Chemical Characterization and Detection of Adulteration in *Olea Europaea* Linn. Oil by ATR-FTIR Method

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Abstract: *Olea europaea* Linn. (Olive; Oleaceae) commonly known as Zaytoon in the Mediterranean region. Olive oil and fruits are an important component of the Mediterranean diet as cooking oil and as a salad. Adulteration in Olive oil is very common with cheap edible oils. Thorough literature shows that there is no study conducted to check adulteration in Olive oil produced in Iraqi Kurdistan. The aim of the present study is to screen genuine Olive oil samples and also develop a method for the detection of adulteration by ATR-FTIR. The detection of adulteration in O. europaea oil was performed by the ATR-FTIR method. The sesame oil (SO), coconut oil (CO), sunflower oil (SFO), and refined olive oil (ROO) were selected as adulterants. For detection of adulteration in *O. europaea* oil, different binary-mixtures of various concentrations of O. europaea oil with sesame oil (SO), coconut oil (CO), sunflower oil (SFO), and refined olive oil (ROO) (0, 5, 10, 20, 30, 40, 50 and 100% v/v) were prepared and FTIR spectra of each concentration were recorded. FTIR results of binary-mixtures were quite difficult to find out adulteration. Hence, on the basis of IR spectra's only it is very difficult to check adulteration in Olive oil. So, we proposed an alternate way and easily to check adulteration in Olive oil. For that, we searched each binary-mixtures in the library of ATR-FTIR (IRAffinity-1S, Shimadzu, Japan), and results are presented in terms of FTIR search score. This method was found fast, easy, and reliable for the detection of cheap edible oils in pure Olive oils.

Keywords: *Olea europaea*, Olive, ATR-FTIR, IRAffinity-1S, Adulteration, Search Score

1. Introduction

Olea europaea Linn. (Olive) belongs to Oleaceae family and also known as Zaytoon in Mediterranean region. Olive oil and fruits are an important component of the Mediterranean diet as cooking oil and as a salad (Ahamad et al., 2019). Olive tree is a globally prevalent plant species and has been described as one of the most important cultivated crops (Abaza et al., 2015). The olive tree is particularly special to mankind due to its recurrent appearances throughout historical and religious texts and its incorporation into traditional herbal medicines (Kaniewski et al., 2012). Olives mainly contain phenolics and lipids, and the main bioactive phenolic is oleuropein and lipids includes oleic acid (Esti et al., 1998). Olive oil mainly contains oleic acid, oleic acid, 3-(octadecyloxy) propyl ester, arachidonic acid, docosahexaenoic acid (DHA), pentadecanoic acid, palmitic acid, and linoleic acid (Ahamad et al., 2020). The beneficial bioactivities of Olive fruits, leaves, and oil may be due to presence above mentioned phytochemicals.

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Olives possesses several beneficial actions in prevention and treatment of human ailments such as prevention of cardiac events and/or Coronary Heart Disease (Buckland et al., 2012; de Lorgeril et al., 1999; Estruch et al., 2013; Dilis et al., 2012), lowers the risk of colorectal cancer (Stoneham et al., 2000; Gimeno et al., 2007) and osteoporosis (Saleh et al., 2011), prevention and treatment of type 1 & 2 diabetes (Guasch-Ferre et al., 2015; Soriguer et al., 2013; Cao et al., 2018; Violi et al., 2015; Carnevale et al., 2017; Bozzetto et al., 2016; Ahamad, 2019), improves lipid profile (Hernaez et al., 2014; Covas et al., 2006), and lowered free radical levels in humans (Carnevale et al., 2014, 2018).

