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Detection and Quantification of Adulteration in Olive Oil using a UV- Spectrophotometric Method

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Abstract: A simple spectrophotometric method for detection and quantification of adulteration of olive oil with sunflower, corn and soybean oils was developed. This was done by measuring the characteristics of the absorption bands between 200 and 400 nm. It was found that max absorbance frequencies related to conjugated diene and triene systems which characterize the chemical composition of sunflower, corn and soybean refined oils was a wave length 268 nm. In order to quantify the adulteration; synthetic mixtures were made by 0.5%, 1%, 5%, 25%, 50% and 75% percents for each of the sunflower, corn and soybean oil in olive oil and the absorbance of each solution was measured at 268 nm against isooctane as a blank. Calibration curves were constructed and rational equations were obtained enabling detection and quantification of adulteration. The minimum detectable present of the examined oils in olive oil was 0.5%. This amount is very acceptable since blow it adulteration will be useless without any meaning.

***Keywords*:** Olive oil, Adulteration, Cheap seeds oils, UV- Spectrophotometric method.

Introduction

Olive oil plays an important role in Palestinian life, economy and diet. Olive oils are marketed according to the process used for their extraction (Codex Stan 2003). Classification of olive oils in base of International Olive Oil Council (IOOC 2001) is virgin olive oils which are produced using only cold pressing techniques, refined olive oil and pomace olive oil (IOOC 2001). Olive oil is more expensive than other seed oils in due to account of its organoleptic for virgin olive oil and their nutritional properties.3 For this reason, they are a potential target for adulteration. The main adulteration issue involves addition of other cheaper oils, such as sunflower oil, soybean and other seed oils. Therefore clearly stress on finding a way for detecting adulterations is very important (Moreno and Mitjavila, 2003; Firestone, 2001).

There are several different methods used in the detection of adulteration (Firestone, 2001; Marini, 2004; Aparicio , 2000). They include the iodine value, saponification value, colorimetric reaction as well as refractive index, density and viscosity measurements. Furthermore Current methodology for analysis of olive oil products includes use of a variety of techniques including gas chromatography (GC), liquid chromatography (LC), mass spectrometry (MS), infrared and near infrared (IR and NIR) spectroscopy, raman and nuclear magnetic resonance (NMR), but determination of triglyceride content by LC is especially useful for verifying the presence of small quantities of other vegetable oils in olive oil (Cert et al, 2000; Andrikopoulos, 1986). Comparison of the triglyceride composition calculated from its fatty acid composition (theoretical) with the triglyceride composition determined by LC (actual) provides a reliable means of detecting seed oils in olive oil (Nagy and Bongiorno , 2005). Frequently IOOC has developed a global method for the detection of extraneous oils in olive oils.1 High linoleic vegetable oils such as sunflower and colza, and some high oleic vegetable oils such as hazelnut, high oleic sunflower and olive pomace oils are detected and finally indicates a typical olive oil is genuine or not genuine (IOOC 2006).

UV spectrometry have also be used (Ballabio, 2006; Haddada , 2007), where the absorption at the wavelengths specified in the method is due to the presence of conjugated diene and triene systems. These absorptions are expressed as specific extinctions (the extinction of 1% solution of the fat in the specified solvent, in a thickness of 1 cm) conventionally indicated by K (also referred to as "extinction coefficient"). The method of analysis

spectrophometric examination in the ultraviolet can provide information on the quality of a fat, its state of preservation and changes brought about in it by technological processes. The analysis of olive oils at certain UV wavelengths can be used to assess the oxidation of the oils and can also indicate the presence of a refined olive oil in virgin olive oil not with other vegetable oils. UV ray absorbance values of refined oils and, particularly, in the 270 nm region are in fact significantly higher than those of virgin and extra virgin olive oils. Recently the extinction coefficients method used as a supplementary help used in detection of the adulterations and classification olive oils. In the present study the main goal is to show that the results of the spectrophotometric examination are precisely and specifically enable to detect the presence of sunflower, corn and soybean oil in olive oil at low percentages. This was done by measuring the characteristics of the absorption bands between 200 and 400 nm, to find what is the wave length where max absorbance frequencies related to conjugated diene and triene systems which characterize the chemical composition of vegetable and refined oils. A low absorption in this region is indicative of a high quality extra virgin olive oil, whereas adulterated/refined oils show a greater level of absorptions in this region.

