



## A Rapid Method for Detection of Refined Olive Oil as Adulterant in Extra Virgin Olive Oil by Differential Scanning Calorimetry

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<http://dx.doi.org/10.13005/ojc/310354>

(Received: April 04, 2015; Accepted: June 19, 2015)

### ABSTRACT

Extra virgin olive oil (EVOO) is expensive oil, due to unique specification and its high quality among other edible oils; it might be mixed with other oils. The aim of this research was to identify the added refined olive oil (ROO) to extra virgin olive oil. One sample of each oil was analyzed, admixtures of EVOO: ROO were prepared at different ratios (50:50, 60:40, 70:30, 80:20, 90:10 and 95:5 w/w). To identify this adulteration, exothermic and endothermic thermograms were investigated. The results indicated that by increasing refined olive oil, onset temperature ( $T_{on}$ ) was increased from -61.08 to -56.16 °C; also, offset temperature ( $T_{off}$ ) decreased from 19.09 to 12.57 °C. Also results showed that when the ROO ratio increased then the enthalpy average values of both exothermic and endothermic curves were declined. Chemical tests were performed in parallel to better understanding these changes in curves.

**Key words:** DSC; Extra virgin olive oil; Refined olive oil; Adulteration; Thermograms.

### INTRODUCTION

Extra virgin olive oil is a product containing high nutritional quality and has great benefits for consumers. Refined olive oil is the most valuable oil because has the potential effects on human health. So, these two types of oils are categorized as functional food oils<sup>1</sup>. It also has excellent organoleptic characteristics (Aroma & Taste) and antioxidant properties<sup>2</sup>. According to trade standard of international olive oil council,

olive oil has the highest oleic acid content after canola oil<sup>3</sup>. Olive oil has a lot of tocopherols like *Capparis spinosal* oil seeds<sup>4</sup>. Extra virgin olive oil is a type of oil which is an extracted from fruits of olive trees using mechanical methods or other tools and instruments. Using thermal actions or any extra process such as decanting or centrifuge that changes extra virgin olive oil specification is absolutely prohibited, extra virgin olive oil is rich in natural antioxidant such as phenols, tocopherols and oleic acid. This fatty acid is more resistant than

other poly unsaturated fatty acids toward free radical at oxidation process<sup>5</sup>.

Great amount of demand for extra virgin oil in the world market and also high price of it made profit-seekers to mix low quality oils with extra virgin oil to reach get benefits. Recently Mediterranean and European passed common legislation to support consumers and producers against fraud<sup>6</sup>.

Various methods are used to detect fraud at extra virgin oil such as Ultraviolet and Visible Spectroscopy (UV/Vis)<sup>7</sup>, Gas Chromatography (GC-MS)<sup>8</sup>, Liquid chromatography, electrothermal atomic absorption spectrometry<sup>9</sup> and wet methods<sup>10</sup>, Nuclear Magnetic Resonance Spectroscopy<sup>11</sup>.

Thermal Analytical techniques such as DSC can also be used to determine melting profile characteristics and crystallization like heat of translation, phase diagram, fatty material contain and fraud identify in fat and butter<sup>12</sup>.

This technique can provide suitable and precise data from fatty acid profiles and triacylglycerol (TAG) at oils and fats<sup>13</sup>. Mainly the DSC instrument can be used for food analysis at quality control and research laboratory. The DSC used mostly for comparing materials at heating but cooling material's studies showed more useful data if thermo dynamically condition is under control. Most methods for fraud detection as mentioned above are expensive and time consuming and could have harmful effect on environment. The DSC instrument have been used by some researchers for fraud detection in olive oil which mixed by other edible oils<sup>14</sup>.

The aim of this research was investigating DSC cooling and heating thermograms for detection, refined olive oil fraud in extra virgin olive oil.

## EXPERIMENTAL

### Sampling

EVOO and ROO were purchased from local market in Roodbar (Gilan province, Iran). One samples of each oil was analyzed. Admixtures of EVOO: ROO were prepared at different ratios

(50:50, 60:40, 70:30, 80:20, 90:10 and 95:5 w/w). Samples were stored in dark bottles without headspace at Room temperature before analysis. All the chemicals and solvents used were of analytical grades purchased from Merck chemical company (Darmstadt, Germany).

### Standard chemical analyses

Peroxide (PV), anisidine (AV) and totox (2PV+ AV) values of the samples were determined according to AOCS method<sup>15</sup>.

