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Effect of gamma irradiation on the total antioxidant capacity and sterols contents in Kaissy variety extra virgin olive oil

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Abstract

Twenty Kaissy variety extra virgin olive oil samples collected from research centre field near Damascus were analyzed by gas chromatography using a flame ionization detector. Fifteen sterols fractions were identified and determined their concentrations: cholesterol, brassicasterol, 24-metilencholesterol, campesterol, campestanol, stigmasterol, Δ7-campesterol, Δ5.23-stigmastadienol, clerosterol, ß-sitosterol, sitostanol, Δ5-avenasterol, Δ5.24-stigmastadienol, Δ7-stigmastenol and Δ7-avenasterol. The total average sterol amounts were in the range 43 to 1240 mg/kg. It has been observed that the concentrations of sterols are consistent with the International Olive Council, IOC Regulations, except the Δ7-stigmastenol which is higher (≥ 0.7%) than the IOC regulation (≤ 0.5%). This might be due to the typical environmental and soil type since Kaissy variety usually grows in red clay soils besides the genetic property classification as more resistant variety and less susceptible toward infection in dry area The total antioxidant components of extra virgin Kaissy variety olive oil extracts were measured for oil immediately pressed and after one year of storage for the average of three samples measurements as hydrophilic and hydrophobic existence species using photochemiluminescence, PCL assay. It has been found that, the total average antioxidant capacity value of immediately pressed Syrian virgin olive oil and after one year of storage was at (179.26, 28.63) and (128.21, 11.92) nmol, as hydrophilic and hydrophobic, respectively. The impact of gamma irradiation sample on the total antioxidant and sterols contents has been shown. The total antioxidants were decreased as the result of storage and after irradiation at three kGy dose in comparison with the control.

1. Introduction

The olive tree with a scientific name (*Olea. europaea L*) and local name zaytoon is very ancient culture in Syria. It is very well known that Syria is one of the culture origins of the olive tree. From Syria, it spread to all Mediterranean basins countries [1]. Universally, Syria occupies the sixth international ranking in olive and olive oil production after Spain, Italy, Greece, Turkey, and Tunisia. Olive fruits contain considerable concentration (1-3%) of fresh pulp weight of hydrophilic and lipophilic (sterols) phenolic compounds which have many biological and antioxidant activities in addition to pectin, organic acids and pigments [2, 3]. Virgin olive oil has a remarkable resistance to oxidation due to the existence of natural antioxidant species due to high level of phenolic fractions. The virgin olive oil contains variable amounts and small quantities of free fatty acids, glycerols, aroma compounds, tocopherols, phenols, sterols etc. [4, 5].

The sterol fractions composition in particular Δ7-stigmastenol in olive oil play consider important parameter for the detection of adulterations and authenticity of the oil in order to meet the international regulatory limits for exporting the oil production to the world market [6].

The content of sterols fractions and natural antioxidants in virgin olive oil plays very important role in the recent years due to the beneficial to human health and also the important indicator of oil quality as a product of major economical importance in the Mediterranean countries which must be meet the international regulations and standard quality before any exporting.

There are many factors affecting the content of compositional fractions and antioxidants capability in virgin olive oil [2]. Among them, gamma irradiation, which is an important method for food sterilization and storage, has shown to be an effective mean of processing olive oil, giving that composition content and biological activities are strongly affected by irradiation [7, 8]. Beside gamma irradiation, other factors affecting olive oil composition have been discussed thoroughly in literature [9]. Abu-Alruz et al. [10] reported the factors affecting Δ7-stigmastenol concentration in Palestinian olive oil. Eleven factors were taken into consideration but the main factors affecting Δ7-stigmastenol concentration are: soil type, region, maturity index and olive fly infection. Quasem et al. reported also the effect acidity on Δ7-stigmastenol of Palestinian olive oil. The results show correlations between Δ7-stigmastenol and acidity and both Δ7-stigmastenol and acidity percentage are increased proportionally [11].

