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Biochemical study of oil extracted from gamma irradiated sunflower (*Helianthus annuus* l.) seeds

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Abstract

The purpose of this study was to evaluate the occurrence of lipid oxidation in sunflower (*Helianthus annuus* L.) oil extracted from seeds which were irradiated with 3, 6 and 9 kGy of gamma radiation and stored for 6 and 12 months. The quality characteristics of extracted sunflower oils acidity value (AV), peroxide value (PV), iodine value (IV), saponification value (SV), thiobarbituric acid value (TBA) and refractive index (RI)), and color values (L*, a*, b* and ΔE- values) were analyzed. Irradiation doses and storage time significantly ($p<0.05$) increased the AV and PV, and only the storage time significantly ($p<0.05$) increased the TBA value and RI, while both irradiation doses and storage time had no significant ($p>0.05$) effect on IV and SV. Storage time had more pronounced effect on AV, PV, TBA and RI than irradiation. Gamma irradiation had a significant ($p<0.05$) effect on all color parameters of sunflower seeds oil including lightness (L*-value), redness (a*-value), yellowness (b*-value), and color difference (ΔE). The studied qualitative characteristics were influence by the used doses of gamma irradiation.

Keywords: Chemical properties, Gamma irradiation, Oil color, Storage period, Sunflower

1. Introduction

Sunflower (*Helianthus annuus* L.) seeds are a rich source of edible oil, and their oil is considered as one of the healthiest and popular oils in the world [1]. Sunflower oil has been valued as a component for spreads in Europe because of its high linoleic fatty acid and absence of linolenic fatty acids [2]. It is often considered premium oil due to its light color, mild flavor, low level of saturated fat and ability to withstand high cooking temperature [3].

Sunflower oil, like other vegetable oils, undergoes extensive oxidation due to storing, marketing, or processing. Reported literatures have found that different processing methods affect the physicochemical properties of vegetable oils [4, 5]. Hydro-peroxide, which is the major oxidation product, decomposes to secondary products. The secondary products adversely affect flavor, aroma, taste, nutritional value and overall quality of food [6, 7].

The effect of irradiation on locally stored foods is of utmost importance and an insight into these aspects of storage will help in understanding the shelf life of foods as well as its effects on sensitive nutrients [8-10]. Irradiation has been shown to cause some physicochemical change such as reducing viscosity, increasing water solubility, acidity, etc. in food [11-15].

At present, there are limited or no studies concerning the quality characteristics and the effects of gamma irradiation of commercial spices of oil seeds produced under Syrian conditions. Therefore, the aim of the present study was to determine the physicochemical properties of oil extracted from Syrian sunflower seeds and to investigate the effect of middle doses (3, 6 and 9 kGy) of gamma irradiation in those properties.

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2. Materials and Methods

2.1. Treatments and analysis performed

Syrian cultivated Sunflower seeds were purchased from local supermarkets and special shop in Damascus, Syria, and exposed to gamma radiation at doses of 3, 6 and 9 kGy in a ^{60}CO package irradiator. The samples were irradiated at place with a dose rate of 7.775 kGy h^{-1}, at room temperature and atmospheric pressure [16]. The oils from control and irradiated sunflower seeds after grinding were extracted by the manual Soxhlet apparatus (Scientific Apparatus Manufacturing Company, Glas-Col Combo Mantle, USA) for 16 h, using distilled AR (analytical grade) n-hexane as the solvent [17]. Physical and chemical properties of oils extracted from irradiated and non-irradiated sunflower seeds samples were performed immediately after extraction, and after 0, 6 and 12 months of storage.

2.2. Chemical analysis of oils

Acidity value (AV) (Oleic acid %) in term of mg KOH g-1 oil, peroxide value (PV) in term of mEq O_2 kg^{-1} oil, iodine value (IV) in g I_2 100 g^{-1}, saponification value (SV) in term of mg KOH g-1 oil sample and the refractive index (RI) at 40 ºC were determined according to standard methods [17]. TBA number (Thiobarbituric acid) in mg MDA kg^{-1} sample was measured according to IUPAC direct method [18].