Experimental

Apparatuses

Spectrophotometer (SP-2000UV) with quartz cuvettes, digital burette (Brand), analytical balance.

Reagents

Sodium hydroxide solution (0.1 M), Ethanol, phenolphthalein indicator, saturated potassium iodide solution, acetic acid 99%, chloroform (A.R), starch solution , sodium thiosulfate Na2S2O3 (0.05 M), Spectrophotometrically pure isooctane.

Oils samples

Twenty one olive oil samples were collected from Tulkarm region in West Bank, corn, soybean and sunflower oil samples were purchased from local market.

Procedure

Free fatty acids

The acidity of olive oil samples were determined according to A.O.C.S Official Method Ca 5a-40.

Peroxidase value

The peroxide value of olive oil samples were determined according to A.O.C.S Official Method Cd 8-53.

Determination the wavelength of maximum absorbance for oil samples

The absorption of 1% of oil in isooctane solution placed in quartz cuvette was measured against a blank of isooctane over the range of 200 nm to 400 nm in intervals of 2 nm. The data were graphed to visualize the highest absorbance to determine the wavelength (λmax).

Construction calibration curves to quantify the adulteration of olive oil

Synthetic mixtures were made of 0.5% , 1%, 5%, 25%, 50% and 75% percents for each of the sunflower, corn and soybean oil in olive oil. The absorption of each mixture was measured at the wavelength (268 nm) where maximum absorbance of vegetable oils not olive oil occurred. This was done according to the procedure described above. Calibration curve for each synthetic mixture versus absorbance were plotted. Rational equations were obtained in order to quantify adulteration of olive oil.

Results and discussion

Olive oil is expected to meet some basic standards, which differentiate it from other oils. It should also be harvested and processed to maintain acceptable quality. Therefore, olive oil produces must verify the quality of their product by chemical testing. These tests ensure the quality of the oil as set by the International Olive Council (IOC). Meeting the IOC standards allows Palestinian farmers to export oil to the international markets. Several factors affect the quality of olive oil, which varies greatly between groves within a single region, and complying with IOC standards requires chemical testing such as the peroxide value, acidity. Twenty one olive oil samples were collected from Tulkarm and Qalqilia regions. Their acidity and peroxide values were determined and results are recorded in **Table 1**. Forty two percent (42%) of the tested samples were extra virgin olive oils.

Table 1: Acidity and Peroxide values for olive oil samples collected from Tulkarm region in West Bank

sample number	sample mass	country	Region	Kind of olive	Irrigated	Oil percent	Soil type	Harvesting date	Storage conditions	Acidity	Peroxide value
OAS01	91.46	Kafr Gammal	mountain	Nabali	no	26%	red	10/25/2012	plastic container	0.44	4.34
OAS02	82.12	East bouquet	mountain	Nabali	no	25%	red	October, 2012	plastic container	0.62	1.16
OAS03	106.5	Kafr Labad	Southeast	Nabali	no	22%	red	October, 2012	plastic container	0.97	2.28
OAS04	95.12	Kafr Zibad	the west(Alkhlaal)	Nabali	no	20%	red	11/10/2012	plastic container	0.65	1.32
OAS05	110.16	Jayyous	mountain	Nabali	no	22%	red	October2012	plastic container	0.29	1.2
OAS06	91.68	Tulkarm	the west	Nabali	no	17%	mountain	11/9/2012	plastic container	4.33	0.28
OAS07	110.05	Tulkarm	Core	Nabali	no	22%	red and white	October, 2012	plastic container	0.59	7.8
OAS08	92.02	Tulkarm	esbet aljarrad	Nabali	no	25%	red	10/19/2012	plastic container	0.44	2.42
OAS09	82.34	Tulkarm	East of Thinnaba	Nabali	no	23%	red	November2012	plastic container	0.41	4.54
OAS010	96.59	Tulkarm	Beit Lid	Nabali	no	20%	red	October, 2012	plastic container	0.92	2.36
OAS011	101.44	Tulkarm	south	Nabali	no	28%	red	October, 2012	plastic container	3.16	4.3
OAS013	100.22	Tulkarm	Qalqilya	Nabali	no	19%	red	October, 2012	plastic container	0.51	5.7
OAS014	98.58	Tulkarm	Qalqilya	Nabali	no	23%	red and white	October, 2012	plastic container	1.04	4.54
OAS015	103.9	Deir al-Ghusun	the middle of monastery Lands	Nabali	no	25%	red with sones	10/10/2012	plastic container	1.47	12
OAS016	92.4	Tulkarm	Thinnaba	Nabali	no	19%	mountain and Rocky	October, 2012	plastic container	0.87	1.35
OAS017	88.23	Qaffin	Thinnaba	Nabali	no	24%	red	October, 2012	plastic container	2.94	3.78
OAS018	96.9	Shweikeh	Thinnaba	Nabali	no	24%	mountain	October, 2012	plastic container	5.33	16.92
OAS019	107.43	Anabta	East of Anabta	Nabali	no	26%	red	October, 2012	plastic container	0.72	5.78
OAS020	105.07	Shweikeh	Back of Shurafa	Nabali	no	25%	red	October, 2012	plastic container	0.89	6.1
OAS021	76.36	Tulkarm	East of Tulkarm (Thinnaba)	Nabali	no	14%	red	10/23/2012	plastic container	0.83	2.4

