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## Evolution of Lipid Oxidation in Syrian Olive Oil under Irradiation and Storage

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#### Authors' contributions

This work was carried out in collaboration between both authors. Authors MAB and AK designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

#### Article Information

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**Original Research Article** 

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#### ABSTRACT

**Aims:** The current study examines variations in Syrian olive oil (SOO) after gamma irradiation treatment and during storage.

**Study Design:** Four irradiation treatments (0, 1, 2 and 3 kGy) were distributed in a completely randomized design with three replicates.

**Place and Duration of Study:** The study was conducted during 2009/2010 growing season of Kaissy olive fruits produced at Deer Al Hajar research station, Damascus, Syria.

**Methodology:** Measurements of selected chemical values involving, acidity value (AV), peroxide value (PV), Thiobarbituric acid (TBA) malondialdehyde value (MDA), iodine value (IV) and saponification (specification) value (SV) and selected physical values namely refractive index (RI) and viscosity were carried out using standard methods.

**Results:** Results revealed that the levels of the analyzed parameters was within the limits established by codex standard for the highest quality category "extra virgin" olive oils (EVOO). Gamma irradiation statistically (P <0.01) influenced AV, PV, IV, SV and viscosity of olive oils. AV,

OV and MDA increased (P <0.01), while SV deceased (P <0.01) by irradiation. The lowest dose gave highest and the highest dose gives the lowest values of AV and PV. Also storage time had a significant influence on all analyzed parameters. AV and PV increased during storage time, and a maximum increase in AV (1.01%) was observed in olive oil that was treated with lowest irradiation dose.

**Conclusion:** Both irradiation treatment and storage time had a significant (P <0.01) effect on most of the measured chemical and physical parameters (AV, PV, MDA value, IV, SV, RI and viscosity) of EVOO.

Keywords: Extra virgin olive oil; Syria; Kaissy cultivar; gamma irradiation.

#### **1. INTRODUCTION**

Olive oil, extracted from Olea europaea tree belonging to family Oleaceae is an important foodstuff constituting a major part of daily food regimes [1]. Extra virgin olive oil (EVOO) and virgin olive oil (VOO) refer to oils that are extracted merely by pressure and other mechanical processes and no other treatments [2]. EVOO and VOO are unique among cooking oils because of their high monounsaturated fatty acid (MUFA) content and the presence of healthpromoting micro-constituents, namely polyphenols, terpenoids, squalene and tocopherols as well as volatile compounds [3,4,5]. In 2011, the European Food Safety Authority (EFSA) published a scientific opinion suggesting that VOO could be considered as a beneficial health agent based on the numerous scientific publications that underline its biological impact [6,7]. EVOO and VOO quality is affected by several factors, such as agronomical practices and processing technology, seasonal conditions, sanitary state of drupes, ripening stage, harvesting and carriage systems, methods and duration of storage, processing technology, cultivar and growing medium [8,9,10].

Treatment of food by specific ionizing radiations to improve microbiological safety and storability is one of the most extensively studied technologies of the 20th century [11]. Gamma irradiation as a phytosanitary treatment of food materials is increasingly recognized throughout the world. It improves the hygienic quality of various foods materials and reduces the losses due to microbial contamination and insect damage [12]. Usually ionizing irradiation used for shelf-life extension of fruit (olive fruits before extraction the oil), fore disinfestations of fruit, since insect can affect the produced quality of produced oils, or for decontamination of the final products (oil for example) [13,14].

However, one of the major concerns with any processing of fatty products, including radiation

processing, is that these processes enhance lipid peroxidation and thereby affect sensory attributes [15]. The susceptibility of irradiated fatty product to oxidative rancidity is related to the nature, proportion, degree of saturation in fatty acids and composition of phospholipids in cell membranes [16,17,18]. Olive oil is a highly valuable agricultural commodity for Syria. However, no data is available on the effect of irradiation on the overall quality of olive oil produced in Syria and other countries [13,14]. Our objective was therefore to evaluate the effect of gamma irradiation processing on the physicchemical properties of olive oils produced from the local "Syrian" olive cultivar, "Kaissy".