Kyçyk et al. [12] reported very recently the sterol composition of virgin olive oil of forty three olive cultivars from the World Olive Germplasm Bank Collection. They found that, the main sterols found in virgin olive oil were: β-sitosterol, Δ5-avenasterol, campesterol and stigmasterol and the total sterol content was ranged from 855 to 2185 mg/kg. Temime et al. [13] reported the sterol profile of Tunisian virgin olive oils produced from Che´toui cultivar, grown under different environmental conditions, using gas chromatography with a flame ionization detector. They found the following sterols as expected in the virgin olive oil: β-sitosterol, Δ5-avenasterol, campesterol and stigmasterol, cholesterol, 24-methylenecholesterol, clerosterol, campestanol, sitostanol, Δ7-stigmastenol and Δ5.24-stigmastadienol but the Δ7-avenasterol was found in all samples with low concentration. All analyzed samples meet the EC Regulation No. 2568 and the total sterol amounts were higher than the minimum limit set by legislation, ranging from 1017 to 1522 mg/kg. They concluded that the sterol content was below the upper legal limit of 4% in all analyzed samples, with a range from 1.2% to 3.2%. These results suggest that, besides the genetic factor, environmental conditions influence the sterolic fraction [13].

Minioti and Georgiou [14] developed a rapid flow injection automated method for the determination of total antioxidant capacity using horseradish peroxidase (HRP) assay for extra virgin olive oil samples in different Greek cultivars and regions. The chemistry involved in this assay is the horseradish peroxidase (HRP) catalysed oxidation of luminol by hydrogen peroxide. Oxidation results in light emission (bioluminescence) that is enhanced using p-iodophenol sensitizer and the rate of sampling is equal to 180 probes per hour [14]. The antioxidant activity of five common compounds found in natural products was determined using luminol CL with Co(II) as EDTA complex [15]. The five antioxidants selected in olive oil were β-carotene, BHT (butylated hydroxytoluene), α-tocopherol, quercetin and l-ascorbic acid (l-AA). The scavenging activity of hydrogen peroxide (SAHP, μ/M) for the five antioxidants studied was (135, l-AA), (26.3, BHT), (3.22, α-tocopherol), (2.78, β-carotene) and (1.22, quercetin) and proved that l-AA and BHT are the most powerful H_2O_2 scavengers followed by α-tocopherol, β-carotene while quercetin is the weakest scavenger under the applied experimental conditions [15].

According to our knowledge, there are no reports in the literature published about Syrian extra virgin Kaissy variety olive oil concerning the sterols contents, antioxidant measurements and the effect of gamma radiation on the oil. This paper covers the above three previous mentioned points including the impact of that on trade, nutrition and health value of the Syrian extra virgin olive oil.

2. Experimental details

2.1 *Sampling*

During crop seasons 2008/2009 and 2009/2010, twenty olive Kaissy variety samples were collected by hand. Each sample was collected from two different trees located at the Deer Al-Hajar (33° 21` N, 36° 28` E, 617 m altitude) research station field near Damascus at the mature stage. The average annual temperature ranges between 19-36°C in July to August and at 3°C from January to February. Finally, the annual rainfall varies from 100 to 150 mm with most rainfall in the winter season.

By the way, the Kaissy table variety occupies 4.78% of the total olive trees planted area in Syria as well as this variety is considered more resistant and less susceptible toward infections in dry areas.

Each collected sample was 3kg weight. After collection, the samples were water cleaned, dried and then subjected to laboratory ground mill where they transformed into oil using cold centrifuge instrument. The olive oil was obtained within 24h. Twenty samples of 250g each were kept individually in dark bottles at 4°C and sealed with parafilm for further analysis and investigations.