Oils are esters of glycerol (**Figure 1**), the simplest triol (tri-alcohol), in which each of the three hydroxyl groups has been converted to an ester. The acid portion of the ester linkage (fatty acids) usually contains an even number of carbon atoms in an unbranched chain of 12 to 24 carbon atoms.

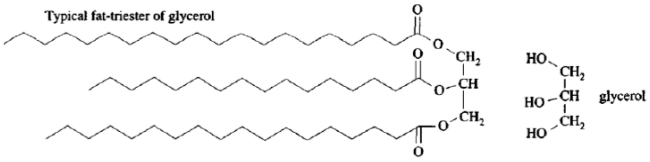


Figure 1 Composition of typical fat-triester of glycerol

The differences among triglycerides are because of the length of the hydrocarbon chains of the acids and the number of position of double bonds (unsaturation). The hydrocarbon chains of the fatty acids may be completely saturated (saturated fat) or may contain one or more double bonds. The geometric configuration of the double bond in fats and oils is normally cis. If the chain includes more than one double bond, the fat is called polyunsaturated. The presence of a double bond puts a kink (**Figure 2**) in the regular zigzag arrangement characteristic of saturated carbons. Because of this kink in the chains, the molecules cannot form a neat, compact lattice and tend to coil, so unsaturated triglycerides often melt below room temperature and are thus classified as oils

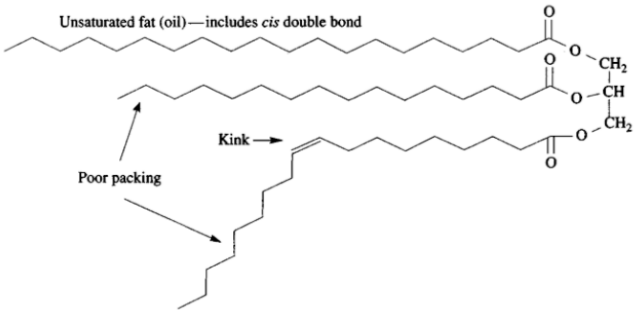


Figure 2 Oil composition includes cis double bond.

The number of carbon atoms in the hydrocarbon chain, followed by a colon and additional numbers indicating the number of double bonds, are used as an abbreviation to designate fatty acids. Thus in the 18-carbon series, C18:0, C18:l, C18:2, and C18:3 represent stearic, oleic, linoleic, and linolenic acids, respectively (**Figure 3**) (Ali et al 2005).