GC/FID and GC/MS techniques are mainly utilized for analysis of fixed oils. GC-based techniques are highly sensitive, selective and accurate for identification and quantification of essential oils, fixed oil and low molecular weight phytochemicals (Ahamad, 2021; Najibullah et al., 2021). However, it has several drawbacks such as it is expensive, time-consuming, and required skilled person to handle the machine (Ahamad et al., 2021). Hence, there is a need to search for alternative analytical tool that could be easy, fast, reliable, sensitive, and accurate. The FTIR coupled with ATR (attenuated total reflectance) spectroscopy provides rapid, sensitive, and accurate alternative analytical methods for analysis of fixed oils (Ahamad et al., 2020). ATR-FTIR spectroscopy also requires no sample preparation steps and it provides fingerprint of sample and can be considered as an alternative way for detection of adulteration in fixed oil. Fixed oils exhibit a complex IR spectrum due to the presence of a large number of constituents in them; hence identification of particular edible oil becomes difficult. However, Olive oil shows some unique IR peaks such as 1161 cm^{-1} (assigned to -C-O stretching vibration and CH_2 bending vibration), 2922 cm^{-1} (assigned to =C-H stretching), and 1743 cm^{-1} (assigned to C=O stretching) and these IR peaks are used to detect adulteration of Olive oil with other edible oils (Almoselhy et al., 2009; Allam et al., 2007). Olive is main crop of Iraqi Kurdistan and there are several producers of its oil. A thorough literature shows that there is no study conducted to check adulteration in Olive oil produced in Iraqi Kurdistan. The aim of present study to screen genuine Olive oil samples and also develop method for detection of adulteration by ATR-FTIR.

2. Materials and Methods

2.1 Collection and Authentication of Olive Oils

Olea europaea edible oils (10 samples) were collected from different places in Kurdistan region and abroad, and these are presented in Table 1. The authentication of Olive oil was performed by ATR-FTIR.

Table 1. Olive oil samples and their place of production/ brand

S. No.	Olive oil samples	Place of production/ brand
	Olive oil samples 1	Erbil, Iraq
	Olive oil samples 2	Shaqlawa, Iraq
	Olive oil samples 3	Afrin, Iraq
	Olive oil samples 4	My family olive oil, Erbil, Iraq
	Olive oil samples 5	Kasho, Sulaymaniyah, Iraq
	Olive oil samples 6	Ranya Olive oil, Ranya, Iraq
	Olive oil samples 7	Kalar Olive oil, Kalar, Iraq
	Olive oil samples 8	Rasan Oilve oil, Halabja, Iraq
	Olive oil samples 9	Safen mountain, Erbil, Iraq
	Olive oil samples 10	Rafael Salgado, Refined Olive oil, Spain

2.2 Screening of *O. europaea* Oil Samples by ATR-FTIR

The authentication of different samples of O. europaea oil was performed by ATR-FTIR (attenuated total reflectance-fourier transform infrared). ATR-FTIR (IRAffinity-1S, Shimadzu, Japan) was utilized to record IR spectra of O. europaea oil. The spectra acquisition was performed in the spectral range of 400 to 4000 cm-1 at room temperature. *O. europaea* oils were placed on ATR surface and FTIR spectra were recorded using 45 scans and 4 cm-1 resolutions. The ATR surface was cleaned using hexane and a background scan was recorded before each sample scan. The FTIR data's obtained for O. europaea oil was also compared with each other. The FTIR spectrum of O. europaea oil was searched in the library (IRAffinity-1S, Shimadzu, Japan) and results were presented as search score.

2.3 Detection of Adulteration in *O. europaea* Oil by ATR-FTIR

The detection of adulteration in *O. europaea* oil was performed by ATR-FTIR method. Sesame oil (SO), coconut oil (CO), sunflower oil (SFO), and refined olive oil (ROO) were selected as solvents of adulteration. For detection of adulteration in *O. europaea* oil, different binary-mixtures of various concentrations of *O. europaea* oil with sesame oil (SO), coconut oil (CO), sunflower oil (SFO), and refined olive oil (ROO) (0, 5, 10, 20, 30, 40, 50 and 100% *v/v*) were prepared and FTIR spectra of each concentration were recorded.