#### 2. MATERIALS AND METHODS

#### 2.1 Production of Olive Oil

Kaissy is the most widespread olive cultivar in Syria and thus was used in this study. Mature and good quality olive fruits were harvested during 2009/2010 growing season at Deer Al Hajar research station, Southeast Damascus, Syria (33° 21' N, 36° 28' E) at 617 m above sea level, under conventional agriculture practices. The climate of the area is sub-Mediterranean with an average annual temperature between 19 and 36°C in July- August and a minimum of 3°C in January- February. Annual rainfall varies from 100 to 150 mm with most of it falling in winter. The supplementary water irrigation was managed to supply 2000 m<sup>3</sup> he<sup>-1</sup> of water per tree over the period from mid-June to mid-September. The oil was extracted at the shortest time possible using a mechanical and physical processes [19]. The physical and chemical properties of Kaissy olive oil are within the limits established by the International Olive Council (IOC) [14]. Olive fruits were crushed with a laboratory hummer crusher and slowlv homogenized for about 30 min at 27°C, before it was centrifuged at 3000 rpm for 3 min without water addition. Finally, oils were decanted and

immediately transferred into dark glass bottles and stored at room temperature for the irradiation treatment and physico-chemical analysis. Each bottle of olive oils (500 g) was considered as a replicate. Olive oil samples were then divided into four groups: group 1 (control) and groups 2, 3 and 4 were irradiated with 1, 2 and 3 kGy of gamma irradiation, respectively.

#### 2.2 Irradiation Treatment

Samples from olive oil (OO) were exposed to gamma radiation at doses of 0, 1, 2 and 3 kGy in a 60CO package irradiator (ROBO, Techsnabexport, Moscow, Russia). Irradiation was carried out in the stationary mode of operation with the possibility of varying dose rate (10.846 to 3.921 kGy h<sup>-1</sup>) depending on the location and the distance from the source (10 to 40 cm). Samples were irradiated at a place, 15 cm from the source with a dose rate of 9.913 kGy/h at room temperature (20 - 25°C) and atmospheric pressure for 6, 12 and 18 min. The absorbed dose was determined using alcoholic chlorobenzene dosimeter [15]. The control and irradiated samples were stored at room temperature (20 – 25°C). Physical and chemical properties of irradiated and non-irradiated samples of oils were performed immediately after irradiation and after 6, 12, 24 and 36 months of storage.

#### 2.3 Chemical and Physical Analysis of Oils

Acidity value (AV, oleic acid %), peroxide value (PV, meq  $O_2$  kg<sup>-1</sup> oil), iodine value (IV, g  $I_2$  100g<sup>-1</sup> oil), and saponification value (SV, mg KOH  $g^{-1}$ oil) were determined according to standard methods [20]. The acid value was obtained by a direct titration with (0.1 N) NaOH and phenolphthalein as an indicator. Ten grams of each sample were magnetically stirred in a total volume of 100 ml distilled water for 30 min was filtered. Ten ml of the filtrate was titrated with (0.1 N) NaOH using 3 drops of a phenolphthalein indicator. For determine lipid peroxidation olive oil sample of 1 q was placed in a 250 ml test flask and homogenized with 20 ml solution of acetic acid (50% acetic acid, 50% chloroform). The mixture was vortexed, incubated in a hot water bath at 50°C for 30 min, and the samples filtered. The filtrate was received into 0,5 ml of potassium iodine (50%), held in dark place for 2 min. Distilled water (100 ml) and 3 drops of starch 1% as an indicator were added, and the mixture was titrated by sodium thiosulfatepentahydrate (0.01 N), added drop wise until the end point. Thiobarbituric acid (TBA) number in term of mg malondialdehyde (MDA)/kg samples was measured according to IUPAC direct method [21], starting with 2 g of olive oil. The viscosity of oils was measured with HAAKE viscometer 6 R plus Model (RTM) using a R2 column at 200 rpm. Viscosity values were determined and expressed as mPa.s. The refractive index of olive oil samples was measured in daylight with an Abbe refractometer (VED Carl Zeiss JENA, German) calibrated against pure water at 25°C.