2.2 *Extraction of Sterols*

The sterols extraction was carried out within very short possible time using 250g Kaissy variety olives similar to the process described by Blatchly et al. [16]. The olive fruits with stones were crushed with hummer crusher and slowly mixed for about 30 min at 27°C, and then the mixture was centrifuged at 3000 rpm for three min without

adding water to extract the oil. Finally, the oil was decanted and transferred immediately into dark bottles and stored in dark at room temperature.
The sterols extraction was carried out as follows: the saponification reaction was made on the mixture of 5g of the oil sample mixed with 0.5 ml of the standard (α-cholestanol) and adding to that 50ml of potassium hydroxide, 2N in ethanol (ethanolic) and then put under reflection at 85°C for about 8 h to reach clearness mixture. Then, the Unsoponified part was separated by separating funnel and washed using ether solvent. This procedure was repeated five times and the obtained material from each step was added together and considered as one lot. Then, the material was filtered on filter paper containing sodium sulfate. The rest of the ether was removed by stream of nitrogen gas (99.999%). Then the sterols were purified using thin layer chromatography TLC and chloroform as carrier solvent, after removing spot having the sterols from the TLC, the material was dissolved in N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) and become ready for analysis.

2.3 *Analytical method*
The sterols were determined at three concentration units: %, mg/100g and mg/Kg and the results will be shown later. The unsaponified fraction was removed by ethyl ether after the extra virgin olive oil samples were saponified first with ethanolic potassium hydroxide solution. The unsaponifiable silanised sterol fractions were very essential method to consider for separating the undesirable interfering compounds in the unsaponifiable fraction before the determination of sterols. The separated fractions were quantified by capillary gas chromatograph on Shimadzu 17A in Assad & Betinjaneh Co. (Syrian Olive Oil), Tartous-Syria with auto-sampler and a flame ionization detector (FID) using an 30m×0.32mm, df= 0.25µm HP-5 capillary column (Agilent Technologies). The temperature program was at 115°C, 1 min up to 250°C with the heating rate 9°C per minute. Then up to 290°C for three minutes with a heating rate of 1.5°C per minute. The injected volume at the column was 0.8 µl at a flow rate of 1.1 ml/min, using helium as carrier gas (100kPa). The detector temperature was 320°C. The sample was dissolved in n-hexane. Quantification was made by addition of 1.0 mg internal standard of α-cholestanol with 5.020g sample. Finally, it should be pointed out here that the results are given as the means of triplicate independent measurements for all applied analysis.

2.4 *Gamma irradiated samples*
The samples of Kaissy variety extra virgin olive oil were irradiated using the ^{60}Co package irradiator source (ROBO, Techsnab export, and Moscow Russia). The irradiation was carried out in stationary mode of operation with possibility of varying dose rate from 10.846 - 30921 kGy/h. The actual applied dose rate was 9.9131 kGy/h, at 20-25°C in air and the distance sample position is (10-40) cm.
Three groups (A, B and C) were irradiated at the following doses: 0 1, 2 and 3 kGy and the results will be mentioned later as the average of three successive measurements in addition to the control. The three investigated Kaissy variety extra virgin olive oil samples were: Group A presents irradiated extra virgin olive oil produced from non-irradiated Kaissy variety olive fruits. Group B presents extra virgin olive oil produced from irradiated Kaissy variety olive fruits. Finally, Group C presents another irradiated Doebli variety extra virgin olive oil obtained from Tartous Company for comparison with Kaissy variety olive oil. The absorbed doses were measured using alcoholic chlorobenzene dosimeter (Oscillotitrator, OK-302/2, Radelkisz, and Budapest, Hungary) [17]. The four treatments (0, 1, 2 and 3 kGy) were distributed in a completely randomized design with three replicates. Data were 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. The degree of significance was denoted as: $p<0.05$ and $p<0.01$.

2.5 *Antioxidant Photochemiluminescence (PCL) Assay*
The PCL assay is easy and rapid to perform and has many advantages over the other assays. It does not require high temperatures to generate radicals and it is more sensitive (nanomolar range) to measure within few minutes (≤ 3 minutes) the scavenging activity of antioxidants against the superoxide radical (O_2^{*-}) which is one of the most reactive oxygen species occurring in human body [18]. While, most of the other methods like (TEAC, TRAP, DPPH, ORAC and FRAP) determine the antioxidant activity in micromolar range and they require minutes or hours [19]. The Photochemiluminescence (PCL) assay principle used in this work is based on the methodology of Popov and Lewin and the reader is advised to refer to the following literature [20-22] for more details. Very briefly, the assay was used to measure the antioxidant activity of plant extracts against superoxide anion radicals ($O_2^{\bullet-}$) generated from luminol, a photosensitizer, when exposed to UV light. In the PCL assay, the photochemical generation of free radicals is combined with the sensitive detection using chemiluminescence