3. Results and Discussion

3.1 Screening of Genuine Olive Oil Samples

ATR-FTIR has emerged as a choice of method for the detection of adulteration of cheap edible oils such as sunflower, sesame, and coconut oils in virgin olive oil. Kurdistan region of Iraq produces large quantities of Olives. There are several Olive oil-producing industries in Kurdistan region. In the present study, ten Olive oil samples (Table 1) were selected randomly and characterized by ATR-FTIR method. The authenticity of Olive oil was determined by their characteristic IR peaks (Figure 1) and score of search results. The genuine Olive oil shows characteristic IR peaks as presented in Figure 1 and Table 2. FTIR spectra of Olive oil samples were overlapped to differentiate any possible adulteration and presented in Figure 2. From Figure 2, it is quite difficult to differentiate between Olive oil samples, however, sample 3 (green clolour) shows variation in the IR spectrum. For simple differentiation between Olive oil samples, a search was performed in the library of FTR-FTIR (IRAffinity-1S, Shimadzu, Japan) and presented in Table 3 (the first three results are given). From Table 3, it is very clear based on the search score that sample no. 3 is highly adulterated with butter (search score: 797) which is produced in Afrin, Iraq with the lowest quality. The best Olive oil samples are: sample no. nine (961), sample no. one (955), and sample no. two (937), respectively. So, Olive oil produced in Safen Mountain, Erbil was found most genuine olive oil, and it was further used in the present study as Extra Virgin Olive Oil (EVOO).

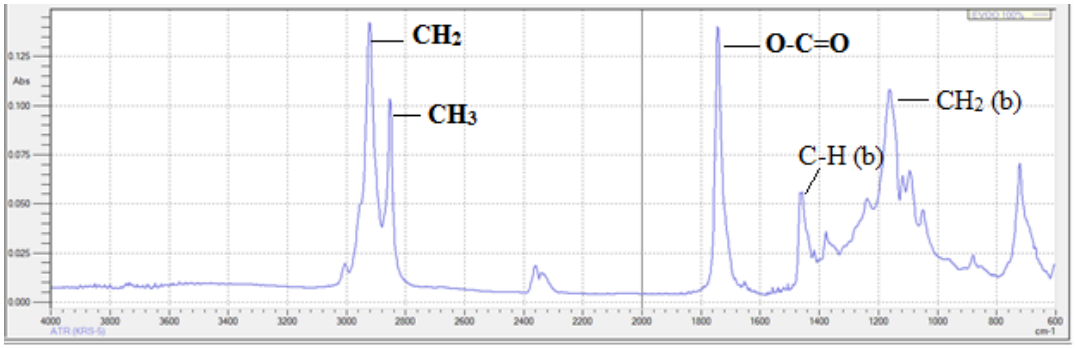

Figure 1: FTIR spectra of extra virgin olive oil (EVOO)

Table 2: Characteristic FTIR peaks, functional groups, and possible type phytochemicals present in *O. europaea* oil

S. No.	IR Peaks (cm^{-1})	Type of vibrations	Type of groups/ phytochemicals
1.	2922	C-H stretching (CH_2)	Alkenes
2.	2852	C-H stretching (CH_3)	Aliphatic compounds in triglycerides
3.	1743	C=O stretching (ketone)	Ester carbonyl groups in triglycerides
4.	1463	C-H bending (CH_2 & CH_3)	Aliphatic compounds
5.	1377	C-H bending	*cis*-Olefinic group
6.	1238	C-O stretching	Ester groups
7.	1161	C-O stretching	Ester groups
8.	1095	C-O stretching	Ester groups
9.	721	-HC-CH-	Olefins

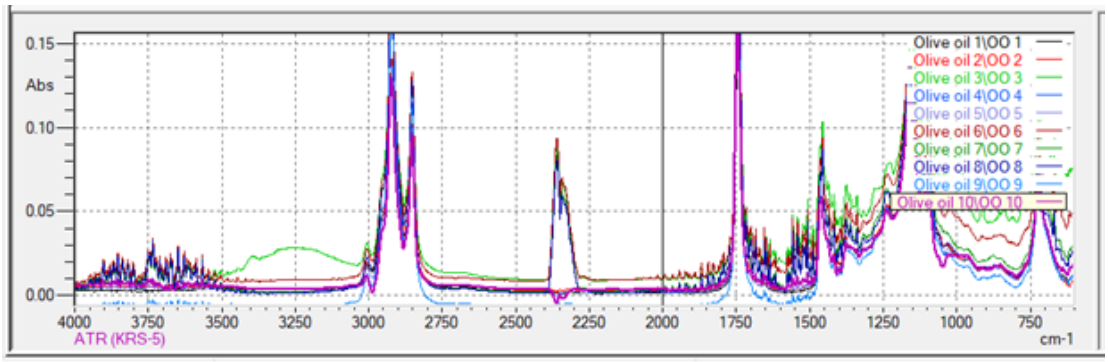

Figure 2: FTIR spectra of olive oil samples (1 to 10)