#### 2.4 Statistical Analysis

The experiment was designed to determine the effects of gamma irradiation on olive oil. All determinations were performed in triplicates. Data was subjected to the analysis of variance test (ANOVA) using the SUPERANOVA computer package (Abacus Concepts Inc, Berkeley, CA, USA; 1998). The P value of less than 0.05 was considered statistically significant.

#### 3. RESULTS AND DISCUSSION

The study was conducted to test the effect of gamma irradiation on the characteristics of olive oil extracted from the olive cultivar, Kaissy during storage. The adopted experimental design was planned to determine the rate of autoxidation of olive oil via irradiation and storage in an ambient temperature. The storage time of the experimental set-up covered the shelf-life of extra virgin or virgin olive oils, usually considered to be more than one year (up to 3 years).

#### 3.1 Effects of Gamma Irradiation and Storage Period on Acid Value of Olive Oil

Table 1 shows the averaged values obtained for AV indices of Syrian olive oil (SOO) and their respective standard deviations after irradiation and during the whole storage time. The initial AV of olive oils (0.53%) is below the maximum levels established by the international regulations [22]. The international olive council [23] has defined and classified the quality of olive oil based on AV as extra- virgin olive oil (max 0.8%), virgin olive oil (max 2.0) and ordinary virgin olive oil (max 3.3). Since the AV of the olive oil was lower than the established limits it could be classified as an extra- virgin olive oil. High AV indicates high free fatty acid which causes oil to become rancid [24].

It is clear that the AV (P < 0.01) changed significantly due to irradiation doses and storage times, however, it was always below the accepted limit for the extra virgin olive oil (Table 1). Following to gamma irradiation treatment, the significant increases in acid value in samples treated with lower doses of gamma irradiation (1 and 2 kGy) were confirmed. The data for AV indicated that the lowest dose gives the highest value and visa versa. This finding could be attributed to the stimulation effect of the lower dose that causes high lipolytic activity of the native lipase [1], while the higher dose causes less stimulation or inhibition effect. After storing the oil samples for 36 months, an increase in AVs of irradiated EVOOs was detected, although the increase in AV was less marked in the control sample comparing with irradiated ones. These results are in agreement with the study performed by Mendez and Falgue [22] who indicated an increase in the acidity of olive oil over time.

To our knowledge, there is no literature about the relationship between the acid value of EVOO and the gamma irradiation treatment. For this reason, it is not possible to compare on the effect of gamma irradiation on OO. Previous work by our group on this parameter in walnut and peanut also showed that the acidity values increased as irradiation dose and storage time increased [15. 18]. It is previously known that the free fatty acid content, resulting from the hydrolysis of triacylglycerol as well as further decomposition of hydroperoxides, is one of the most important indicators of oil deterioration during processing. Therefore, controlling the level of FFA within a reasonable range would prevent the breakdown of fats [25]. The increasing acidity in oil after irradiation may be attributed to the degradation of large lipid molecules producing smaller molecules including free fatty acids [15,18].

#### 3.2 Effect of Gamma Irradiation and Storage Period on Peroxide Value of Olive Oil

Peroxide values (PVs) of irradiated and nonirradiated control samples of extra virgin olive oils (EVOO) at different storage periods are presented in Table 1. The PV in control sample was 2.28 meq  $O_2 \text{ kg}^{-1}$  oil (Table 1). The PV was taken as a measure of primary oxidation compounds produced in the oxidation of olive oil samples. According to the national [26] and Codex Standard, the max allowable peroxide value for extra virgin, virgin and ordinary olive oils is 20 meq  $O_2 \text{ kg}^{-1}$  oil Codex Standard [27]. As shown in Table 1, gamma irradiation increased the level of peroxide values, which was significant (P < 0.01) in all irradiated samples. The highest increase in peroxide value was shown in oil irradiated with 1 kGy (6.54 meg  $O_2$  kg<sup>-1</sup> oil) and the lowest increase in oil irradiated with 3 kGy (3.89 meg  $O_2$  kg<sup>-1</sup> oil). Previously, an increase in the peroxide value was attributed to interaction of gamma radiation with fat molecules, which triggered oxidation, dehydration and polymerization reactions [28]. A study of peanuts irradiated to 7 kGy revealed approximately a five- fold increase in the peroxide value [29]. Exited ions produced as a result of irradiation could have lead to the formation of free radicals which might have resulted in an increased peroxide value in the oil of the irradiated samples [30]. Also, as shown in Table 1. the peroxide values of EVOO samples stored for 0, 6, 12, 24 and 36 months are under the max allowable limits. Generally, the PVs were observed to increase with storage. The higher value in PV is, however, not acceptable since this is a reflection on the deteriorating quality of olive oil [31].

