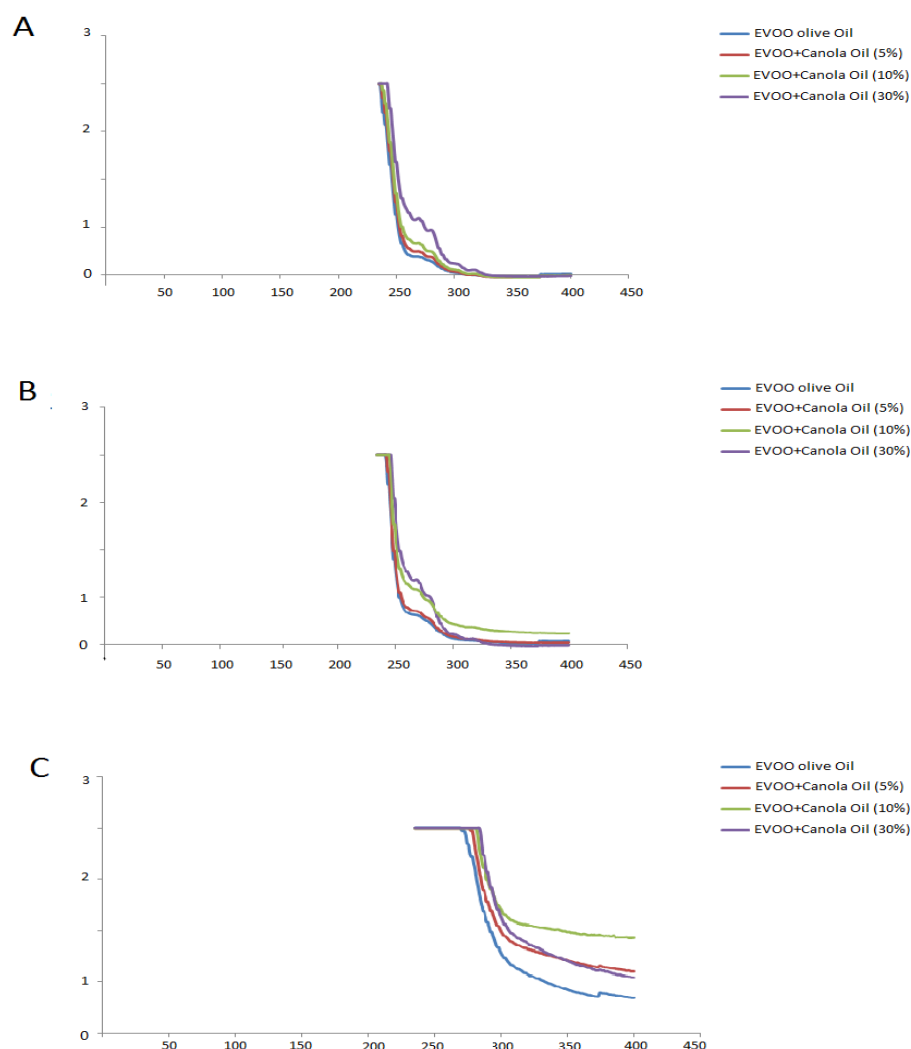


Figure 1. UV-VIS spectra (240-400nm) of the extra virgin olive oil mixed with the different percentages of canola oil. The UV absorbance of the EVOO (blue) and its canola-mixed samples containing canola oils 5% (red), 10% (light green) and 30% (violet) were analyzed in the thermal conditions with three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

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 $1600\text{--}1750\text{ cm}^{-1}$ (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