process. This reaction is induced by optical excitation of a photosensitizer S that results in the generation of the superoxide radical ($O_2^{\bullet-}$) [21]:

$$S + h\nu + O_2 \rightarrow S^{\bullet+} + O_2^{\bullet-}$$

The free radicals are visualised with the chemiluminescent detection reagent luminol. It works as photosensitizer as well as oxygen radical detection reagent. The PCL assay has been applied by many research groups for its advantages in comparison with different assays [18, 20-24]. Kits of chemicals for determination of antioxidant capacity of water-soluble substances (ACW) and antioxidant capacity of lipid-soluble substances (ACL) were obtained from Analytik Jena. The photochemiluminescence (PCL) apparatus used in this work is from Analytik Jena AG (Jena, Germany). The applied protocols for ACW and ACL have been mentioned in details in the following published literature [25, 26].

3. Resulats and Discussion

It should be mentioned first that the present work is focused only on Kaissy variety extra virgin olive oil. In addition to Kaissy variety, Syria has many other olive varieties including Zaity, Sorani, Doebli, Khoderi, Abo satl, Mosssabi, Dan and Jolt **.** Table 1 shows the Kaissy variety extra virgin olive oil sterols fractions determined at three different concentration units at % (w/v), and mg/Kg, ppm, respectively.

Table 1: Kaissy variety extra virgin olive oil sterols fractions determined at three different concentration units at %, mg/100g and mg/Kg, respectively

Sterols fractions	**Conc. (%)**	**Conc. (mg/kg)**
Colesterol	0.25	3.59
Brassicasterol	0.01	0.19
24-Metilencholesterol	0.08	1.10
Campesterol	3.00	43.45
Campestanol	0.08	1.09
Stigmasterol	0.64	9.20
Δ7-Campesterol	0.07	1.00
Δ5.23-Stigmastadienol	0.05	0.79
Clerosterol	1.24	17.96
ß-Sitosterol	85.68	1240.24
Sitostanol	0.32	4.58
Δ5-Avenasterol	7.07	102.38
Δ5.24-Stigmastadienol	0.58	8.46
Δ7-Stigmastenol	0.73	10.79
Δ7-Avenasterol	0.60	8.64
Total sterols contents	100.4	1453.46

3.1. Sterols compositions and concentrations

Fifteen fractions were identified and determined their concentrations: cholesterol, brassicasterol, 24-metilencholesterol, campesterol, campestanol, stigmasterol, Δ7-campesterol, Δ5.23-stigmastadienol, clerosterol, ß-sitosterol, sitostanol, Δ5-avenasterol, Δ5.24-stigmastadienol, Δ7-stigmastenol and Δ7-avenasterol. The three important sterols concentration for Δ5-avenasterol, β-sitosterol and campesterol were at 7.07, 85.68 and 3.00%, respectively. These sterols present almost 96% of the total sterols which are usually found in extra virgin olive oil. The chemical components with a total of almost 100% have been identified. It is well known that β-sitosterol is the most abundant phytosterol in virgin olive oil; its level shows more than 75% of total sterols and between 76.8 % and 84.4 % determined in Tunisian olive oil [12]. Therefore, our reported results (85.68%) are consistent with reported literature and International Olive Council, IOC Regulations [25-27]. Δ5-avenasterol and campesterol are the most representative and characteristic sterols in virgin olive. Their percentage in Syrian extra virgin olive oil is about 10% which is in good agreement with the Tunisian extra virgin olive oil: Δ5-avenasterol (4.1– 19%) and campesterol (1.9–2.9%) [12].