#### 3.3 Effect of Gamma Irradiation and Storage Period on MDA Value of Olive Oil

Malondialdehyde value (MDA) of control sample of extra virgin olive oil (EVOO) was 0.0018 mg MAD kg<sup>-1</sup> oil (Table 1). Gamma irradiation significantly increased the level of MDA in all irradiated samples. The highest increase in MDA was shown in EVOO treated with 3 kGv (0.0027 mg MAD kg<sup>-1</sup> oil). In our experiment, statistical (P <0.05) interaction was found between MDA values and the storage period. After 36 months of storage, MDA values of EVOO treated with 0, 1, 2 and 3 kGy were 0.0062, 0.0039, 0.0035, and 0.0040 mg MAD kg<sup>-1</sup> oil, respectively. However, long storage increased MDAs value with time (P <0.05) reaching 0.0062 mg MAD/kg oil in control sample, a value that in olive oil not caused rancid notes [32]. MDA increased upon storage in accord with double bond shifts in fatty acids and production of aldehydes through decomposition of hydroperoxides formed during oxidation of oils [33]. In the modified formulation, after 36 months irradiation treatments MDAs did not even reach 0,5 µg/kg, that lower than the limit to detect rancidity (1 µg/kg) [34].

| Treatment                 | Control                      | 1 kGy                       | 2 kGy                        | 3 kGy                        |  |  |  |  |
|---------------------------|------------------------------|-----------------------------|------------------------------|------------------------------|--|--|--|--|
| Storage period/(Months)   |                              |                             |                              |                              |  |  |  |  |
| Acid value (% oleic acid) |                              |                             |                              |                              |  |  |  |  |
| 0                         | 0.53±0.03 <sup>cC</sup>      | 0.77±0.03 <sup>dA</sup>     | 0.58±0.04 <sup>bA</sup>      | 0.48±0.05 <sup>aA</sup>      |  |  |  |  |
| 6                         | 0.52±0.01 <sup>aC</sup>      | 0.81±0.09 <sup>cB</sup>     | 0.72±0.01 <sup>bB</sup>      | 0.53±0.01 <sup>aC</sup>      |  |  |  |  |
| 12                        | 0.43±0.01 <sup>aA</sup>      | 0.82±0.01 <sup>dB</sup>     | 0.70±0.004 <sup>cB</sup>     | 0.51±0.01 <sup>bB</sup>      |  |  |  |  |
| 24                        | 0.42±0.02 <sup>aA</sup>      | 0.85±0.06 <sup>dB</sup>     | 0.75±0.03 <sup>cC</sup>      | 0.59±0.01 <sup>bD</sup>      |  |  |  |  |
| 36                        | 0.50±0.01 <sup>aB</sup>      | 1.01±0.01 <sup>dC</sup>     | 0.78±0.02 <sup>cD</sup>      | 0.72±0.02 <sup>bE</sup>      |  |  |  |  |
| P-level                   | **                           | **                          | **                           | **                           |  |  |  |  |
|                           | P                            | eroxide value (meq          | O₂ kg <sup>-1</sup> oil)     |                              |  |  |  |  |
| 0                         | 2.3±0.6 <sup>aA</sup>        | 6.5±0.9 <sup>dC</sup>       | 4.8±0.1 <sup>cB</sup>        | 3.9±0.5 <sup>bA</sup>        |  |  |  |  |
| 6                         | 2.8±0.9 <sup>aA</sup>        | 4.1±0.1 <sup>cB</sup>       | 4.3±0.1 <sup>dB</sup>        | 3.9±0.1 <sup>bA</sup>        |  |  |  |  |
| 12                        | 3.4±0.1 <sup>bB</sup>        | 2.8±0.2 <sup>aA</sup>       | 2.9±0.2 <sup>aA</sup>        | 4.1±0.1 <sup>cA</sup>        |  |  |  |  |
| 24                        | 13.4±0.8 <sup>bC</sup>       | 13.1±1.0 <sup>bD</sup>      | 12.4±0.1 <sup>aC</sup>       | 12.5±0.4 <sup>aB</sup>       |  |  |  |  |
| 36                        | 14.9±0.3 <sup>bD</sup>       | 14.9±0.1 <sup>bE</sup>      | 14.4±0.2 <sup>aD</sup>       | 14.3±0.3 <sup>aC</sup>       |  |  |  |  |
| P-level                   | **                           | **                          | **                           | **                           |  |  |  |  |
| MDA value (mg MDA/kg oil) |                              |                             |                              |                              |  |  |  |  |
| 0                         | 0.0018±0.0001 <sup>aA</sup>  | 0.0026±0.0001 <sup>cB</sup> | 0.0023±0.0001 <sup>bC</sup>  | 0.0027±0.0001 <sup>cD</sup>  |  |  |  |  |
| 6                         | 0.0019±0.0001 <sup>aAB</sup> | 0.0021±0.0001 <sup>aA</sup> | 0.0021±0.0001 <sup>aAB</sup> | 0.0021±0.0001 <sup>aBC</sup> |  |  |  |  |
| 12                        | 0.0020±0.0001 <sup>aB</sup>  | 0.0020±0.0001 <sup>aA</sup> | 0.0020±0.0001 <sup>aA</sup>  | 0.0019±0.0001 <sup>aA</sup>  |  |  |  |  |
| 24                        | 0.0022±0.000a <sup>C</sup>   | 00021±0.0001 <sup>aA</sup>  | 0.0022±0.0001 <sup>aBC</sup> | 0.0022±0.000 <sup>aC</sup>   |  |  |  |  |
| 36                        | 0.0062±0.0001 <sup>cD</sup>  | 0.0039±0.000 <sup>bC</sup>  | 0.0035±0.000 <sup>aD</sup>   | 0.0040±0.0001 <sup>bE</sup>  |  |  |  |  |
| P-level                   | **                           | **                          | **                           | **                           |  |  |  |  |