2.3. Color measurement

The color of sunflower oil was measured using AvaSpec Spectrometer Version 1, 2 June 2003 (Avantes, Holland) and expressed as color L* (lightness), a* (redness), and b* (yellowness) values. Reflectance values were obtained at wave length of 568 nm by exposing the samples to the illuminant [14]. The reported results (L*, a*, b*) are the mean of 9 determination.

2.4. Statistical analysis

The four treatments 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: $p<0.05^*$, $p<0.01^{**}$ [19].

3. Results and discussion

3.1. Effect of gamma irradiation and storage period on acidity value of sunflower seed oil

The acidity values of oil extracted from gamma irradiated and non-irradiated sunflower seeds are shown in Table 1. The value of acidity for oil extracted from non-irradiated control samples of sunflower seeds was 0.88 mg KOH g^{-1} oil. This value is higher than the range (0.11 - 0.51 mg KOH g^{-1} oil) that was reported in the literature for sunflower seed oil [20-22].

Gamma irradiation treatment increased significantly ($p<0.05$) the acid values, which is due to the conversion of triglycerides [23]. These results show that the oils under investigation are less stable towards radiations and storage, resulting in high rancidity. Similar findings were reported for the acid values of sunflower and soy bean oil [15] and pumpkin seed [24], which increased significantly with high gamma radiation (5 and 20 kGy).

The acid value of the oil extracted from non-irradiated sunflower seed samples increased ($p<0.01$) during the storage. After 12 months of storage, the acid value of oil extracted from irradiated sunflower seeds was lower ($p<0.05$) than those of non-irradiated ones. Previous work on this parameter in walnut and peanut also showed that the acidity values increased as irradiation dose and storage time increased [9, 16]. The increasing acidity in oil after irradiation may be attributed to the degradation of large lipid molecules producing smaller molecules including free fatty acids.

3.2. Effect of gamma irradiation and storage period on peroxide value of sunflower seed oil

Table 1 shows the effect of gamma irradiation, and storage time on peroxide value (PV) of sunflower seed oils. As shown in Table 1, the PV of oil extracted from non-irradiated control sample of sunflower seeds was 2.45 meq O_2 kg^{-1} oil. This value was much lower than that reported in the literature for sunflower oil (4.13 - 10.26 meq O_2 kg^{-1} oil) [20, 23, 25]. However, the peroxide value of sunflower seed oil found in this study (2.45 meq O_2 kg^{-1} oil) is below the maximum acceptable value of 10 meq O_2 kg^{-1} oil set by Codex Alimentarius Commission for seed oils [26]. The low peroxide value of sunflower oil indicated in the present study, suggests that it would be less susceptible to rancidity than other vegetable oils [27].

In this study, both storage time and irradiation affected peroxide value of sunflower seed oil. Peroxide values were increased significantly ($p<0.01$) in oil extracted from seeds exposed to the highest irradiation dose (9 kGy). Also the PV of oil extracted from irradiated and non-irradiated sunflower seed samples stored for 12 months was higher ($p<0.01$) than that of extracted from the same seeds at zero time.

Present results of the effects of gamma irradiation on PV are in agreement with those of Lutfullah *et al*. [23] for sunflower seeds, Al-Bachir [10] for pistachio, and Abd El-Aziz and Abd El-Kalk, [24] for pumpkin seeds. Makhoul *et al*. [28] reported that, the PV increased in magnitude during the storage of sunflower oil. Also, Chemat *et al*. [4]. reported that the peroxide value of sunflower oil was increased when it was treated by ultrasound wave.