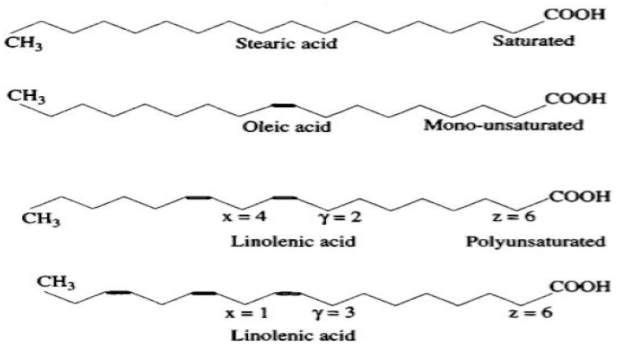


Figure 3 The 18-carbon series, C18:0, C18:l, C18:2, and C18:3 represent stearic, oleic, linoleic, and linolenic acids, respectively

Olive oil has several complex chemical components (Fiorino and Nizzi-Griffi 1992) The composition of olive oil can give valuable information in understanding their functional, quality and nutritional properties (Aparicio and Aparicio-Ruíz 2000). It also can be used as a fingerprint for reliable identification and classification of olive oils according to the olive variety and the geographic origin (Tsimidou and Karakostas 1993). **Table 2** shows that percent of oleic acid is the highest among fatty acids percent in olive oil, while polyunsaturated fatty acids present in minor. This is in contrast to other vegetable oils, such as corn, soyabeen and sunflower which they have higher percents of polyunsaturated fatty acids like linoleic and linolenic

Table 2: Fatty acid composion of olive oil

	Type of Oil (%)			
Type of fatty acid	**Olive Oil**	**Corn oil**	**Soya been**	**Sun-flower**
Stearic acid (C18:0)	**0.5-5**	**2.0-5.0**	**0.3- 4.1**	**1 – 7**
Oleic acid (C18:1)	**55 – 83**	**19.0-49.0**	**2.4- 23.3**	**14 - 40,**
Linoleic (C18:2)	**3.5 – 21**	**34.0-62.0**	**2.6-52.2**	**48 – 74**
Linolenic (C18:3)	**0-1.5**	**0-1**	**3.5-5.6**	**0.09-0.12**
Absorb-ance at 268 nm	**0.17**	**1.97**	**2.5**	**1.49**

Authenticity or good quality of olive oil correlates directly with its international marketability (Mailerand Beckingham 2006). Olive oil is more expensive than other seed oils in due to account of their organoleptic for virgin olive oil and it nutritional properties. For this reason, they are a potential target for adulteration. The main adulteration issue involves addition of other cheaper oils, such as sunflower oil, soybean and other seed oils. Therefore clearly stress on finding a way for detecting adulterations is very important. There are several different methods used in the detection of adulteration, they include: the iodine value, saponification value, colorimetric reaction as well as refractive index, density and viscosity measurements. Among the quickest methods to test for the purity of the oil is to use a UV spectrophotometric technique, which gives some indication of olive oil “virginity”. The UV absorption is used to identify oils which are old or which have been refined. The test measures changes in the structure of fatty acids, something which occurs during ageing or heating of oil. The test may be used by people who are purchasing the oil to ensure it is fresh and not adulterated with refined olive oil. A solution of 1% of olive oil in isooctane was prepared. The absorbance was measured at 200 nm to 400 nm against isooctane as a blank in order to find the maximum absorbance for olive oil. The same was done for solutions of sunflower, corn and soybean in isooctane. **Figure 4** show the absorption data for an olive oil sample NO. OAS014 (acidity 1.04, peroxide value 5.54) and solutions of sunflower, corn, soybean:

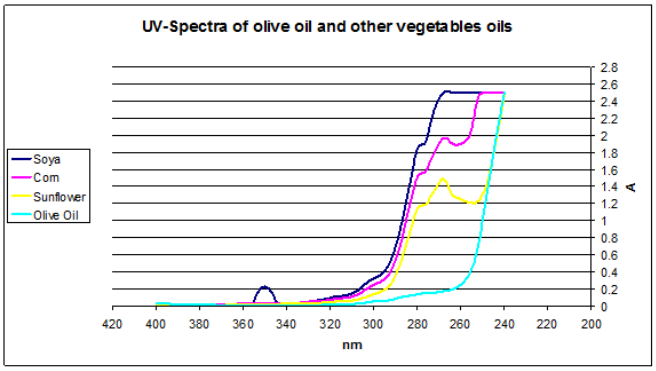
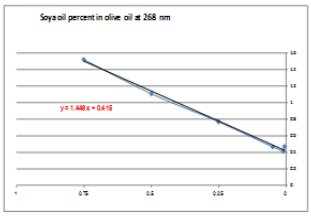