| Table 1. Effects of gamma irradiation and storage period on acid, peroxide and TBA values of |  |  |  |  |  |  |
|--|--|--|--|--|--|--|
| olive oil  |  |  |  |  |  |  |

<sup>abc</sup> Means values in the same row not sharing a superscript are significantly different. <sup>ABC</sup> Means values in the same column not sharing a superscript are significantly different.

NS: not significant.

\* Significant at p<0.05; \*\* Significant at p<0.01

#### 3.4 Effect of Gamma Irradiation and Storage Period on Iodine Value of Olive Oil

The effect of gamma irradiation (0, 1, 2, and 3 kGy) and storage time (0, 6, 12, 24, and 36 months) on iodine value (IV) of extra virgin olive oil (EVOO) are illustrated in Table 2. The iodine value of non-irradiated control sample of EVOO was 89.68 g  $I_2$  100 g<sup>-1</sup> oil (Table 2). Iodine value can be used to demonstrate un-saturation that is characteristic of olive and many other vegetable oils. It has been revealed that a low iodine value is considered as an indicator of poor oil quality [35]. The results indicated that 1 and 3 kGy doses of gamma radiation have significant (P <0.01) effect on IV of EVOO. The higher IV (89.68 g  $I_2$  100 g<sup>-1</sup> oil) were acquired from the oil of the sample which is not exposed to irradiation; and relating to the irradiation dosages, these values decreased significantly (P < 0.01) in EVOO samples treated with 1 and 3 kGy, and finally the lowest values (84.48 g l<sub>2</sub> 100 g<sup>-1</sup> oil) recorded in sample treated with 1 kGy gamma irradiation. Similar findings were reported for the iodine value of sunflower and soybean oil [36] and pumpkin oil [37] which decreased significantly with gamma irradiation (from 1 to 20 kGy). The decrease in IV due to irradiation may be attributed to the saturation of the saturated fatty acids double bonds [15].