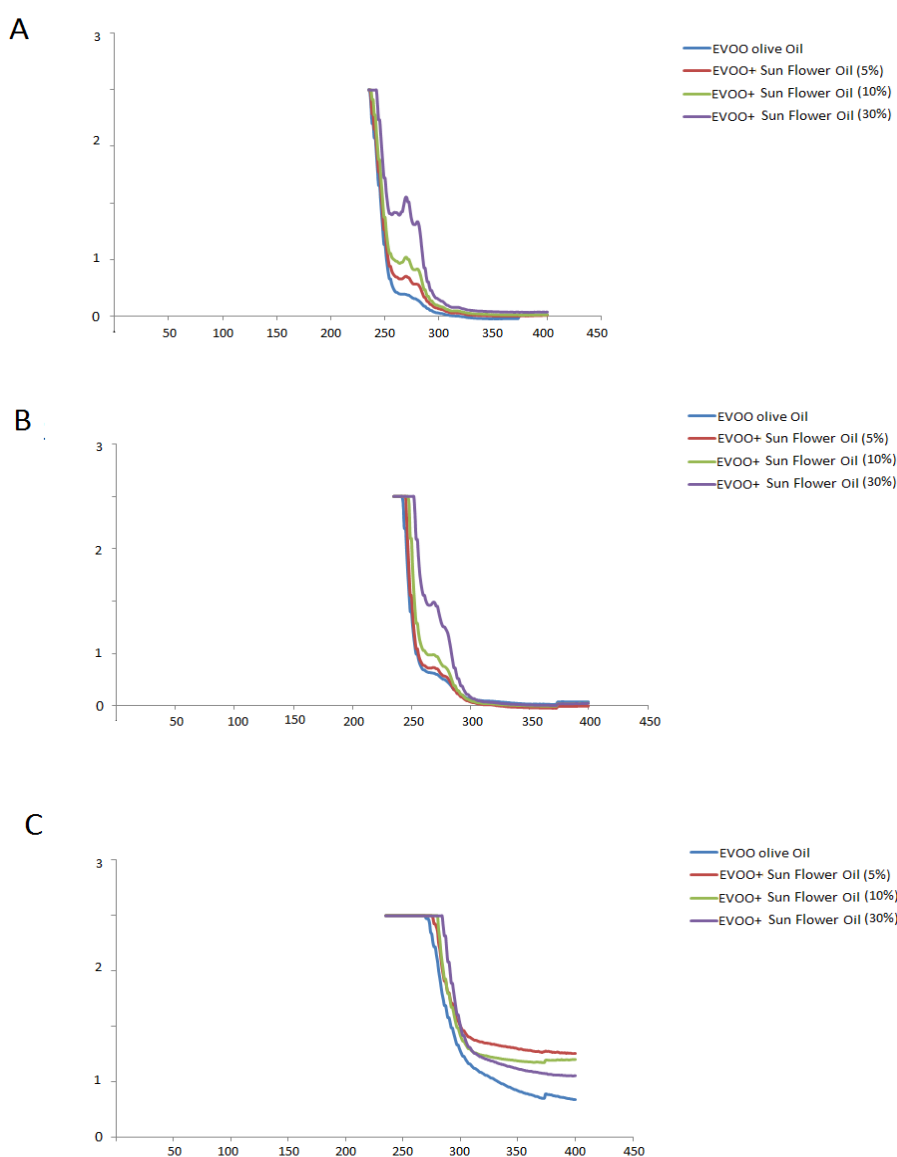


Figure 2. UV-VIS spectra (240-400nm) of the extra virgin olive oil mixed with the different percentages of Sun flower oil. The UV absorbance of the EVOO (blue) and its sun flower-mixed samples containing sun flower oils 5% (red), 10% (light green) and 30% (violet) were analyzed in the thermal conditions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