Table 3: Identification of genuine olive oil based upon search score in ATR-FTIR library

Olive oil samples	Search results (first three)					
	Identified as	Score	Identified as	Score	Identified as	Score
Sample 1	Olive oil	955	Sesame oil	944	Butter	932
Sample 2	Olive oil	937	Sesame oil	929	Butter	918
Sample 3	Butter	797	Polyvinyl stearate	789	Olive oil	782
Sample 4	Olive oil	909	Sesame oil	902	Butter	893

Sample 5	Olive oil	871	Butter	867	Sesame oil	864
Sample 6	Olive oil	894	Sesame oil	886	Butter	877
Sample 7	Olive oil	892	Sesame oil	885	Butter	873
Sample 8	Olive oil	895	Sesame oil	887	Butter	874
Sample 9	Olive oil	961	Sesame oil	953	Butter	939
Sample 10	Olive oil	912	Sesame oil	905	Butter	893

3.2 Detection of Adulteration in Olive Oil

Extra Virgin Olive Oil (EVOO) produced in Safen Mountain Erbil was adulterated with cheap edible oils such as sunflower oil (SFO), sesame oil (SO), coconut oil (CO), and refined olive oil (ROO). For detection of adulteration in *O. europaea* oil, different binary-mixtures of various concentrations of O. europaea oil with sesame oil (SO), coconut oil (CO), sunflower oil (SFO), and refined olive oil (ROO) (0, 5, 10, 20, 30, 40, 50 and 100% v/v) and results were presented in Figure 3. The IR spectra of edible oils and EVOO were found almost similar and it is difficult to differentiate based on IR peaks as seen in Figure 3. Hence, a simple method was developed to check adulteration in EVOO with edible oils and refined Olive oil. In this method, a search was performed in the library of FTR-FTIR (IRAffinity-1S, Shimadzu, Japan) for each adulteration concentration and presented in Table 4 to 7.

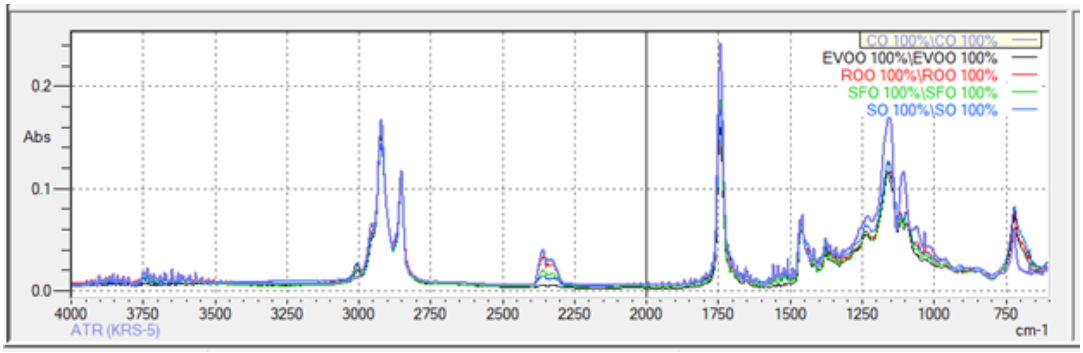

Figure 3: FTIR spectra of extra virgin olive oil (EVOO), refined olive oil (ROO), sunflower oil (SFO), sesame oil (SO), and coconut oil (CO)

3.3 Adulteration with Sunflower Oil (SFO)

Sunflower oil (SFO) has been used as common edible oil and found as a common adulterant in Olive oil because of its low price. The Olive oil was adulterated with SFO ranging from 5 to 100% and results were presented in Figure 4. From Figure 4, it is quite difficult to found out adulteration in Olive oil. Hence, on the basis of IR spectra's only it is very difficult to check adulteration in Olive oil. Hence, we proposed an alternate way and easy to check adulteration in Olive oil. For that, we searched each binary-mixtures in the library of FTR-FTIR (IRAffinity-1S, Shimadzu, Japan), and the results are presented in Table 4. From Table 4, it is quite easy to see guess adulteration on the basis of search score. As we see, the search score of EVOO (100%) was 973 and in the case of adulteration of EVOO with SFO (5%) the search score was 940, and this downfall in search score continue upto 50% addition of SFO in EVOO. The SFO (100%) shows the first preference as Sunflower oil with a score of 963 compared to Olive oil (954).