The Δ7-stigmastenol is higher (≥ 0.73%) than the IOC regulation (≤ 0.5%) [27]. There are many factors affecting the percentage of Δ7-stigmastenol above the regulations. One of these roles is due to the soil type and atmospheric region conditions. The olive oil was produced from trees planted in research centre located 30 km south of Damascus (dry area) in red clay soil (about 90% are non-soluble materials in water). This observation

was pointed out by [10] and our results are consistent with their observation. By the way, the soil type and geographic area affect the Δ7-stigmastenol level to about 50% in addition to infection mode, harvest time, pressed temperature, olive variety [10]. Finally Kaissy variety is also classified as more resistant variety and less susceptible toward infection in dry area [1]. It can be said that this high amount of Δ7-stigmastenol is not due to adulterations and authenticity of the oil but is due to soil type, environmental and geographical conditions.

3.2. Gamma irradiation

Table 2 presents group A, the irradiated extra virgin olive oil produced from non-irradiated Kaissy variety olive fruits. The results are the average of three separate measurements. The chemical components with a total of almost 100% have been identified.

Table 2: Irradiated extra virgin olive oil produced from non-irradiated Kaissy variety olive fruits

Sterols fractions	Average A Control (%)	Average A 1 KGy (%)	Average A 2 KGy (%)	Average A 3 KGy (%)
Colesterol	0.25	0.25	0.22	0.24
Brassicasterol	0.01	0.01	0.01	0.01
24-Metilencholesterol	0.08	0.05	0.05	0.08
Campesterol	3.00	3.04	3.04	3.13
Campestanol	0.08	0.07	0.08	0.08
Stigmasterol	0.64	0.70	0.63	0.65
Δ7-Campesterol	0.07	0.05	0.05	0.05
Δ5.23-Stigmastadienol	0.05	0.07	0.07	0.06
Clerosterol	1.24	1.19	1.20	1.24
ß-Sitosterol	85.68	86.70	86.76	86.52
Sitostanol	0.32	0.49	0.43	0.36
Δ5-Avenasterol	7.07	5.68	5.86	6.03
Δ5.24-Stigmastadienol	0.58	0.65	0.62	0.62
Δ7-Stigmastenol	0. 72	0.39	0.37	0.36
Δ7-Avenasterol	0.60	0.69	0.63	0.58
Total	100.4	100.03	100.02	100.01

No significant changes have been observed as the results of irradiation apart from Δ5-avenasterol, the concentration is lower than the control after irradiation at three different doses. Note that the concentration of Δ5-avenasterol is decreased with the increasing radiation dose. This means that the irradiation of extra virgin olive oil produced from non-irradiated Kaissy variety olive fruits has no effect. The previous Δ7-stigmastenol discussion can be extended to this section. Figure 1 shows a clear comparison between the control and the irradiated samples.