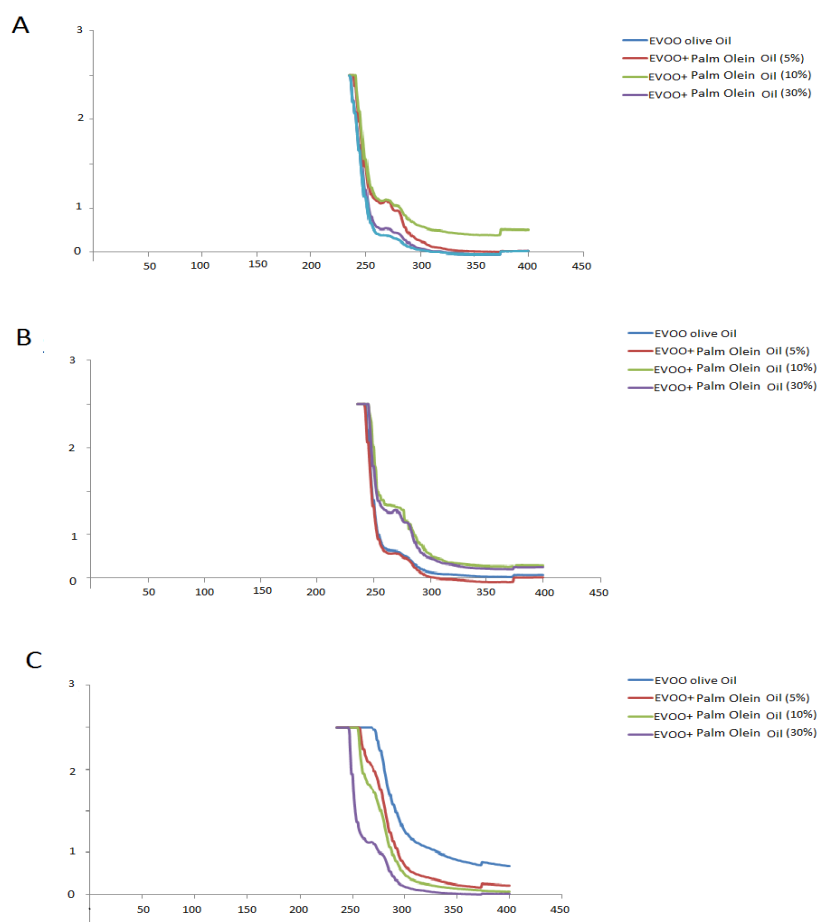


Figure 3. UV-VIS spectra (240-400nm) of the extra virgin olive oil mixed with the different percentages of Palm Olein oil. The UV absorbance of the EVOO (blue) and its palm-mixed samples containing palm olein oils 5% (red), 10% (light green) and 30% (violet) were analyzed in the thermal conditions with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

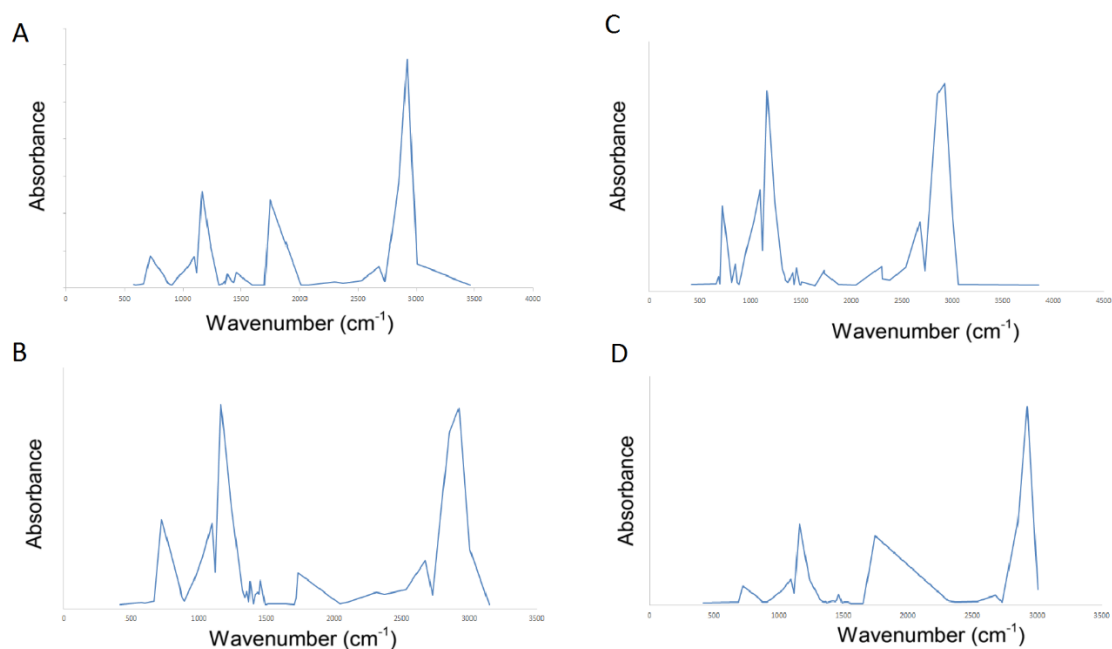


Figure 4. FT-IR spectra (500-4000 cm^{-1}) of A) extra virgin olive oil, B) EVOO with canola oil, C) EVOO with sunflower oil, D) EVOO with Palm Olein oil.

