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The detection and quantification of sunflower adulteration of olive oil by UV spectroscopy method

Mahfouz Al-Bachir[1*], and Yasser Othman[1]

[1] Department of Radiation Technology, Atomic Energy Commission of Syria, Damascus, P.O. Box 6091, Syrian Arab Republic

Abstract

Extra virgin olive oil is so expensive which makes tempting to adulterate them with other lower price vegetable oils to achieve more profit. The aim of this study is to develop a simple, direct, and reliable UV-spectrophotometric procedure for identification and quantification of the adulteration of pure Syrian kaissy olive oil (SKOO) with sunflower oil (SO). A simple procedure has been developed for detection, classification and quantification of the adulteration of pure Syrian kaissy olive oil (SKOO) with sunflower oil (SO). The oxidative degradation was investigated by UV-visible spectroscopy and used to monitor the changes using peroxide values. The results indicated that the K232 (2.31), K270 (0.38), and ΔK (0.01) values of genuine SKOO, which are lower than the 2.5, 0.22 and 0.01, the maximum values established by the national and international regulations for extra virgin olive oil. The obtained results demonstrated the increase in the adulteration percentage increased the K232, K270 and ΔK values the values of K232, K270 and ΔK values in SKOO mixtures with SO. The concentration of the adulterant of sunflower oil in olive oil showed a positive correlation with K232 ($R2 = 0.9886$; $p< 0.01$), K270 ($R2 = 0.9984$; $p< 0.01$) and ΔK ($R2 = 0.9992$; $p< 0.01$). Equation to predict percent of adulteration from K232, K270 and ΔK values were developed. The obtained results demonstrated the feasibility of detecting adulterations of SKOO with SO.

Keywords*:* Adulteration, Olive oil, Syria, Oxidation, UV spectrophotometer

1.Introduction

The International Olive Oil Council (IOOC) defines olive oil as the oil obtained solely from the fruit of the olive tree (Olea europaa L.) with the exclusion of oils obtained using solvents or re-esterification processes and of any mixture of other oils [16]. The chemical tests and the organoleptic properties categorize olive oils into extra virgin, virgin, and ordinary virgin olive oil indicating its edible quality and marketable values [1]. Hence, purity of olive oil is a very significant issue. In fact, the FDA allows producers of olive oil to place a health claim on their products because there is some scientific evidence to support a risk reduction of coronary heart disease by consuming a higher proportion on monounsaturated fat in one's diet [11].

Olive oil is more expensive than other seed oils in due to account of its organoleptic for virgin olive oil and their nutritional properties.

Its price is higher compared to the rest of edible vegetable oils. For these reasons, olive oil is one of the oils that have been subjected to adulteration by other vegetable oils for long time [4, 10, 19].

The history of adulteration detection of extra virgin olive oil (EVOO) is quite long. Characterization classification, authentification and adulteration of olive oils has been described using various analytical methods and chemo metrical techniques [18].

The initial tests were manly based on values of iodine, saponification, density, viscosity, refractive index etc [1, 19]. Principle components analysis (PCA) is widely used for the evaluation of olive oil and virgin olive. The use of PCA treated data enables the detection of adulterant levels [22, 24, 25].

Olive oil contains more oleic acid and less linoleic and linolenic acids than other vegetable and seed oils [3, 20]. Therefore, the ratio of linoleic and linolenic

Corresponding authors: Dr. Mahfouz Al-Bachir, Syria, Damascus, P.O. Box 6091, Syria,
E-mail: ascientific@aec.org.sy

acid in olive oil can be used as a way to detect its adulteration with other vegetable oils, which have a higher content of linoleic and linolenic acids amount of oleic acid in comparison to olive oil [12]. Furthermore UV and fluorescence spectroscopy has been intensively used to study the origin and contents of olive and vegetable oils [10]. EEC Regulation 2568/91 (1991) outlines the methods for measuring olive oil purity, using a UV spectrophotometric technique [27].

The detection of the type and the quantitive contents of the adulterant is not a simple task. Actually, no rapid and universal method exists that is officially recognized for all the authenticity issues [5]. Consequently, fats and accurate characterization of pure olive oil and commercial mixtures represents an important challenge [13]. The aim of this study is to develop a simple, direct, and reliable UV-spectrophotometric procedure for identification and quantification of the adulteration of pure Syrian kaissy olive oil (SKOO) with sunflower oil (SO).

2. Materials and methods

Altogether 9 olive oil samples produced at 5 different production seasons, namely, 2007 (2 samples), 2008 (3 samples), 2009 (2 sample), 2015 (1 sample), and 2016 (1 sample). Olive oil was obtained from olive fruits of Kaissy cultivar from the orchards located at Deer Al Hajar research station, southeast Damascus, Syria (33o 21´ N, 36o 28´ E) at 617 m above sea level, under conventional agriculture practices. The oil was extracted at the shortest time possible using mechanical and physical processes [9]. Olive fruits were crushed with hummer crusher and slowly mixed for about 30 min at 27 oC, Then, the past mixed was centrifuged at 3000 rpm for 3 min without addition of water to extract the oil. Olive oils were extracted and stored at our lab.