### Fatty acid composition by gas chromatography (GC)

Gas liquid chromatography was used to determine the component fatty acids. Fatty acids were converted into the methyl esters using transesterification methods of oils with sodium methoxide as catalyst according to AOCS method<sup>16</sup>. Agilent-technologies 6890 N gas chromatograph equipped with a flame ionization detector and a capillary column (120m X25 mm ID-BPX 700. 250) (USA). Capillary column with temperature programming was used to determine the fatty acid composition according to AOCS method<sup>17</sup>. Helium was used as the carrier gas.

### DSC analysis

Samples of oil (8–10 mg) were weighed into aluminum pans; covers were sealed into place and analyzed with a Setaram instrumentation differential scanning calorimeter, 69300 caluire (France). Indium (melting temperature 156.6 p C,  $H_f = 28.45$  J/g) and n-dodecane (melting temperature - 9.65 p C,  $H_f = 216.73$  J/g) were used to calibrate the instrument and an empty pan was used as reference. Oil samples were equilibrated at 30 p C for 3 min and then cooled at -80 p C at the rate of 2 p C/min, equilibrated at -80 p C for 3 min and then heated from -80 p C to 30 p C at 2 p C/min. Dry nitrogen was purged in the DSC cell at 50 cm<sup>3</sup>/min. Thermograms were analyzed with Universal Analysis Software (Version 3.9A, TA Instruments) to obtain enthalpy ( $H$ , J/g),  $T_{on}$  (p C) and  $T_{off}$  (p C) of the transitions (intersection of baseline and tangent at the transition) and peak height ( $H_p$  W/g). Range of the transitions was calculated as temperature difference between  $T_{on}$  and  $T_{off}$  and at least triplicate analyses were carried out per sample. Statistical analysis was performed using SPSS 19 software, paired t-test model.

## RESULT AND DISCUSSION

Differences between samples were significant ( $p < 0.05$ ) for all samples. Standard deviations and mean values of  $T_{on}$ ,  $T_{off}$  and enthalpy in admixture refined olive oil and extra virgin olive oil, were tabulated in table 1. The results showed that by increasing refined olive oil  $T_{on}$  increased, and  $T_{off}$  decreased; in addition enthalpy in exothermic were tabulated in table 1 and endothermic processes decreased. Peroxide value and anisidine value were tabulated in table 1 and by increasing refined olive oil changed. Peroxide

value decreased and anisidine value increased.

Once, the ratio of ROO was increased, the amount of oleic acid (C18:1) increased, and the amount of linoleic acid (C18:2) was decreased, too.

The results of an investigation showed that the major peak was previously associated with the crystallization of highly unsaturated TAG, while the minor one was attributed to the crystallization of more saturated TAG fractions of EVOO, These results coincides with those reported by former studies. In an investigation Tan *et al.*, (2000) are revealed a