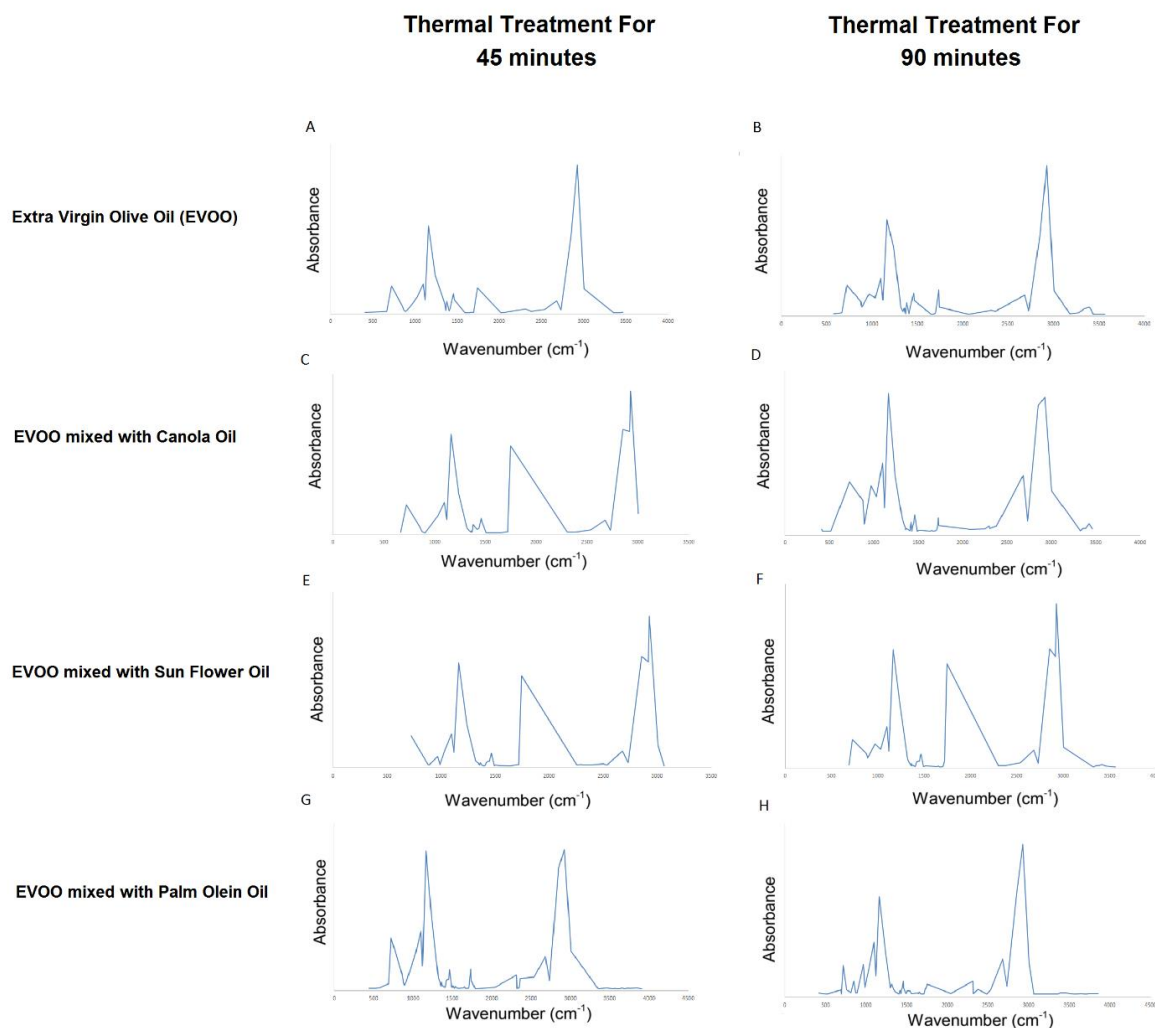


Figure 5. FT-IR spectra (500-4000 cm^{-1}) of the A,B) extra virgin olive oil, C, D) EVOO with canola oil, E, F) EVOO with sunflower oil, G, H) EVOO with Palm Olein oil under thermal treatments.