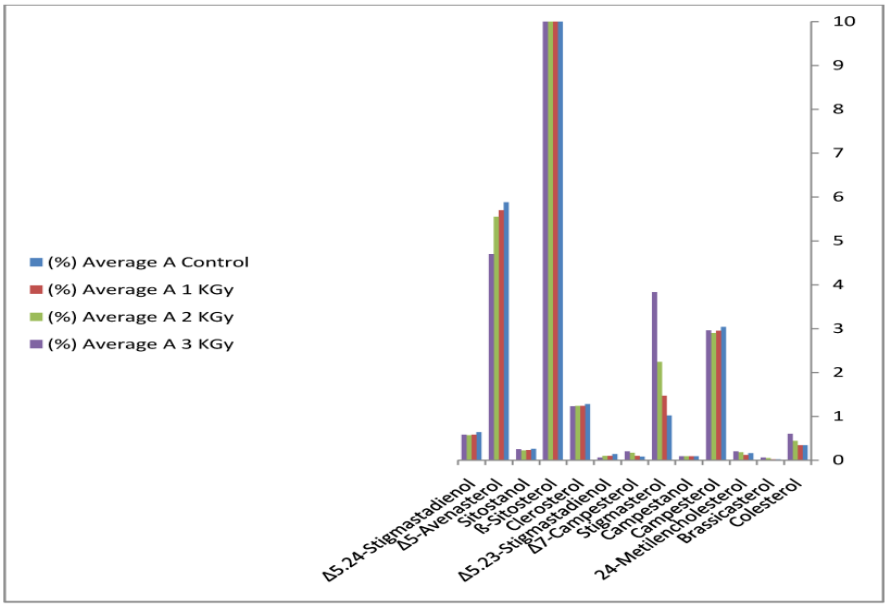

Figure 1: A clear consistence between the control and the irradiated samples, apart from Δ5-avenasterol, the concentration is lower than the control after irradiation at three different doses

Table 3 presents group B, extra virgin olive oil produced from irradiated Kaissy variety olive fruits. The results are the average of three separate measurements. The chemical components with a total of almost 100% have been identified. No significant changes have been observed as the results of irradiation apart from the Stigmasterol, the concentration is higher than the control after irradiation at three different doses. Note that the concentration of Stigmasterol is increased with radiation dose increasing. This means that the irradiation of extra virgin olive oil produced from irradiated Kaissy variety olive fruits has an effect on Stigmasterol only. The Δ5-avenasterol concentration has been reduced to about 20% in comparison with the control. Figure 2 shows a clear comparison between the control and the irradiated samples.

Table 3: Extra virgin olive oil produced from irradiated Kaissy variety olive fruits

Sterols fractions	Average B Control (%)	Average B 1 KGy (%)	Average B 2 KGy (%)	Average B 3 KGy (%)
Colesterol	0.34	0.34	0.44	0.60
Brassicasterol	0.02	0.02	0.05	0.06
24-Metilencholesterol	0.16	0.12	0.18	0.20
Campesterol	3.04	2.95	2.90	2.96
Campestanol	0.09	0.09	0.09	0.09
Stigmasterol	1.02	1.47	2.24	3.83
Δ7-Campesterol	0.08	0.10	0.17	0.20
Δ5.23-Stigmastadienol	0.14	0.10	0.10	0.06
Clerosterol	1.28	1.24	1.24	1.23
ß-Sitosterol	86.27	86.47	85.53	84.33
Sitostanol	0.26	0.23	0.22	0.25
Δ5-Avenasterol	5.88	5.70	5.55	4.70
Δ5.24-Stigmastadienol	0.64	0.58	0.57	0.58
Δ7-Stigmastenol	0.39	0.25	0.25	0.26
Δ7-Avenasterol	0.40	0.39	0.48	0.34
Total	100.01	100.05	100.01	99.69

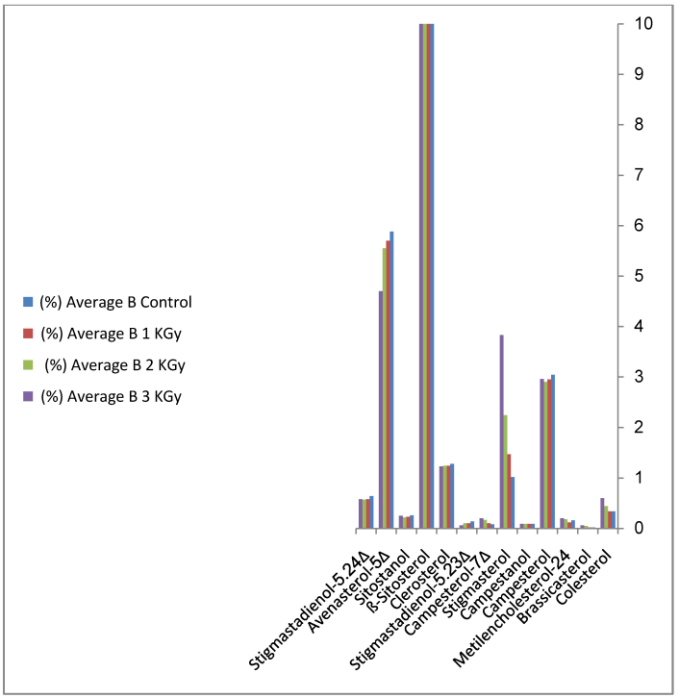

Figure 2: A clear consistence between the control and the irradiated samples apart from the stigmasterol, the concentration is increased with radiation dose increasing

Table 4 presents group C, irradiated Doebli variety extra virgin olive obtained from Tartous Company for comparison with Kaissy variety olive oil. The results are the average of three separate measurements. The chemical components with a total of almost 100% have been identified.