During the first year of storage (up to 12 months), there was a significant (P <0.01) decrease in the iodine value of control and irradiated EVOO. After that and during the second year of storage (up to 24 months), there was a significant increase (P <0.01) in the iodine value of control and irradiated EVOO. The IV of the analyzed oils after irradiation at 0, 1, 2 and 3 kGy and stored for 0, 6, 12 and months ranged from 81.21 to 92.89 g  $I_2$  100 g<sup>-1</sup> oil and did not exceed the standard limit of (75 – 94 g  $I_2$  100 g<sup>-1</sup> oil) indicating that these oils could be labeled as virgin olive oil quality [23].

#### 3.5 Effect of Gamma Irradiation and Storage Period on Saponification Value of Olive Oil

Saponification value (SV) was an indicatior of the molecular weight of triglycerides in oil and a high

proportion of SV in the used extra virgin olive oil (EVOO) (204.70  $\pm$  1.08 mg KOH g<sup>-1</sup> oil) (Table 2). Also, the high SV is an indication that the EVOO may be suitable for soap, shampoo and paints making [38]. Higher SV indicates high proportion of lower fatty acids since SV is inversely proportional to the average molecular weight on chain length of the fatty acids [39]. As shown in Table 2, SVs decreased (P < 0.01) from an initial value of 204.68 to a value of 202.15 and 199.61 mg KOH g<sup>-1</sup> oil after irradiation at a doses of 1 and 2 kGy, respectively. These results are in agreement with the results obtained by several investigators. Al-Bachir [18] reported that a marked decreased in SV was noticed for oil extracted from irradiated peanut seeds with 1, 2 and 3 kGy.

The SV of EVOO decreased upon storage, which indicated that large original molecules of oils containing long-chain fatty acids degraded to smaller molecules as a result of oxidation and cleavage of bonds [16].

#### 3.6 Effect of Gamma Irradiation and Storage Period on Refractive Index of Olive Oil

The refractive index (RI) of extra virgin olive oil (EVOO) is measured for all samples and mean values with standard deviations per samples are

shown in Table 3. RI value of EVOO was 1.4667 (Table 3). Tanilgan et al. [40] pointed out that refractive index value of varieties of oils ranged from 1.467-1.469. Refractive index values for oil were reported as 1.468-1.470 according to Karleskind and Wolff [41]. National and codex standard define the refractive index at 20°C for extra virgin, virgin and ordinary olive oils to range between 1.4677 and 1.4705 values [27].

There were no differences in the values of refractive index between irradiated and nonirradiated EVOO samples, indicating that gamma irradiation up to 3 kGy did not exert a significant negative effect on these parameters of the EVOO. Our results are in accordance with the previously reported findings of Bhatti et al. [42] who did not observe any significant change in refractive indices between the controls and irradiated almonds. During storage (after 6, 12, 24 and 36 months), the refractive indices values changed significantly (P < 0.01) in both irradiated and non-irradiated control samples of EVOO.

All samples in our study had refractive index values ranged between 1.4656 and 1.4686 at 25°C. It is quite expected a physical constant to be within the range, as long as there is no purity change of the sample. Our results, although with fewer differences, were similar to those of found in the literature [40,41].