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 $1200\text{-}1400\text{ cm}^{-1}$ (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 $700\text{-}800\text{ cm}^{-1}$ and decrease in the range of $1200\text{-}1400\text{ cm}^{-1}$ (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 $1200\text{-}1400\text{ cm}^{-1}$ (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

vegetable oils).

References

1. Zamora R, Alba V, Hidalgo FJ. Use of high-resolution ^{13}C nuclear magnetic resonance spectroscopy for the screening of virgin olive oils. *Journal of the American Oil Chemists' Society*. 2001;78(1):89-94.
2. Sánchez J, Harwood JL. Biosynthesis of triacylglycerols and volatiles in olives. *European Journal of Lipid Science and Technology*. 2002;104(9-10):564-73.
3. Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. *New England Journal of Medicine*. 2013;368(14):1279-90.
4. Tsimidou M, Karakostas K. Geographical classification of Greek virgin olive oil by non-parametric multivariate evaluation of fatty acid composition. *Journal of the Science of Food and Agriculture*. 1993;62(3):253-7.
5. Rohman A, Man YC. Fourier transform infrared (FTIR) spectroscopy for analysis of extra virgin olive oil adulterated with palm oil. *Food research international*. 2010;43(3):886-92.
6. Yang H, Irudayaraj J. Comparison of near-infrared, Fourier transform-infrared, and Fourier transform-Raman methods for determining olive pomace oil adulteration in extra virgin olive oil. *Journal of the American Oil Chemists' Society*. 2001;78(9):889.
7. Mendes TO, da Rocha RA, Porto BL, de Oliveira MA, dos Anjos VdC, Bell MJ. Quantification of extra-virgin olive oil adulteration with soybean oil: a comparative study of NIR, MIR, and Raman spectroscopy associated with chemometric approaches. *Food analytical methods*. 2015;8(9):2339-46.
8. Gonzalez-Fernandez I, Iglesias-Otero M, Esteki M, Moldes O, Mejuto J, Simal-Gandara J. A critical review on the use of artificial neural networks in olive oil production, characterization and authentication. *Critical reviews in food science and nutrition*. 2019;59(12):1913-26.
9. Didham M, Truong VK, Chapman J, Cozzolino D. Sensing the Addition of Vegetable Oils to Olive Oil: The Ability of UV-VIS and MIR Spectroscopy Coupled with Chemometric Analysis. *Food Analytical Methods*. 2019:1-7.
10. Downey G, McIntyre P, Davies AN. Detecting and quantifying sunflower oil adulteration in extra virgin olive oils from the Eastern Mediterranean by visible and near-infrared spectroscopy. *Journal of Agricultural and Food chemistry*. 2002;50(20):5520-5.
11. da Silveira R, Vágula JM, de Lima Figueiredo I, Claus T, Galuch MB, Junior OOS, et al. Rapid methodology via mass spectrometry to quantify addition of soybean oil in extra virgin olive oil: a comparison with traditional methods adopted by food industry to identify fraud. *Food Research International*. 2017;102:43-50.
12. Uncu O, Ozen B. A comparative study of mid-infrared, UV-Visible and fluorescence spectroscopy in combination with chemometrics for the detection of adulteration of fresh olive oils with old olive oils. *Food Control*. 2019;105:209-18.
13. Woodcock T, Downey G, O'Donnell CP. Confirmation of declared provenance of European extra virgin olive oil samples by NIR spectroscopy. *Journal of Agricultural and Food Chemistry*. 2008;56(23):11520-5.
14. Bevilacqua M, Bucci R, Magrì AD, Magrì AL, Marini F. Tracing the origin of extra virgin olive oils by infrared spectroscopy and chemometrics: A case study. *Analytica chimica acta*. 2012;717:39-51.
15. Gurdeniz G, Ozen B. Detection of adulteration of extra-virgin olive oil by chemometric analysis of mid-infrared spectral data. *Food chemistry*. 2009;116(2):519-25.
16. Kulling S, Bunzel D, Frommherz L, Molkentin J, Lehmann I, Engert S, et al. The Setup of the National Reference Centre for Authentic Food (NRZ-Authent) in Germany. *European Journal of Lipid Science and Technology*. 2019;121(12):1900023.
17. Lizhi H, Toyoda K, Ihara I. Discrimination of olive oil adulterated with vegetable oils using dielectric spectroscopy. *Journal of Food Engineering*. 2010;96(2):167-71.