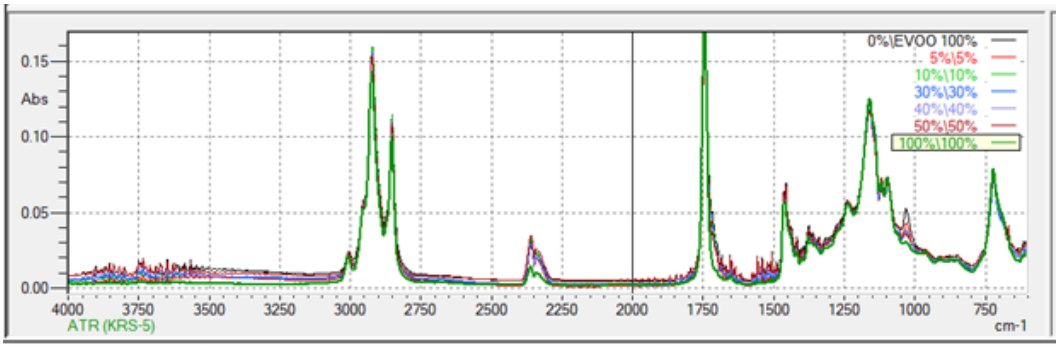

Figure 4: FTIR spectra of extra virgin olive oil (EVOO, 100%) and its adulteration with Sunflower oil (5 to 100% v/v)

Table 4: Detection of adulteration in extra virgin olive oil after adulteration with Sunflower oil (0 to 100% v/v) and their corresponding search results in ATR-FTIR library

Adulteration (%)	Search results (first three)					
	Identified as	Score	Identified as	Score	Identified as	Score
0	Olive oil	973	Sesame oil	961	Butter	942
5	Olive oil	940	Sesame oil	933	Butter	920
10	Olive oil	939	Sesame oil	931	Butter	917
20	Olive oil	936	Sesame oil	930	Butter	914
40	Olive oil	934	Sesame oil	931	Butter	912
50	Olive oil	931	Sesame oil	930	Butter	909
100	Sunflower oil	963	Olive oil	954	Butter	928

3.4 Adulteration with Coconut Oil (CO)

Coconut oil (CO) is an edible oil obtained from coconut fruits and is sometimes found as an adulterant in Olive oil because of its low price. The Olive oil was adulterated with CO ranging from 5 to 100% and results were presented in Figure 5. From Figure 5, it is quite difficult to found out adulteration in Olive oil. Hence, on the basis of IR spectra only it is very difficult to check adulteration in Olive oil. Hence, we proposed an alternate easy way to check adulteration in Olive oil. For that, we searched each binary-mixtures FTR-FTIR (IRAffinity-1S, Shimadzu, Japan) library, and the results were given in Table 5. From Table 5, it is quite easy to see check adulteration on the basis of the search score. As we see, the search score of EVOO (100%) was 973 and in the case of adulteration of EVOO with CO (5%) the search score was 966, and this downfall in score continue upto 50% addition of CO in EVOO. The CO (100%) shows the first preference as Coconut oil with a score of 938 compared to Olive oil (860).

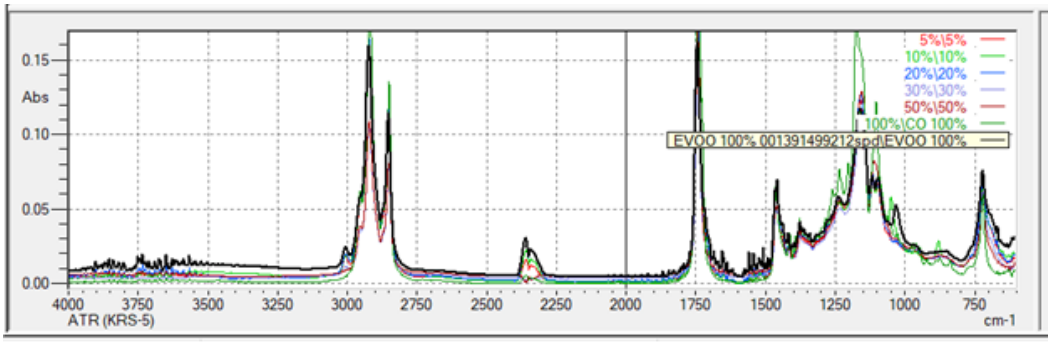

Figure 5: FTIR spectra of extra virgin olive oil (EVOO, 100%) adulteration with Coconut oil (CO) 5 to 100% v/v