The lipid oxidation was attributed to the combination of free radicals with O_2 to form hyroperoxides [29]. The oxidative rates of oleic acid, linoleic acid and linolenic acid are 1:12:25 [7]. Sunflower oil has approximately 70% linoleic acid and is highly susceptible to lipid oxidation [6]. This the reason why the peroxide value increased after exposed to irradiation.

Also the peroxide value is a measure of content of hydroperoxide, which is primary oxidation product. These results from decomposition of hydroperoxide to secondary oxidation products are due to irradiation [30]. It is obvious that gamma irradiation accelerated the oxidative degradation of lipids giving rise to the formation of hexanal, which in turn affected product sensory quality [31].

3.3. Effect of gamma irradiation and storage period on TBA value of sunflower seed oil

Data for TBA number (Thiobarbituric acid) of oil extracted from irradiated and non-irradiated sunflower seeds are shown in Table 1. The TBA value of oil extracted from non-irradiated control samples of sunflower seeds was 0.022 mg malonaldehyde (MDA) kg^{-1} oil. The results indicated that, irradiation doses (3, 6 and 9 kGy) of gamma irradiation had no significant ($p>0.05$) effect on TBA of sunflower seeds oil. Present oxidation results of sunflower oil are in general agreement with those of Javanmard *et al*. [32] who showed that oxidation value at irradiation dose (up to 5 kGy) had no significant change in lipid oxidation of chicken meat. On other hand, these findings are directly contradictory to those reported in previous studies which found significant changes in the TBA counts of fermented sausage after irradiation [13]. Since there are some differences in the experimental designs among the previous studies, such as the source of irradiation, the properties of the raw materials, and the preparation methods of end products, etc. it is not easy to compare the results of all previous studies with those of this study. However, these contradictory findings may reveal the complexity in understanding of chemical characteristics of irradiated products, which rich in lipid [33].

As the storage time increased, TBA as indicator to lipid oxidation of sunflower oil increased (Table 1). This is in agreement with the study of [28] who reported that, the TBA of sunflower oil increased upon storage in accord with double bond shifts in fatty acids and production of aldehydes though decomposition of hydroperoxides formed during oxidation of oils.

3.4. Effect of gamma irradiation and storage period on iodine value of sunflower seed oil

The sunflower seed oil had an iodine value of 114.78 ± 2.77 g of I_2 100 g^{-1} oil (Table 2), indicating a high degree of un-saturation. The iodine value gives a proximate amount of the unsaturated fatty acids in any sample oil thereby, providing a comparative idea of the saturated fatty acid components [34]. This value was higher than 88 g of I_2 100 g^{-1} oil reported by Samarth and Mahanwar [22]. but lower than (132.31 g of I_2 100 g^{-1} oil that indicating by El-kady *et al*.[35] for Egyptian sunflower oil.

Akinhanmi *et al*. [36] concluded that oils are classified into drying, semi drying and non-drying according to their iodine values. The values obtained in this studies indicates that sunflower seed oils is drying oil since it is higher than 100.

The results indicated that, both storage time (6 and 12 months) and irradiation doses (3, 6 and 9 kGy) of gamma irradiation had no significant ($p>0.05$) effect on iodine values of sunflower seeds oil. Contrast findings were reported by Zeb and Ahmad [15] for the iodine value of sunflower and soybean oil which decreased significantly with high gamma radiation (1, 5 and 20 kGy). Similar results indicated [24] for the iodine value of pumpkin oil which decreased significantly with 1, 3, 6 and 10 kGy of gamma radiation. Another study reported a decrease in iodine value upon irradiation [37]. It was reported that both irradiation with 1, 2 and 3 kGy of gamma irradiation and storage for 12 months significantly ($p<0.05$) the iodine value of almond kernel [8]. pistachio kernel [10] and peanut seed [9].

Table 1. Effect of gamma irradiation and storage period on biochemical properties of Sunflower oil.