| Treatment   | Control                   | 1 kGy                     | 2 kGy                      | 3 kGy                     |  |  |  |
|---|---------------------------|---------------------------|----------------------------|---------------------------|--|--|--|
| Storage period/(Months)                                 |                           |                           |                            |                           |  |  |  |
| Iodine value (g l <sub>2</sub> 100 g <sup>-1</sup> oil) |                           |                           |                            |                           |  |  |  |
| 0   | 89.68±1.11 <sup>bC</sup>  | 84.48±1.13 <sup>aB</sup>  | 90.40±1.80 <sup>bC</sup>   | 86.14±1.55 <sup>aC</sup>  |  |  |  |
| 6   | 85.92±0.91 <sup>abB</sup> | 84.38±0.95 <sup>abB</sup> | 86.51±1.55 <sup>bB</sup>   | 84.09±1.34 <sup>aB</sup>  |  |  |  |
| 12  | 81.22±0.63 <sup>aA</sup>  | 81.21±1.92 <sup>aA</sup>  | 82.52±1.70 <sup>aA</sup>   | 82.53±0.73 <sup>aA</sup>  |  |  |  |
| 24  | 89.64±1.31 <sup>aC</sup>  | 92.89±0.32 <sup>bC</sup>  | 91.01±1.56 <sup>abC</sup>  | 91.70±1.77 <sup>abD</sup> |  |  |  |
| 36  | 88.60±0.93 <sup>aC</sup>  | 85.03±0.08 <sup>bB</sup>  | 85.77±1.17 <sup>bB</sup>   | 86.29±0.00 <sup>bC</sup>  |  |  |  |
| P-level   | **                        | **                        | **                         | **                        |  |  |  |
| Saponification value (mg KOH g <sup>-1</sup> oil)       |                           |                           |                            |                           |  |  |  |
| 0   | 204.70±1.08 <sup>cC</sup> | 202.15±1.11 <sup>bE</sup> | 199.61±1.17 <sup>aD</sup>  | 204.70±1.14 <sup>cE</sup> |  |  |  |
| 6   | 199.40±0.95 <sup>aB</sup> | 199.65±0.98 <sup>aD</sup> | 199.95±1.05 <sup>aD</sup>  | 199.76±0.61 <sup>aD</sup> |  |  |  |
| 12  | 198.92±0.06 <sup>aB</sup> | 198.50±0.80 <sup>aC</sup> | 198.21±0.99 <sup>aC</sup>  | 198.59±0.55 <sup>aC</sup> |  |  |  |
| 24  | 196.13±0.10 <sup>aA</sup> | 195.27±1.15 <sup>aB</sup> | 196.18±0.61 <sup>ªB</sup>  | 195.70±0.13 <sup>aB</sup> |  |  |  |
| 36  | 195.91±0.12 <sup>bA</sup> | 193.97±0.08 <sup>aA</sup> | 194.35±0.70 <sup>abA</sup> | 193.89±1.74 <sup>aA</sup> |  |  |  |
| P-level   | **                        | **                        | **                         | **                        |  |  |  |

Table 2. Effects of gamma irradiation and storage period on iodine value, and saponificationvalue of olive oil

<sup>abc</sup> Means values in the same row not sharing a superscript are significantly different.

ABC Means values in the same column not sharing a superscript are significantly different.

NS: not significant; \* Significant at p<0.05;

