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RESEARCH ARTICLE

PHYSICOCHEMICAL PROPERTIES OF OIL EXTRACTS FROM GAMMA IRRADIATED ALMOND (PRUNUS AMYGDALUS L.)

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Abstract

Almond nuts were exposed to radiation doses of 1, 2 and 3 kGy of gamma irradiation covering the range for insect/pest disinfestations and for microbial load. Fatty acid composition, acidity value, peroxide value, iodine value specification number, TBA value and color of almond oil extracted from un-irradiated and irradiated sample were determined, immediately after irradiation and after 12 months of storage. Results showed that non-irradiated almond oil was characterized by high amount of unsaturated fatty acids. The fatty acid profile slightly changed due to irradiation. The major change in fatty acid composition was the decrease in the quantity of fatty acids (C16:0, C18:0 and C18:1) and the increase in the quantity of fatty acid (C18:2). Irradiation reduced acidity value, peroxide value, iodine value and specifications value number of almond lipid. Whereas, the TBA value was almost unaffected. Color parameters L*, a* and b* showed a small but statistically significant ($p<0.05$) increase in all irradiated samples.

Keywords: Almond nut, Color characteristics, Fatty acids, Gamma irradiation, Lipid oxidation.

Introduction

Oils from tree nut are both edible and non-edible depending on the type of the nuts. These oils are often available as raw materials for chemical and industrial applications (Atsu Barku *et al*., 2012). Nuts provide an interesting nutritional supply due to their high nutritive and energetic value (Griel and Kris-Etherton 2006). However, their high fatty content makes them unattractive for new consumers demanding "light", low fatty foods. Among nuts, almonds have a significant economical importance (Atsu Barku *et al*., 2012). Almond nut oil is reported as a possible source of nutritional oil (Agunbiade and Olanlokun, 2006). This is recently receiving much interest due to its many attributed beneficial effects, such as its ability to lower cholesterol, specifically by reducing low-density lipoprotein (LDL) cholesterol, while preserving the beneficial high-density lipoprotein (HDL) (Lovejoy *et al*., 2002).

The possibility of using gamma irradiation to improve the microbiological and fungal quality of different foods has been studied (Bhatti *et al*., 2013). The need to eliminate undesirable microorganisms from food products must always be balanced with the maintenance of product quality (Taipina *et al*., 2009). In many cases, food irradiation is limited due to fatty acid

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decomposition and subsequent off flavor formation in the foodstuff (Taipina *et al*., 2009; Sommers *et al*., 2004). However, as other nuts, almond oil contains high levels of unsaturated fatty acids and are prone to lipid oxidation resulting to the production of hydroperoxides that can be toxic, at high concentrations as well as odorous volatile compounds when irradiated (Mexis and Kontominas, 2009a). Beside, unsaturated fatty acids, radiolysis usually leads to essential acids loss, with nutritional impairment. Irradiation also produces free radicals as a result of radiolysis (Sajilata and Singhal, 2006; Mexis and Kontominas, 2009b).

Until now, there have been no previous studies reported on irradiated or non irradiated Syrian almond seeds (var Baladi). As gamma irradiation is investigated to affect the physicochemical characteristics and nutritive quality of several foods. In this regard, the present work was undertaken to determine the effective gamma irradiation doses required for insect disinfestations and microorganisms decontamination, and the effect on physicochemical properties of oil extracted from irradiated and non-irradiated almond kernels.

Materials and methods

Sample preparation

Samples of almond kernels cv. Baladi (crop year 2010/2011) were exposed to gamma radiation at doses of 0, 1, 2 and 3 kGy in a ^{60}CO package irradiator (dose rate 8.488 kGy h-1). The oils from control and irradiated almond kernels 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 (AOAC, 2010). Physical and chemical properties of oils extracted from irradiated and non-irradiated almonds kernels samples were performed immediately after irradiation, and after 12 months of storage, until the new season.