Treatments	Control	3 KGY	6 KGY	9 KGY	P- level
Storage period /(Months)		**Acid value (mg KOH g^{-1} Oil)**			
0	0.88±0.02^{cB}	0.94±0.02^{bB}	0.91±0.02^{bcB}	1.23±0.03^{aC}	**
6	1.40±0.36^{bB}	1.60±0.30^{abB}	1.69±0.21^{abB}	2.08±0.08^{aB}	NS
12	4.59±0.38^{aA}	4.42±0.55^{aA}	3.23±0.66^{bA}	3.22±0.62^{bA}	*
P-level	**	**	**	*	
		Peroxide Value (meqO_2 kg^{-1} Oil)			
0	2.45±0.07^{bB}	2.42±0.07^{bB}	2.42±0.17^{bC}	2.76±0.07^{aB}	**
6	2.70±0.35^{aAB}	2.99±0.40^{aA}	2.76±0.06^{aB}	3.08±0.39^{aB}	NS
12	3.16±0.25^{cA}	3.28±0.08b^{cA}	3.47±0.07^{abA}	3.67±0.16^{aA}	**
P-level	**	**	**	**	
		TBA value (mg MDA kg^{-1} oil)			
0	0.022±0.001^{aC}	0.022±0.001^{aC}	0.020±0.001^{cC}	0.021±0.001^{bB}	NS
6	0.024±0.001^{aB}	0.023±0.001^{aB}	0.023±0.002^{aB}	0.024±0.001^{aA}	**
12	0.026±0.001^{aA}	0.026±0.001^{aA}	0.025±0.001^{aA}	0.025±0.001^{aA}	NS
P-level	**	**	**	**	

abc 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 $p<0.01$.

Table 2. Effect of gamma irradiation and storage period on biochemical properties of Sunflower oil.

Treatment	Control	3 KGY	6 KGY	9 KGY	P- level
Storage period/ (Months)		**Iodine number (g I_2 100 g^{-1} Oil)**			
0	114.78±2.77^{aA}	114.33±2.57^{aA}	117.22±0.64^{aA}	114.15±1.06^{aA}	NS
6	116.12±1.85^{aA}	115.99±1.22^{aA}	116.48±1.09^{aA}	115.99±1.85^{aA}	NS
12	112.65±1.56^{aA}	113.84±4.00^{aA}	114.65±3.20^{aA}	115.61±3.68^{aA}	NS
P-level	NS	NS	NS	NS	
		Saponification value (mg KOH g^{-1} Oil)			
0	194.02±0.86^{aA}	191.72±2.02^{aA}	192.86±1.08^{aA}	191.98±2.14^{aA}	NS
6	190.98±6.89^{aA}	192.14±5.78^{aA}	193.84±3.78^{aA}	193.12±2.02^{aA}	NS
12	193.21±3.13^{aA}	194.60±1.46^{aA}	195.66±0.55^{aA}	194.56±1.75^{aA}	NS
P-level	NS	NS	NS	NS	
		Refractive Index (nD 25 °C)			
0	1.464±0.0001^{aC}	1.464±0.0001^{aC}	1.464±0.0001^{aC}	1.464±0.0001^{aC}	NS
6	1.470±0.0001^{aA}	1.471±0.0002^{aA}	1.471±0.0001^{aA}	1.471±0.0001^{aA}	**
12	1.468±0.0001^{aB}	1.468±0.0001^{aB}	1.468±0.0002^{aB}	1.468±0.0001^{aB}	NS
P-level	**	**	**	**	

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* Significant at $p<0.05$; ** Significant at $p<0.01$.