Table 5: Detection of adulteration in extra virgin olive oil after adulteration with Coconut oil (0 to 100% v/v) and their corresponding search results in ATR-FTIR library

Adulteration (%)	Search results (first three)					
	Identified as	Score	Identified as	Score	Identified as	Score
0	Olive oil	973	Sesame oil	961	Butter (CO)	942
5	Olive oil	966	Sesame oil	912	Butter (CO)	887
10	Olive oil	943	Sesame oil	923	Butter (CO)	914
20	Olive oil	940	Sesame oil	945	Butter (CO)	936
40	Olive oil	930	Sesame oil	931	Butter (CO)	924
50	Butter (CO)	938	Olive oil	919	Sesame oil	884
100	Coconut oil (CO)	942	Olive oil	860	Sesame oil	849

3.5 Adulteration with Sesame Oil (SO)

Sesame oil (SO) is an edible oil obtained from *Sesamum indicum* (Pedaliaceae), and has high nutritional values; also used as a laxative, demulcent and emollient. Pharmaceutically, it is used as a vehicle for intramuscular oily injections. It is a common adulterant to Olive oil. In the present study, the Olive oil was adulterated with SO ranging from 5 to 100% and results were presented in Figure 6. From Figure 6, it is quite difficult to found out adulteration in Olive oil. Hence, on the basis of IR spectra only it is very difficult to check adulteration in Olive oil. Hence, we proposed an alternate easy way to check adulteration in Olive oil. For that, we searched each binary-mixtures of Olive oil with SO in FTR-FTIR (IRAffinity-1S, Shimadzu, Japan) library, and results were given in Table 6. From Table 6, it is quite easy to see check adulteration on the basis of the search score. As we see, the search score of EVOO (100%) was 973 and in the case of adulteration of EVOO with SO (5%) the search score was 932, and this downfall in score continue upto 50% addition of SO in EVOO (Search score 884 for Olive oil). The SO (100%) shows the first preference as Sesame oil with a score of 937 compared to Olive oil (875).

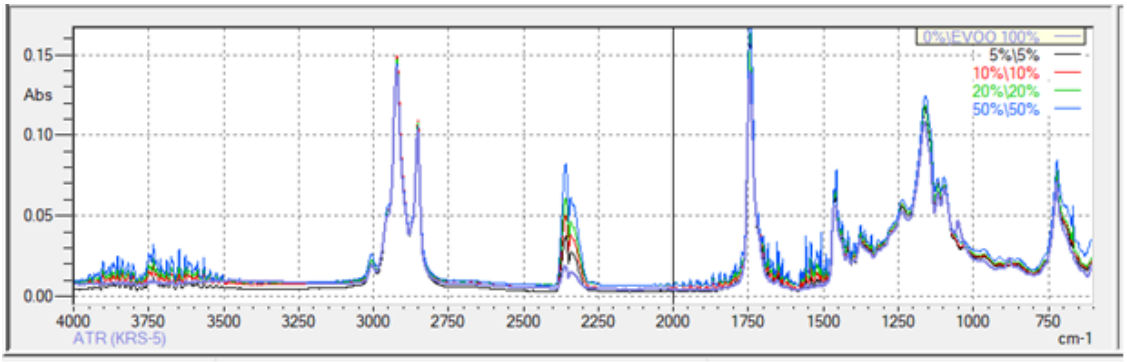

Figure 6: FTIR spectra of extra virgin olive oil (EVOO, 100%) adulteration with Sesame oil (SO) 5 to 100% v/v

Table 6: Detection of adulteration in extra virgin olive oil after adulteration with sesame oil (0 to 100% v/v) and their corresponding search results in ATR-FTIR library