No significant changes at all have been observed as the results of irradiation. It can be seen that the Δ7-stigmastenol concentration is within the acceptable International regulation [21]. The reason is that, the Doebli variety is planted in lime soil in the coastal area with high rainy and humidity level (annual average rainfall, 1050 mm, humidity, 50-90%, altitude 1100 m).
Therefore, some Syrian virgin olive oil is naturally has high Δ7-stigmastenol concentration due to the soil and geographical area as mentioned earlier. Other variety like Doebli is consistent with the International regulation. Figure 3 shows a clear comparison between the control and the irradiated samples. No obvious changes are observed with this olive variety. It can be emphasized once more that low irradiated doses are not sufficient for any significant changes.

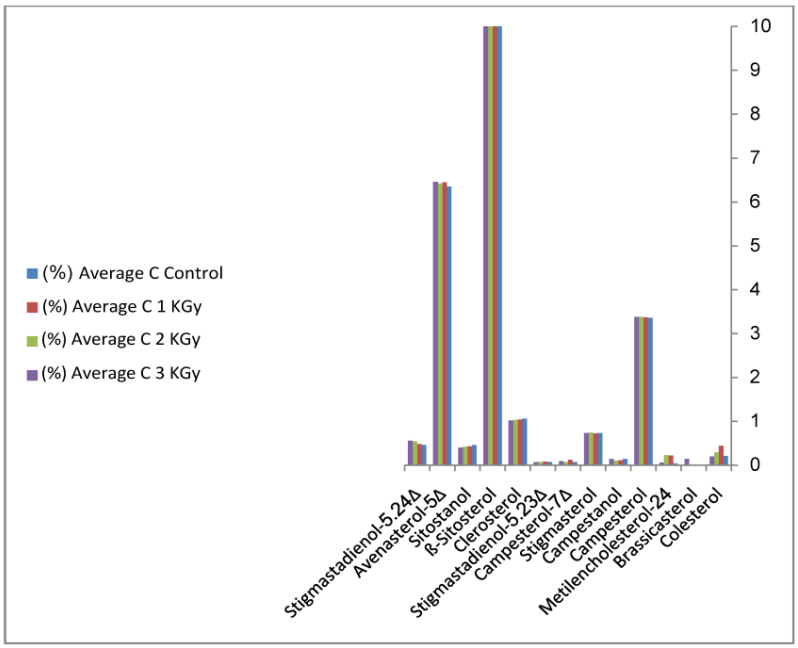

Figure 3: A clear comparison between the control and the irradiated samples. No obvious changes are observed with this olive variety

Table 4: Irradiated Doebli variety extra virgin olive obtained from Tartous Company

Sterols fractions	Average C Control (%)	Average C 1 KGy (%)	Average C 2 KGy (%)	Average C 3 KGy (%)
Colesterol	0.21	0.44	0.29	0.20
Brassicasterol	0.01	0.01	0.01	0.14
24-Metilencholesterol	0.04	0.22	0.23	0.06
Campesterol	3.36	3.37	3.38	3.38
Campestanol	0.14	0.11	0.10	0.14
Stigmasterol	0.73	0.72	0.74	0.73
Δ7-Campesterol	0.07	0.12	0.07	0.09
Δ5.23-Stigmastadienol	0.07	0.08	0.07	0.07
Clerosterol	1.06	1.04	1.03	1.02
ß-Sitosterol	85.67	85.10	85.30	85.50
Sitostanol	0.46	0.43	0.42	0.40
Δ5-Avenasterol	6.35	6.45	6.42	6.46
Δ5.24-Stigmastadienol	0.46	0.48	0.54	0.56
Δ7-Stigmastenol	0.49	0.48	0.49	0.50
Δ7-Avenasterol	0.86	0.89	0.90	0.89
Total	99.98	99.94	99.99	100.14