Finally, the oils were decanted and immediately transferred into dark glass bottles and stored at room temperature. Sunflower oil was purchased from local super market. Adulterated samples of pure SKOO of all harvest seasons were mixed with sunflower oil at different blending ratios (2:98, 4:96, 6:94, 8:92, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10). All measurements were made in triplicate in 2017.

2.1. UV-visible analysis

Absorbance of UV was used to detect abnormal oxidation compounds in olive, sunflower and mixtures oil according to norms established by IOC [2]. K232 and K270 were calculated from absorption at 232 and 270 nm. ΔK was calculated from 266, 268 and 274 nm absorptions, while R value was calculated as K232 divided by K270 with T70 UV-Visible spectrophotometer (PG Instruments Limited, England) using a 1% solution of oil in cyclohexane and a path length of 1 cm. 250 mg of oil was dissolved in 25 mL of cyclohexae and the extinction of the solution is then determined at the specified wavelengths with reference to pure solvent. Specific extinction are calculated from the spectrophotometer reading (UVWin5.0 program) using quartz cell with 1 cm optical path.

2.2. Statistical analysis

The data was managed in a Microsoft Excel worksheet. Mean and standard deviation of all UV-visible parametric variables were calculated. Analyses were performed by using SPSS for windows (Version 17.0.1, 2001, SPSS Inc., Chicago, USA). A p value of <0.05 was considered significant. Multiple regression analysis was performed to detect the relation between the variables; the coefficients of determination (R2) for each regression model were calculated.

3. Results and discussions

3.1. K232 and K270 extinction coefficients

The conjugated diene content (K232 value), the conjugated triene content (K270 value), ΔK value and R value (K232/K270) of the SKOO samples was determined for adulterant 15 mixtures samples with sunflower oil SO with percentage 0, 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%. Table 1 shows the means and standard deviations of 9 olive oil samples in three replicates for K232, K270, ΔK and R parameters of SKOO mixtures with SO.

The K232 (2.31), K270 (0.38), and ΔK (0.01) values of genuine SKOO, which are lower than the 2.5, 0.22 and 0.01, the maximum values established by the national and international regulations for extra virgin olive oil [27]. These results are in agreement with the results of Hashempour et al. (2010) [14] on Zard", "Coratina", Frangivento", "Beledy" and Arbequina" grown in Iran, that reporting the values of extinction coefficients were (K232 <2.5; K270 <0.22).

Also, these results are in agreement with those of other authors [1, 7]. Based on the above results, analyzed of all samples of olive cultivars grown in Tunisia and Palestine showed very low values of the evaluated parameters (K232 <2.5; K270 <0.22).

Table 1 shows that the pure sunflower oil have higher value of K232 (5.18), K270 (2.86), and ΔK (0.36) and lower R (1.81) than that of genuine SKOO. This makes it easy to judge if there is an adulteration of olive oil or not; since high absorbance at this wave length (K232, K270 and ΔK values) and low value of R means that olive oil is subjected to adulteration of sunflower oil of the examined oils [4].

The UV spectrophotometric study provides information about the degree of olive oil oxidation and therefore its quality [11].

The absorbency at 232 nm is caused by hydroperoxides (primary stage of oxidation) and conjugated dinenes (intermediate stage of oxidation). The absorbency at 268 nm is caused by carbonylic compounds (secondary stage of oxidation) and conjugated trinenes (technological treatments) [4, 8].

When the oil is treated with a decolorizing agent (i.e. an absorbent earth) during refining process, conjugated trienin 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 270 nm [4].

Oxidation can cause double bond position shifts in polyunsaturated fatty acids. When linoleic acid is oxidized to form hydroperxides, a shift in one of the double bonds occurs producing a a conjugated diene that can be measured by UV absorbance at 232 nm [21].

Fraudulent olive oil admixtures are usually chemically corrected to meet international standards, thus requiring more complex analysis to be recognized as adulteration. Nevertheless, even when official analytical methods are applied to screen olive oil samples, olives, biological differences, due to geographical origin and genetic aspects, sometimes generate problems distinguish between sophistications and authentic extra virgin olive oils [26].

Table 1. Mean values of specific extinction coefficient parameters measured on the UV spectrometer for adulterated 9 Syrian kaissy olive oil sample.