3.5. Effect of gamma irradiation and storage period on saponification value of sunflower seed oil

The sunflower seed oil had a saponification value of 194.02 ± 0.98 mg KOH g^{-1} oil (Table 2). Similar results were reported for Egyptian sunflower seed oil (192.6 mg KOH g^{-1} oil) by [35]. Previous studies conducted by [8] revealed a high sapnnification value (194.84 mg KOH g^{-1} oil) of almond oil and (193.50 mg KOH g^{-1} oil) of pistachio oil [10]. Higher value of saponofication indicates the presence of appreciable quantity of longer chain acids. The results clearly indicated that sunflower seed oil contained mainly higher molecular fatty acids weight i.e C18 and above [38]. Saponification value of oil is an index of average molecular weight of the triglyceride composition of the oil. Values above 200 mg KOH g^{-1} oil indicate the presence of fatty acids of low or fairly low molecular weight, while values below 190 mg KOH g^{-1} oil is an indication that high molecular weight fatty acids is present [39]. The results indicated that both storage time (6 and 12 months) and irradiation doses (3, 6 and 9 kGy) of gamma irradiation had no significant ($p>0.05$) effect on saponification value of sunflower seeds oil.

3.6. Effect of gamma irradiation and storage period on refractive index of sunflower seed oil

Refractive index of fat is an important characteristics for determining the purity or quality of substances. Refractive index of sunflower oil extracted from irradiated and non irradiated seeds are presented in Table 2. The refractive index value of oil extracted from non-irradiated control samples of sunflower seeds was 1.464, which was slightly lower than 1.475 that reported by [21] for sunflower oils extracted from other variety.

Regarding irradiation exposure, the increase in the radiation doses did not have significant ($p>0.05$) effects on the refractive index of sunflower seed oil. Our results are in accordance with the previously reported finding of [40] who also did not observe any significant change in refractive indices between the control and irradiated sunflower oils. The results indicated that storage periods had an effect on refractive index of sunflower seeds oil. A significant ($p<0.05$) increase in refractive index of sunflower oil extracted from irradiated and non-irradiated samples during storage may be due to the exclusion of some saturated fatty acid and / or compounds which could affect this property [36].The refractive index depend on thermal degradation and percentage of polar compounds formed during oxidation and hydrolytic reactions [41]. Earlier research work Lutfullah *et al*. [23] indicated that 1, 5, 10, 15 and 20 kGy of gamma irradiation doses did not have significant effects on the refractive index of sunflower and soybean oil.

3.7. Effect of gamma irradiation and storage period on color of sunflower seed oil

Color parameters of oil extracted from non-irradiated and irradiated sunflower seeds such as lightnes (L*-value), redness (a*-value), yellowness (b*-value), and color difference (ΔE), were determined (Table 3). Gamma irradiation and storage time had a significant ($p<0.05$) effect on all color parameters of sunflower seeds oil. Irradiation doses of 6 kGy and 9 kGy affected the lightness (L* value) of sunflower seed oil with as they presented higher values ($p<0.01$) than control at time zero. After storage, the L*-values of all oil samples decreased. At the end of storage time, the L*-value of oil extracted from sunflower seeds irradiated with 3 kGy were higher ($p<0.05$), while the L*-value of oil extracted from sunflower seeds irradiated with 9 kGy were lower ($p<0.05$) than those of control ones. Redness (a*-value) was decreased with increasing gamma irradiation dose. Higher doses (6 kGy and 9 kGy) had significant effects ($p<0.05$) on a* value. After storage, the a*-values of all oil samples increased ($p<0.05$), and at the end of storage time the a*-value of oil extracted from irradiated sunflower seeds were higher ($p<0.05$) than those of control ones.

Lower doses of gamma irradiation (3 kGy and 6 kGy) increased significantly ($p<0.05$) the b*-value, while the higher dose 9 kGy decreased significantly ($p<0.05$) the b*-value of sunflower oil than those of control ones.

Color difference (ΔE) was decreased with lower doses (3 kGy and 6 kGy) and had significant effect ($p<0.05$) on ΔE. After storage, the ΔE of all oil samples increased. At the end of storage time the ΔE of oil extracted from sunflower seeds irradiated with 3 kGy and 6 kGy were lower ($p<0.05$), while the ΔE of oil extracted from sunflower seeds irradiated with 9 kGy was higher ($p<0.05$) than those of control ones.