\*\* Significant at p<0.01

| Treatment                  | Control                      | 1 kGy                        | 2 kGy                       | 3 kGy                       |  |  |  |  |
|----------------------------|------------------------------|------------------------------|-----------------------------|-----------------------------|--|--|--|--|
| Storage period/(Months)    |                              |                              |                             |                             |  |  |  |  |
| Refractive index (nD 25°C) |                              |                              |                             |                             |  |  |  |  |
| 0                          | 1.4667±0.0001 <sup>aB</sup>  | 1.4665±0.0001 <sup>aB</sup>  | 1.4670±0.0001 <sup>aD</sup> | 1.4669±0.0001 <sup>aD</sup> |  |  |  |  |
| 6                          | 1.4658±0.0002 <sup>bA</sup>  | 1.4659±0.0001 <sup>bA</sup>  | 1.4652±0.0001 <sup>aA</sup> | 1.4656±0.0001 <sup>bA</sup> |  |  |  |  |
| 12                         | 1.4659±0.0001 <sup>aA</sup>  | 1.4659±0.0000 <sup>aA</sup>  | 1.4659±0.0001 <sup>aB</sup> | 1.4659±0.0001 <sup>aB</sup> |  |  |  |  |
| 24                         | 1.4670±0.0000 <sup>dAB</sup> | 1.4668±0.0001 <sup>cC</sup>  | 1.4665±0.0001 <sup>bC</sup> | 1.4662±0.0001 <sup>aC</sup> |  |  |  |  |
| 36                         | 1.4685±0.0003 <sup>abC</sup> | 1.4685±0.0001 <sup>abD</sup> | 1.4682±0.0001 <sup>aE</sup> | 1.4686±0.0001 <sup>bE</sup> |  |  |  |  |
| P-level                    | **                           | **                           | **                          | **                          |  |  |  |  |
| Viscosity (mPa.s)          |                              |                              |                             |                             |  |  |  |  |
| 0                          | 140.00±1.00 <sup>aB</sup>    | 140.00±0.00 <sup>aB</sup>    | 140.67±0.58 <sup>bB</sup>   | 142.33±0.58 <sup>bB</sup>   |  |  |  |  |
| 6                          | 142.67±0.58 <sup>aC</sup>    | 143.67±0.58 <sup>aC</sup>    | 143.33±0.58 <sup>°C</sup>   | 143.00±1.00 <sup>aB</sup>   |  |  |  |  |
| 12                         | 151.33±0.58 <sup>aD</sup>    | 150.67±0.58 <sup>aD</sup>    | 151.33±0.58 <sup>aD</sup>   | 150.33±0.58 <sup>aC</sup>   |  |  |  |  |
| 24                         | 130.00±1.00 <sup>aA</sup>    | 130.33±0.58 <sup>aA</sup>    | 130.00±0.00 <sup>aA</sup>   | 129.67±0.58 <sup>aA</sup>   |  |  |  |  |
| 36                         | 156.67±1.53 <sup>ªE</sup>    | 158.00±1.00 <sup>aE</sup>    | 158.33±0.58 <sup>aE</sup>   | 158.33±0.58 <sup>aD</sup>   |  |  |  |  |
| P-level                    | **                           | **                           | **                          | **                          |  |  |  |  |

# Table 3. Effect of gamma irradiation and storage period on refractive index and viscosity of olive oil

<sup>abc</sup> Means values in the same row not sharing a superscript are significantly different.

ABC Means values in the same column not sharing a superscript are significantly different. NS: not significant; \* Significant at p<0.05; \*\* Significant at p<0.01</p>

#### 3.7 Effect of Gamma Irradiation and Storage Period on Viscosity of Olive Oil

In optics to know the rheological properties of extra virgin olive oil (EVOO), we studied the influence of gamma irradiation and storage time on viscosity. The viscosity of EVOO was measured for all samples and mean values with standard deviations per samples are shown in Table 3. The viscosity of non-irradiated control sample of EVOO was 140.00 mPa.s (Table 3). Viscosity is a physical characterization constant mostly depending on the temperature and to some extent to the compositional differences of the vegetable oils. Viscosity is an important parameter for the design of industrial processes. It can also be used to evaluate quality of fats and oils in frying [43].

Gamma irradiation treatments (P >0.01 except for 0 month of storage) show a non significant effect on viscosity of EVOO during the different periods of storage (0, 6, 12, 24 and 36 months). While, storage time showed a significant (P <0.01) effect on viscosity of EVOO treated with different dose of gamma irradiation (0, 1, 2 and 3 kGy). All samples of EVOO (irradiated with different doses and stored for different times) showed a higher viscosity (between 140.00 and 158.33 mPa.s) than most vegetable oils (mean value 50 – 100 mPa.s) as was reported by Besbes et al. [44]. This could be explained by the content of saturated and non-saturated fatty acids [45].

#### 4. CONCLUSION

Considering the acid (0.48 to 0.77%), peroxide (2.28 to 6.45 meq  $O_2$  kg<sup>-1</sup>), MDA (0.0018 to 0.0027 mg MDA kg<sup>-1</sup> oil), iodine (84.48 to 90.40 g iodine 100 g<sup>-1</sup> oil), the specification values (199.61 to 204.60 g KOH g<sup>-1</sup> oil), and the Refractive index (1.4665 to 1.4670) in olive oil extracted from irradiated and non-irradiated fruits, irradiation had an acceptable initial effects on quality. Generally, the chemical characteristics remained below the maximum desirable limits set by Codex.

The results of this study revealed that both irradiation treatment (up to 3 kGy) and storage time (up to 36 months) had a significant (P <0.01) effect on most of the measured chemical and physical parameters (AV, PV, MDA value, IV, SV, RI and viscosity) of EVOO.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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