Adulteration percentage %	**K value**			
	K232	**K270**	**ΔK**	**R**
0	2.31±0.20	0.38±0.17	0.01±0.01	7.36±3.58
2	2.34±0.21	0.43±0.18	0.02±0.01	6.52±2.88
4	2.35±0.19	0.47±0.18	0.03±0.01	5.80±2.17
6	2.54±0.55	0.54±0.19	0.03±0.01	5.12±1.58
8	2.40±0.16	0.54±0.18	0.04±0.01	4.83±1.22
10	2.40±0.19	0.56±0.21	0.04±0.02	4.74±1.39
20	2.60±0.43	0.84±0.21	0.08±0.01	3.21±0.60
30	3.28±0.86	1.18±0.28	0.12±0.03	2.80±0.43
40	3.54±0.66	1.34±0.21	0.15±0.02	2.64±0.16
50	3.89±0.48	1.58±0.19	0.18±0.02	2.47±0.25
60	3.99±0.58	1.79±0.24	0.22±0.03	2.24±0.18
70	4.37±0.49	2.11±0.19	0.26±0.01	2.07±0.11
80	4.61±0.40	2.32±0.23	0.29±0.02	1.99±0.13
90	4.84±0.39	2.55±0.16	0.33±0.02	1.90±0.07
100	5.18±0.31	2.86±0.08	0.36±0.01	1.81±0.07
P-Value	**	**	**	**

** Significant at p<0.01.

3.2. Linearity/curvilinearity of the UV absorbance to-storage time (years) relationship

In the present study, detection of adulteration of genuine Syrian Kaissy Olive Oil (SKOO) with SO up to the concentration of 90% was investigated by measuring the characteristics of the absorption bands at 232, 266, 278, 270 and 274 nm.

Figures, 1, 2 and 3 shows the change in K232, K270 and ΔK values specific extinction coefficient at different percentage of adulteration. The K232, K270 and ΔK values for salad mixtures it increased. Based on the results, parameters for detecting adulteration were examined. Note that, the increase in the adulteration percentage increased the K232, K270 and ΔK values the

values of K232, K270 and ΔK values in SKOO mixtures with SO are increased with the presence of adulterants in SKOO. From the results presented in Figures, 1, 2 and 3, it could be concluded that the analysis of K232, K270 and ΔK value does provide satisfactory results and could be used as a basis for detecting the adulteration. UV spectroscopy is used to identify oils which are old, adulterated or which have been refined [4, 17]. The extinction coefficients method used as a supplementary help used in detection of the adulterations and classification olive oils. The greater the value of K232, the greater the concentration of conjugated dienes, whereas K270 is proportional to the concentration of conjugated trienes [8]. The quality of the olive oil is studied by measuring the characteristics of the absorption bands between 200 and 300 nm. These are frequencies related to conjugated diene and trien systems. 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 absorption in the region [4,6].

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 absorption in this region [5]. The significant correlation between K232 and peroxide is inspected as both parameters reflect primary oxidation products of the oil and therefore positive correlation was observed and was previously reported [23]. The relationship between the changes in the conjugated diene (K232), triene (K270) and K value with percentage of adulterations were studied by regression analysis. The concentration of the adulterant of sunflower oil in olive oil showed a positive correlation with K232 (R2 = 0.9886; p< 0.01) (Figure 1), K270 (R2 = 0.9984; p< 0.01) (Figure 2) and ΔK (R2 = 0.9992; p< 0.01) (Figure 3).

In a better multiple linear regression model, a K232, K270 and ΔK values prediction equation for SKOO were proposed:

Sunflower oil (%) = 33.372 X K232 value – 74.665 (s.e.e. 10.83 %, R2 = 0.9886) **[1]**

Sunflower oil (%) = 40.677 X K270 value – 14.853 (s.e.e. 6.56 %, R2 = 0.9984) **[2]**

Sunflower oil (%) = 284.92 X ΔK value – 3.0285 (s.e.e. 5.19 %, R2 = 0.9993) **[3]**

At K232 specific extinction coefficient value of 2.5, using equation [1], the predicated SO%, were 8.77%. At K270 specific extinction coefficient value of 0.22, using equation [2], the predicated SO%, were -5.90%. At ΔK value of 0.01, using equation [3], the predicated SO%, were -0.18%.

Very few studies of olive oil adulteration have been conducted with Middle East Arab regions, and adulteration percentage equations using Ultra-visible spectroscopy method dimensions in this production area have not previously been published. This is the first study examining the relationship between K232, K270 and ΔK values and adulteration percentage of SKOO.