18. Zabaras D. Olive oil adulteration with hazelnut oil and analytical approaches for its detection. *Olives and Olive Oil in Health and Disease Prevention*: Elsevier; 2010. p. 441-50.
19. Dourtoglou V, Dourtoglou T, Antonopoulos A, Stefanou E, Lalas S, Poulos C. Detection of olive oil adulteration using principal component analysis applied on total and regio FA content. *Journal of the American Oil Chemists' Society*. 2003; 80(3):203-8.
20. Sun X, Lin W, Li X, Shen Q, Luo H. Detection and quantification of extra virgin olive oil adulteration with edible oils by FT-IR spectroscopy and chemometrics. *Analytical Methods*. 2015;7(9):3939-45.
21. Jiménez-Carvelo AM, Osorio MT, Koidis A, González-Casado A, Cuadros-Rodríguez L. Chemometric classification and quantification of olive oil in blends with any edible vegetable oils using FTIR-ATR and Raman spectroscopy. *LWT*. 2017;86:174-84.
22. Merás ID, Manzano JD, Rodríguez DA, de la Peña AM. Detection and quantification of extra virgin olive oil adulteration by means of autofluorescence excitation-emission profiles combined with multi-way classification. *Talanta*. 2018; 178:751-62.
23. Torrecilla JS, Rojo E, Dominguez JC, Rodríguez F. A novel method to quantify the adulteration of extra virgin olive oil with low-grade olive oils by UV-Vis. *Journal of agricultural and food chemistry*. 2010; 58(3):1679-84.
24. Ok S. Detection of olive oil adulteration by low-field NMR relaxometry and UV-Vis spectroscopy upon mixing olive oil with various edible oils. *Grasas y Aceites*. 2017;68(1):173.
25. Šmejkalová D, Piccolo A. High-power gradient diffusion NMR spectroscopy for the rapid assessment of extra-virgin olive oil adulteration. *Food Chemistry*. 2010; 118(1):153-8.
26. Agiomyrgianaki A, Petrakis PV, Dais P. Detection of refined olive oil adulteration with refined hazelnut oil by employing NMR spectroscopy and multivariate statistical analysis. *Talanta*. 2010;80(5):2165-71.
27. Mannina L, Patumi M, Proietti N, Bassi D, Segre AL. Geographical characterization of Italian extra virgin olive oils using high-field ¹H NMR spectroscopy. *Journal of Agricultural and Food Chemistry*. 2001;49(6):2687-96.
28. Xu Z, Morris RH, Bencsik M, Newton MI. Detection of virgin olive oil adulteration using low field unilateral NMR. *Sensors*. 2014;14(2):2028-35.
29. Dais P, Hatzakis E. Quality assessment and authentication of virgin olive oil by NMR spectroscopy: a critical review. *Analytica Chimica Acta*. 2013;765:1-27.
30. Zhang X, Qi X, Zou M, Liu F. Rapid authentication of olive oil by Raman spectroscopy using principal component analysis. *Analytical letters*. 2011;44(12): 2209-20.
31. Jiménez MS, Velarte R, Gomez MT, Castillo JR. Multielement determination using on-line emulsion formation and ICP-MS/FAAS for the characterization of virgin olive oils by principal component analysis. *Atomic Spectroscopy-Norwalk Connecticut*. 2004;25(1):1-12.
32. Nosratpour M, Farhoosh R, Sharif A. Quantitative indices of the oxidizability of fatty acid compositions. *European Journal of Lipid Science and Technology*. 2017; 119(12):1700203.
33. Amereih S, Barghouthi Z, Marowan O. Detection and quantification of adulteration in olive oil using a uv-spectrophotometric method. 2014.
34. ergen I. Metode spectroscopice de investigare si control a compozitiei alimentelor. Editura Agroprint, Timisoara (Spectroscopic methods of investigation and control of food composition). 2009:113-9.
35. Torrecilla JS, Rojo E, Domínguez JC, Rodríguez F. Linear and non linear chemometric models to quantify the adulteration of extra virgin olive oil. *Talanta*. 2010;83(2):404-9.
36. Fuentes E, Báez ME, Bravo M, Cid C, Labra F. Determination of total phenolic content in olive oil samples by UV-visible spectrometry and multivariate calibration.