**Table 1: DSC data obtained from DSC thermograms of EVOO, ROO and their admixtures<sup>A</sup>**

Treatments	$T_{on}$ ( $T^{\circ}C \pm SD^b$ )	$T_{off}$ ( $T^{\circ}C \pm SD$ )	"T" ( $T^{\circ}C \pm SD$ )	Enthalpy <sub>Exo</sub> (Enthalpy J/kg $\pm SD$ )	Enthalpy <sub>Endo</sub> (Enthalpy J/kg $\pm SD$ )
100%EVOO	-61.80 $\pm$ 0.01 <sup>a</sup>	19.09 $\pm$ 0.11 <sup>a</sup>	80.8 9 $\pm$ 0.10 <sup>a</sup>	10.60 $\pm$ 0.03 <sup>g</sup>	32.53 $\pm$ 0.08 <sup>h</sup>
5% ROO	-61.39 $\pm$ 0.03 <sup>f</sup>	17.10 $\pm$ 0.07 <sup>b</sup>	78.49 $\pm$ 0.04 <sup>g</sup>	10.37 $\pm$ 0.08 <sup>f</sup>	26.97 $\pm$ 0. 12 <sup>g</sup>
10% ROO	-61.23 $\pm$ 0.06 <sup>e</sup>	16.95 $\pm$ 0.06 <sup>bc</sup>	60.15 $\pm$ 0.00 <sup>f</sup>	10.16 $\pm$ 0.03 <sup>e</sup>	26.15 $\pm$ 0.03 <sup>e</sup>
20% ROO	-59.05 $\pm$ 0.01 <sup>d</sup>	16.77 $\pm$ 0.12 <sup>c</sup>	78.18 $\pm$ 0.11 <sup>e</sup>	9.89 $\pm$ 0.03 <sup>d</sup>	25.80 $\pm$ 0.03 <sup>f</sup>
30%ROO	-58.61 $\pm$ 0.07 <sup>c</sup>	16.53 $\pm$ 0.15 <sup>d</sup>	75.14 $\pm$ 0.08 <sup>d</sup>	9.89 $\pm$ 0.13 <sup>d</sup>	24.69 $\pm$ 0.04 <sup>e</sup>
40% ROO	-58.52 $\pm$ 0.01 <sup>c</sup>	15.03 $\pm$ 0.05 <sup>e</sup>	73.55 $\pm$ 0.04 <sup>c</sup>	9.48 $\pm$ 0.03 <sup>b</sup>	22.92 $\pm$ 0.02 <sup>d</sup>
50% ROO	-56.34 $\pm$ 0.03 <sup>b</sup>	14.91 $\pm$ 0.09 <sup>e</sup>	71.25 $\pm$ 0.06 <sup>b</sup>	9.27 $\pm$ 0.05 <sup>a</sup>	20.30 $\pm$ 0.11 <sup>b</sup>
100% ROO	-56.15 $\pm$ 0.00 <sup>g</sup>	12.57 $\pm$ 0.06 <sup>f</sup>	68.72 $\pm$ 2.06 <sup>h</sup>	9.72 $\pm$ 0.01 <sup>c</sup>	16.04 $\pm$ 0.04 <sup>a</sup>

$\Delta T$ , Temperature difference between  $T_{on}$  and  $T_{off}$ .

<sup>A</sup> Same letters within each column do not significantly differ ( $n = 3$ ,  $p < 0.05$ ).

Abbreviations: EVOO, Extra virgin olive oil; ROO, Refined olive oil; Exo, Exothermic; Endo, Endothermic

**Table 2: Fatty acids composition of extra virgin olive oil, refined olive oil and their admixture**

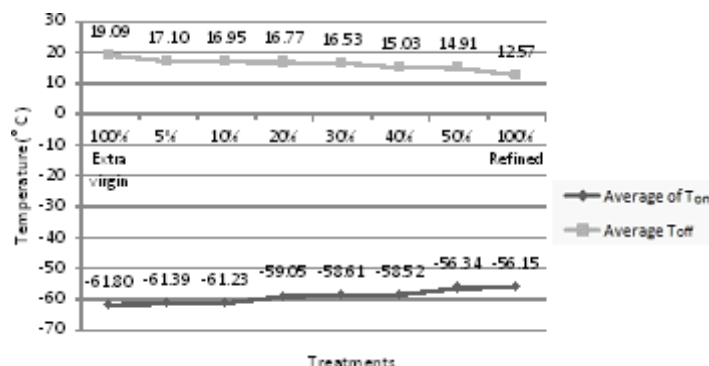
FA	100% EVOO	5% ROO	10% ROO	20% ROO	30% ROO	40% ROO	50% ROO	100% ROO
C <sub>14</sub> :0	0.01	0.01	0.04	0.015	0	0	0.05	0.02
C <sub>16</sub> :0	15.13	14.9	14.43	14.46	13.23	13.72	13.42	12.25
C <sub>16</sub> :1	0.92	1	0.84	0.99	0.67	1.27	0.77	0.92
C <sub>18</sub> :0	2.3	2.4	2.08	2.44	2.3	1.93	2.28	2.8
C <sub>18</sub> :1	62.04	63.79	64.19	64.35	68.47	68.49	69.39	69.9
C <sub>18</sub> :2	13.42	12.71	12.87	12.31	11.1	11.7	11.17	9.4
C <sub>18</sub> :3	1.05	1.08	1.08	1.09	1.4	1.47	1.52	1.63
others	5.13	4.11	3.47	4.34	3.19	3.42	3.4	3.58
PV	11.9	11.8	11.6	10.8	9.12	8.8	7.6	7
AV	7.71	7.78	7.8	7.84	8.63	8.88	8.9	9.04
Totox	31.51	31.38	31	29.44	26.87	26.48	24.1	23.04