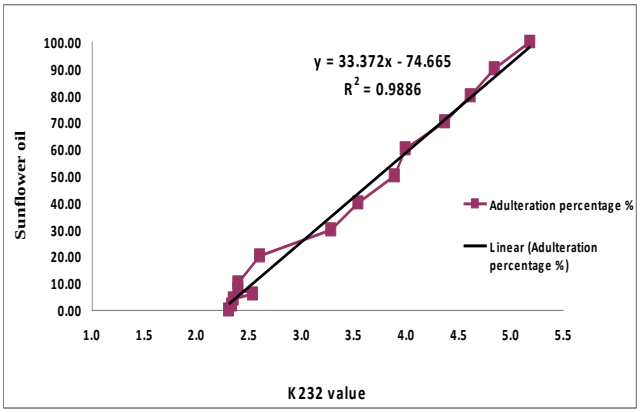

Figure 1. Linear dependence between the concentration of the adulterant and the specific extinction coefficients UV at 232 nm (K232) of Syrian kaissy olive oil.

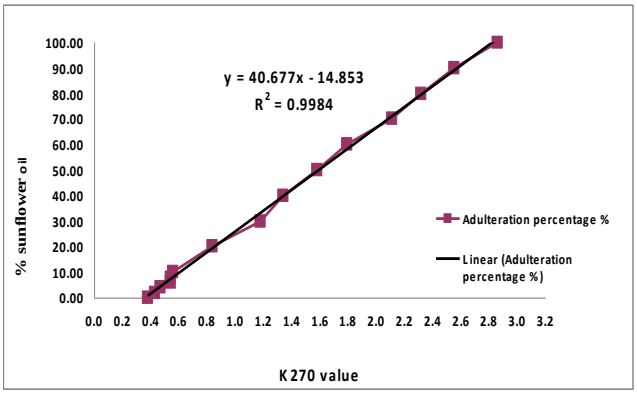

Figure 2. Linear dependence between the concentration of the adulterant and the specific extinction coefficients UV at 270 nm (K270) of Syrian kaissy olive oil.

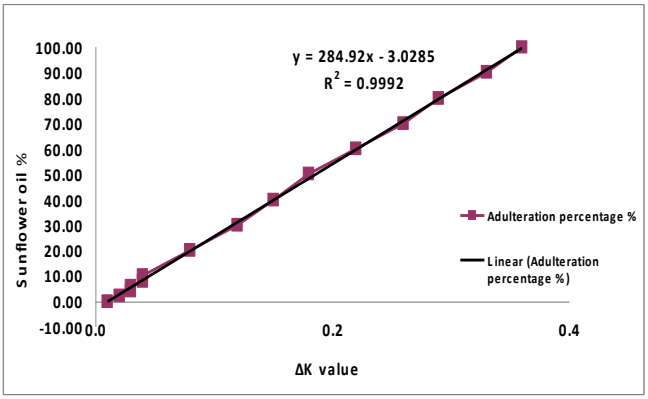

Figure 3. Linear dependence between the concentration of the adulterant and the specific extinction coefficients UV of delta-K value of Syrian kaissy olive oil.

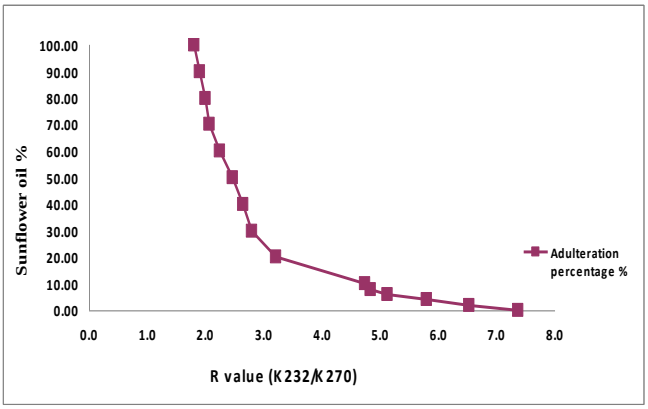

Figure 4. Exponential dependence between the concentration of the adulterant and the specific extinction coefficients UV of R value (K232/K270) of Syrian kaissy olive oil.

Therefore, the present study provides the most recent adulteration estimates by Ultra-visible spectroscopy method of SKOO. We developed equation to predict percent of adulteration from K232, K270 and ΔK values, because we were interested in developing a prediction equation for this measure of SKOO adulteration as an outcome for pathways study.

The strongly positive correlation found between K232, K270 and ΔK values and total percentage adulteration in SKOO suggested that Ultra-visible spectroscopy method could be one useful indicator for defining adulteration problem. Future research should assess the validity of Ultra-visible spectroscopy analysis among more culturally diverse samples.

4. Conclusion

Comparative analysis shows that pure olive oils exhibit a lower K232, K270 and ΔK values and a higher R value compared to the adulterated.

These changes could be associated with the presence of adulterants in salad olive oils. These preliminary results suggests that the UV-spectrophotometric indexes are correlated and could be useful for a fast detection of adulteration and discriminating among olive oils of different commercial categories. Regression equation can be derived from the output, to determine the intermediate percentage of adulteration.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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