- Food Analytical Methods. 2012;5(6):1311-9.
37. Domenici V, Ancora D, Cifelli M, Serani A, Veracini CA, Zandomenighi M. Extraction of pigment information from near-UV vis absorption spectra of extra virgin olive oils. *Journal of agricultural and food chemistry*. 2014;62(38):9317-25.
38. Gonçalves TR, Rosa LN, Gonçalves RP, Torquato AS, Março PH, Gomes STM, et al. Monitoring the oxidative stability of monovarietal extra virgin olive oils by UV-Vis spectroscopy and MCR-ALS. *Food Analytical Methods*. 2018;11(7):1936-43.
39. Lerma-García MaJs, Simo-Alfonso EF, Chiavaro E, Bendini A, Lercker G, Cerretani L. Study of chemical changes produced in virgin olive oils with different phenolic contents during an accelerated storage treatment. *Journal of agricultural and food chemistry*. 2009;57(17):7834-40.
40. Dupuy N, Le Dréau Y, Ollivier D, Artaud J, Pinatel C, Kister J. Origin of French virgin olive oil registered designation of origins predicted by chemometric analysis of synchronous excitation– emission fluorescence spectra. *Journal of agricultural and food chemistry*. 2005;53(24):9361-8.
41. Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. Methods for testing antioxidant activity. *Analyst*. 2002; 127(1):183-98.
42. Farmer EH. Peroxidation in relation to olefinic structure. *Transactions of the Faraday Society*. 1946;42:228-36.
43. Tapp HS, Defernez M, Kemsley EK. FTIR spectroscopy and multivariate analysis can distinguish the geographic origin of extra virgin olive oils. *Journal of agricultural and food chemistry*. 2003;51(21):6110-5.
44. Nunes CA. Vibrational spectroscopy and chemometrics to assess authenticity, adulteration and intrinsic quality parameters of edible oils and fats. *Food Research International*. 2014;60:255-61.
45. Sinelli N, Casiraghi E, Tura D, Downey G. Characterisation and classification of Italian virgin olive oils by near-and mid-infrared spectroscopy. *Journal of Near Infrared Spectroscopy*. 2008;16(3):335-42.
46. Casale M, Oliveri P, Casolino C, Sinelli N, Zunin P, Armanino C, et al. Characterisation of PDO olive oil Chianti Classico by non-selective (UV–visible, NIR and MIR spectroscopy) and selective (fatty acid composition) analytical techniques. *Analytica chimica acta*. 2012;712:56-63.
47. Maggio RM, Kaufman TS, Del Carlo M, Cerretani L, Bendini A, Cichelli A, et al. Monitoring of fatty acid composition in virgin olive oil by Fourier transformed infrared spectroscopy coupled with partial least squares. *Food Chemistry*. 2009;114(4): 1549-54.
48. Didham M, Truong VK, Chapman J, Cozzolino D. Sensing the Addition of Vegetable Oils to Olive Oil: The Ability of UV–VIS and MIR Spectroscopy Coupled with Chemometric Analysis. *Food Analytical Methods*. 2020;13(3):601-7.