Adulteration (%)	Search results (first three)					
	Identified as	Score	Identified as	Score	Identified as	Score
0	Olive oil	973	Sesame oil	961	Butter	942
5	Olive oil	932	Sesame oil	924	Butter	911
10	Olive oil	919	Sesame oil	913	Butter	899
20	Olive oil	908	Sesame oil	903	Butter	890
40	Olive oil	903	Sesame oil	890	Butter	878
50	Sesame oil	885	Olive oil	884	Butter	869
100	Sesame oil	937	Olive oil	875	Butter	855

3.6 Adulteration with Refined Olive Oil (ROO)

Refined Olive Oil (ROO) was also used as an adulterant to Olive oil in the present study. Adulteration in Olive oil with ROO ranging from 5 to 100%, and results were presented in Figure 7. From Figure 7, it is quite difficult to found out adulteration in Olive oil. Hence, on the basis of IR spectra only it is very difficult to check adulteration in Olive oil. Hence, we proposed an alternate easy way to check adulteration in Olive oil. For that, we searched each binary-mixtures of Olive oil with ROO in ATR-FTIR (IRAffinity-1S, Shimadzu, Japan) library, and results were given in Table 7. From Table 7, it is quite easy to see check adulteration on the basis of search score. As we see, the search score of EVOO (100%) was 973 and in the case of adulteration of EVOO with ROO (5%) the search score was 967, and this downfall in score continues upto 50% addition of SO in EVOO (Search score 951 for Olive oil). The ROO (100%) shows the first preference as Olive oil with a score of 946 compared to EVOO (Search score of 973).

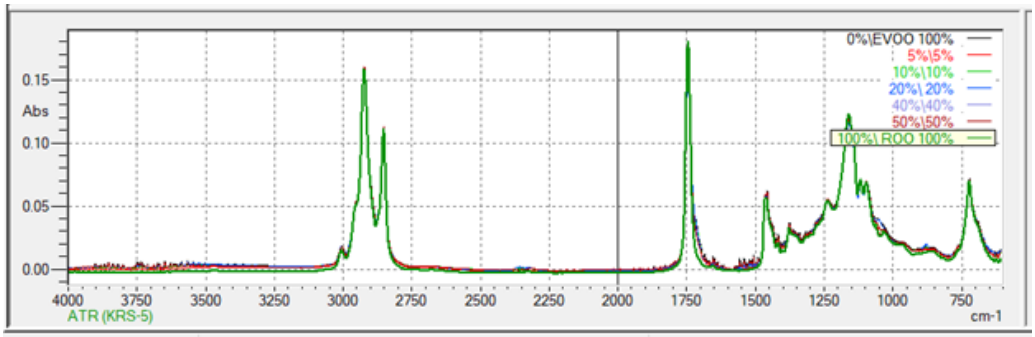

Figure 7: FTIR spectra of extra virgin olive oil (EVOO) adulteration with refined olive oil (ROO) 0 to 100% v/v

Table 7: Detection of adulteration in extra virgin olive oil after adulteration with refined olive oil (ROO) (0 to 100% v/v) and their corresponding search results in ATR-FTIR library

Adulteration (%)	Search results (first three)					
	Identified as	Score	Identified as	Score	Identified as	Score
0	Olive oil	973	Sesame oil	961	Butter	942
5	Olive oil	967	Sesame oil	955	Butter	937
10	Olive oil	958	Sesame oil	950	Butter	933
20	Olive oil	958	Sesame oil	945	Butter	937
40	Olive oil	953	Sesame oil	949	Butter	929
50	Olive oil	951	Sesame oil	949	Butter	929
100	Olive oil	946	Sesame oil	937	Butter	919

4. Conclusion

Olive is an important medicinal plant that is used all over the world. The olive tree is a prevalent plant species and one of the important cultivated crops of the Mediterranean region. Olives and their phenolic compounds are reported to possess a number of biological and pharmacological activities including antidiabetic, antiobesity, antioxidant, anti-atherogenic, antihypertensive, anti-inflammatory, and hepatoprotective. In the present research, an alternate method of detection of adulteration in Olive oil was developed on the basis of the search score obtained from the FTIR library. This method was found suitable, easy, fast, and reliable for the detection of adulteration in Extra Virgin Olive oil.

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