Abbreviations: EVOO, extra virgin olive oil; ROO, Refined olive oil

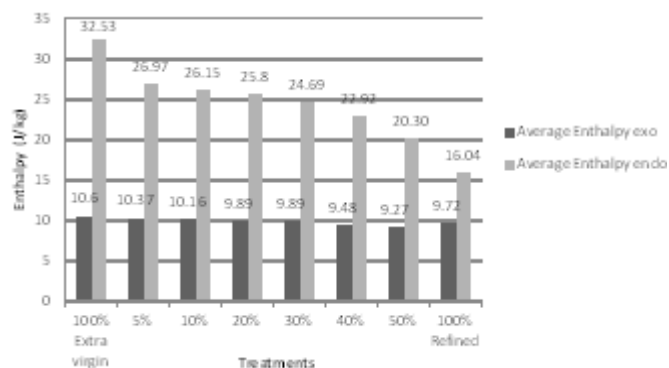
Abbreviations: PV, peroxide value (meq/ kg oil); AV, Anisidine value

more ordered crystal structure, having TAG chains more compactly associated, could be formed as a result of ROO addition to EVOO, as reflected by the higher crystallization enthalpy. The shift of crystallization toward lower temperatures may be due to the addition of more unsaturated lipid fractions of ROO in EVOO, since lower crystallization

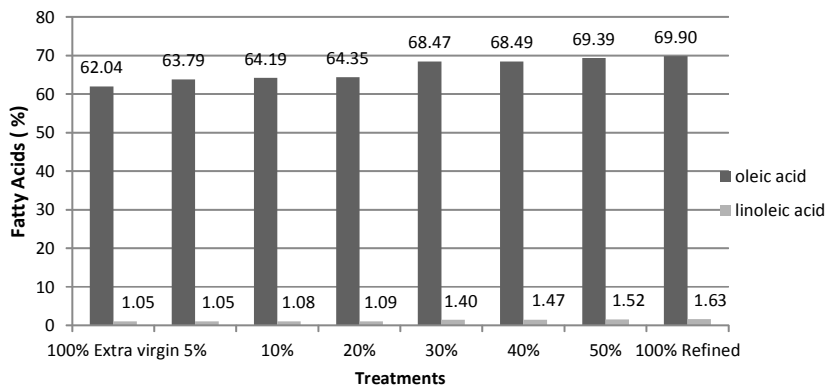
temperature onset and significantly narrowed transition range were previously reported for oils with higher degree of unsaturation. In a research conducted by C.P Tan *et al.*,(1999) , Changes in the sample that are associated with the absorption or evolution of heat cause a change in the different heat flow, which is then recorded as a peak. Naturally



**Fig. 1: Comparison between average of  $T_{on}$  and  $T_{off}$  (°C) in admixtures refined olive oil and extra virgin olive oil**



**Fig. 2: Comparison between average of enthalpy<sub>exo</sub> and enthalpy<sub>endo</sub> (J/kg) in admixtures refined olive oil and extra virgin olive oil**



**Fig. 3: Comparison between means of oleic and linoleic acids in refined olive oil, extra virgin olive oil and their admixtures**

occurring fats and oils, such as edible oil composed of a wide variety of triacylglycerols, melt/crystallize over a wide range of temperatures. Thermal properties of edible oils are closely related to those of triacylglycerols<sup>18</sup>.

### CONCLUSION

In this research, by adding more refined olive oil to extra virgin olive oil, DSC factors were altered and based on these changes by adding more amounts of refined olive oil to extra virgin,  $T_{on}$  increased and  $T_{off}$  decreased. This matter is subject to changes in fatty acids, since by adding more refined olive oil, the amount of oleic acid, which is a monounsaturated fatty acid was increased, and the amount of linoleic acid, which is polyunsaturated fatty acid, decreased.  $T_{on}$  relates to melting

monounsaturated triacylglycerols and  $T_{off}$  relates to crystallization of polyunsaturated triacylglycerols. Furthermore, since proxides are primary products and unstable, which could be converted to secondary products like aldehyde and ketones, therefore in this case the proxide value was decreased and anisidine value increased. Moreover, since the refining process was accomplished in vacuum and high temperature, the volatile peroxide could be easily removed; consequently the peroxide value in refined olive oil is much lower than extra virgin olive oil.

### ACKNOWLEDGMENTS

The authors are so grateful to the Laboratory Complex of I.A.U., science and research branch for valuable technical assistance.

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