There is little or no information available in the literature in the effect of gamma irradiation on the Hunter,s color values of sunflower. However, for other plant materials, and in studying the effect of irradiation treatment on color properties of licorice, Al-Bachir [12] noted that gamma irradiation had no effect on the lightness (L*-value) and redness (a*-value), but the yellowness (b*-value) was less intense of licorice root powders extract. Irradiation affected the colors of the herbal cosmetic products [42] almond [8] peanut [9] and pistachio [10].

Table 3. Effect of gamma irradiation and storage period on color change of sunflower oil.

Treatment	Control	3 KGY	6 KGY	9 KGY	P-level
Storage period/(Months)			L		
0	65.25±0.03^{cA}	65.15±0.02^{cA}	66.94±0.71^{bA}	68.74±0.86^{aA}	**
6	61.90±0.49^{cB}	63.08±0.43^{bB}	62.28±0.16^{cB}	67.49±0.11^{aB}	**
12	51.38±0.09^{bC}	52.62±0.13^{aC}	51.70±0.42^{bC}	49.64±0.16^{cC}	**
P-level	**	**	**	**	
			a		
0	25.25±0.04^{aB}	25.16±0.04^{aB}	24.31±0.19^{bB}	23.29±0.29^{cC}	**
6	22.10±0.74^{aC}	16.64±5.21^{bC}	23.02±0.83^{aC}	26.10±0.21^{aB}	**
12	30.20±0.11^{cA}	32.68±0.13^{bA}	34.90±0.10^{aA}	35.04±0.11^{aA}	**
P-level	**	**	**	**	
			b		
0	29.85±1.19^{cB}	35.25±0.59^{aB}	31.57±0.53^{bB}	26.83±0.29^{aC}	**
6	42.14±1.40^{bA}	41.96±1.08^{bA}	46.24±3.39^{aA}	42.29±0.54^{bA}	**
12	28.01±0.40^{bB}	32.58±0.45^{aC}	31.76±0.38^{aB}	27.88±0.67^{bB}	**
P-level	**	**	**	**	
			ΔE		
0	78.82±0.86^{bC}	76.70±0.21^{aB}	77.42±0.47^{bB}	79.13±0.55^{aC}	**
6	82.14±1.40^{bB}	81.96±1.08^{bA}	86.24±3.39^{aA}	82.29±0.54^{bA}	*
12	84.06±0.23^{bB}	81.50±0.24^{aC}	83.62±0.15^{aB}	87.36±0.35^{bB}	**
P-level	**	**	**	**	

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NS: not significant.
* Significant at $p<0.05$; ** Significant at $p<0.01$.

4. Conclusion

Sunflower seed oil exhibits good physical and chemical properties that enable it ranks good among edible oils. The overall physicochemical properties of sunflower oil treated extracted from seeds irradiated with up to 9 kGy and stored for up to 12 months were determined. It was found that, for all analyzed samples, the acid value ranged from 0.88 to 4.59 %, peroxide value ranged from 2.07 to 3.56 mequiv.g O_2 kg^{-1} of oil, iodine value ranged from 112.65 to 117.22 g iodine 100 g^{-1} oil, specification value ranged from 190.12 to 195.66 g KOH g^{-1} oil, and Refractive index 1.464 to 1.468, These falls within the recommended codex for edible sunflower oils (maximum acid value of 10 mg KOH g^{-1} oil, maximum peroxide level of 10 mg KOH g^{-1} oil, peroxide value of 10 mequiv.g O_2 kg^{-1} of oil, iodine value ranged from 94 to 122 g iodine 100 g^{-1} oil, specification value ranged from 182 to 194 g KOH g^{-1} oil, and Refractive index 1.467 to 1.471.

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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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