3.3. Antioxidant measurements
Table 5 shows the antioxidant measurements for integral water and lipid soluble antioxidant of non-irradiated Kaissy extra virgin olive oil pressed immediately after olive ripening (control). Table 5 shows the total water-soluble antioxidant (ACW) and lipid soluble antioxidant (ACL) capacity as total phenolic values at 28.63 and

179.26 nmol for hydrophilic and hydrophobic fractions, respectively. After irradiation at 3 KGy, the total antioxidants were decreased dramatically. This leads to deep investigations in order to know the reason for that and this will be considered in future work. No significant changes have been observed at 1 and 2 KGy doses.

Table 5: The antioxidant measurements for integral water and lipid soluble antioxidant of non-irradiated kaissy extra virgin olive oil pressed immediately after olive ripening (control)

Samples	Total Water-soluble antioxidant (ACW) nmol* as total phenolic	Total Lipid-soluble antioxidant (ACL) nmol* as total phenolic
Non-irradiated Kaissy extra virgin olive oil pressed just after ripening (control)		
0-1	28.50	179.10
0-2	27.60	176.00
0-3	29.80	182.70
Irradiated Kaissy extra virgin olive oil at 3kGy		
3-1	0.510	5.30
3-2	0.439	7.50
3-3	0.443	4.45

* ±SD (0.5-1.2 nmol)

Table 6 shows the total average water-soluble antioxidant (ACW) olive oil and the total average Lipid-soluble antioxidant (ACL) olive oil after one year of storage as hydrophilic and hydrophobic existence species using photochemiluminescence, PCL assay, respectively. It has been found that, the total average antioxidant capacity value of immediately pressed Syrian virgin olive oil and after one year of storage was decreased from (179.26, 28.63) in Table 5 to (128.21, 11.92) nmol, in Table 6. Therefore, it is advised to consume the oil freshly for having extra level of antioxidants fractions necessary for our health.

Table 6: Total average water-soluble antioxidant (ACW) olive oil and the total average lipid-soluble antioxidant (ACL) olive oil after one year of storage as hydrophilic and hydrophobic existence species

Sample	1	2	3
Blank (nmol)	0.12773	0.11781	0.13680
Standard (µl)	10	20	30
Average of three measurements (ACW/ACL)	10.51/128.23	11.74/130.65	13.52/125.77
Total Average Water-soluble antioxidant (ACW) olive oil after one year of storage	11.92 nmol* as total phenolic compounds		
Total Average Lipid-soluble antioxidant (ACL) olive oil after one year of storage	128.21 nmol* as total phenolic compounds		

*The results are given as the means and the standard deviation, ±SD of three independent measurements

Finally, the health and nutrition value of extra virgin olive oil are considerable and they are due to high concentration of polyphenols (sterols). These species are very excellent source for anti-inflammatory properties. One of the key polyphenols chemical fractions exist in the Syrian extra virgin olive oil is the hydroxytyrosol antioxidant content which protects our blood vessels cells from any damage and enhances the antioxidant defense system from being damaged by reactive oxygen molecules. Virgin olive oil controls also blood cholesterol level due to its sufficient concentration of essential fatty linoleic and oleic acids and antioxidants content as explained earlier. It reduces the risk of cardiovascular disease and cancers by decreasing the penetration rate of fatty acids in blood vessels cells [28, 29].

Conclusions

It can be emphasized that low irradiated doses are not sufficient for any significant changes in the concentration of sterols in the investigated extra virgin olive oil. It is recommended to consume the olive oil freshly in order to have high level of antioxidants. Syrian extra virgin olive oil meets the international standard regulation limits and ready for exporting the oil production to the world market in spite of high concentration of Δ7-stigmastenol fraction which is due to the soil type and geographic area but not to the results of adulterations and authenticity of the oil.

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