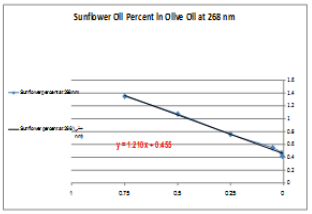
Figure 4 UV- Absorption spectra of olive oil and other vegetable oils (soya, corn and sunflower).

The absorbency at 232-245 nm is caused by hydroperoxides (primary stage of oxidation) and conjugated dienes (intermediate stage of oxidation). The absorbency at 268 nm is caused by carbonylic compounds (secondary stage of oxidation) and conjugated trienes (technological treatments). When the oil is treated with a decolorizing agent (i.e. an absorbent earth) during the refining process, conjugated trienoic compounds are formed. These compounds have a maximum absorption situated at approximately 270 nm; this means that refined oils have higher values of absorbance at 268 nm. The absorption in the U.V. is related to the presence of conjugate double bonds. In the oils, due to oxygen fixation in linolenic and linoleic acids’ double bond position, hydroperoxides arise. During more advanced oxidation states, products are generated with conjugate diene systems carbon- oxygen. The maximum absorption in this case ranges between wavelength of 260-280 nm. A conjugate triene system presents a triple band with a maximum absorption at 268 nm and another at 232-245nm. The absorption curve in the U.V. of oil is very much influenced by the oxidation products, some of which provoke an increase of the absorption at 232 nm and

others at 270 nm. **Figure 4** shows that sunflower, corn and soyabeen have higher absorbance at 268 nm than that of olive oil. This makes it easy to judge if there is an adulteration of olive oil or not; since high absorbance at this wave length (268 nm) means that olive oil is subjected to adulteration of one or more type of the examined oils. In order to quantify the adulteration; synthetic mixtures were made by 0.5%, 1%, 5%, 25%, 50% and 75% percents for each of the sunflower, corn and soybean oil in olive oil and the absorbance of each solution was measured at 268 nm against isooctane as a blank. Calibration curves A, B and C presented in **Figure 5** were constructed and rational equations were obtained enabling detection and quantification of adulteration.



A



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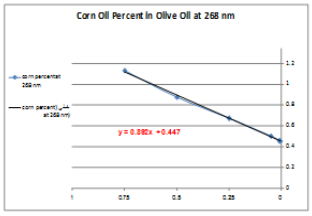

C

Figure 5 Calibration curves represent absorbance versus percent of adulteration (x-axis) of : A soybean, B sunflower and C corn in olive oil and their rational equations enabling detection and quantification of adulteration.

Using calibration curves in **Figure 5** enable detection of adulteration of olive oil to percent of 0.5 % mixture of the examined oil at considerable amount of absorbance. **Table 3** shows this minimum detectable present of the examined oils in olive oil and absorbance values. This amount is very acceptable since blow it adulteration will be useless without any meaning.

Table 3 The minimum detectable present of the examined oils in olive oil and absorbance values.

Kind of Oil	Min. detectable present	Measured A
Corn	**0.5%**	**0.452**
Soya	**0.5%**	**0.474**
Sunflower	**0.5%**	**0.423**

Conclusion

Detection an adulteration of olive oil can be done by several tests. The proposed method in this work is a simple spectrophotometric method depends on measuring the absorbance of 1% of suspected sample solution in isooctane at a wave length of 268 nm. If the absorbance measured is higher than 0.2 this an indication that the sample is subjected to adulteration with other seeds and vegetables oils since olive oil at this wave length doesn't show high absorption values at 268 nm referring to the lack of poly unsaturated fatty acids. Using calibration curves of absorbance versus percent of seed oils in olive oil; one can quantify the amount of adulteration, where the minimum detectable present of the examined oils in olive oil is less than 0.5%.

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