Physicochemical characteristics of oils

Fatty acids (FA) profile of oils

The lipid fraction of almond oil samples was extracted and FA methyl esters (FAME) were prepared according to Al-bachir and Zeinou (2009). The FA profile was determined by gas chromatography in a GC- 17 A Shimadzu chromatograph (Shimadzu Corp., Koyoto, Japan) equipped with a flame ionization detector and a capillary column (CBP20-S25- 050, Shimadzu, Australia). The selected chromatographic conditions were oven temperature 190 ^{o}C, detector temperature 250 ^{o}C, injector temperature 220 ^{o}C; N2 was used as a carrier gas with split ratio 29:1, the sample volume injected was 1 µl. Peak areas were integrated and converted to FA percentages (direct area normalization) by means of the CLASS – VP 4.3 program (Shimadzu Scientific Instruments, Inc., Columbia, MD). The FA identification was carried out by retention times and by addition of standards.

Chemical analysis

Acidity value (Oleic acid %), Peroxide Value, Iodine value, Specification number and TBA number (Thiobarbituric acid) of almond oil were determined according to standard methods (AOAC, 2010). The acid value was calculated as mg KOH g-1 oil. The peroxide value was calculated in mEqO2 kg-1 oil. Specification number was calculated in mg KOH g-1 oil. The TBA value was calculated in mg MDA kg-1 oil sample (AOAC, 2010).

Color measurement

The color of almond oil was measured using AvaSpec Spectrometer Version 1, 2 June 2003 (Avantes, Holland) and expressed as colour 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 (Kwon *et al.*, 2009). The reported results (L*, a*, b*) are the mean of 9 determination.

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). A separation test on treatment means was conducted using Fisher's least significant differences (LSD) methods at 95% confidence level (Snedecor and Cochran, 1988).

Results and discussion

Effect of gamma irradiation and storage on fatty acid composition of oil

Fatty acids composition of almond oils as a function of irradiation and storage time at room temperature is shown in Table 1. Data presented in table 1 illustrates that fatty acids of non-irradiated (control) almond oil are characterized by high amount of unsaturated fatty acids, oleic acid C18:1 (70.10%), linoleic acid C18:2 (21.07%), low amount of saturated fatty acids, palmitic acid C16:0 (6.91%), and stearic acid C18:0 (1.42%) and small concentration of palmitolic acid C18:1 (0.44%). The fatty acids composition of almond oils reported in this study is in general agreement with that reported in literature (Bhatti *et al*., 2013; Kodad and Company, 2008; Miraliakbari and Shahidi, 2008; Mexis *et al*.,2009). However, unsaturated fatty acids like oleic, linolenic and linolenic acids are fundamental in the human diet as they can't be produced by animal metabolism (Shahidi and Wanasundara, 1998). It is apparent that higher the un-saturation of fatty acids the higher is their oxidation potential.

Table1. *Effect of gamma irradiation and storage period on fatty acids content (%), saturated fatty acids (SFA), unsaturated fatty acids (UFA), and (UFA/SFA) contents of almond oil*

Treatment Storage period (months)	Control	1 KGY	2 KGY	3 KGY	LSD 5%
		C16:0			
0	6.97±0.17	6.95±0.05	6.99±0.03	6.88±0.02	**0.17**
12	6.84±0.05	6.88±0.01	6.93±0.02	6.88±0.03	**0.06**
LSD 5%	**0.28**	**0.08**	**0.06**	**0.05**	
		C16:1			
0	0.44±0.04	0.41±0.04	0.39±0.03	0.36±0.02	**0.06**
12	0.38±0.07	0.36±0.06	0.47±0.15	0.34±0.01	**0.16**
LSD 5%	**0.12**	**0.12**	**0.24**	**0.03**	
		C18:0			
0	1.42±0.01	1.47±0.01	1.47±0.01	1.45±0.03	**0.03**
12	1.46±0.04	1.53±0.01	1.44±0.01	1.45±0.02	**0.04**
LSD 5%	**0.06**	**0.02**	**0.02**	**0.05**	
		C18:1			
0	70.10±0.18	69.97±0.03	69.92±0.06	70.10±0.02	**0.18**
12	69.66±0.09	69.30±0.08	69.78±0.05	69.53±0.07	**0.14**
LSD 5%	**0.31**	**0.14**	**0.13**	**0.12**	
		C18:2			
0	21.07±0.04	21.19±0.06	21.24±0.01	21.21±0.03	**0.07**
12	21.66±0.07	21.94±0.05	21.48±0.03	21.80±0.08	**0.11**
LSD 5%	**0.12**	**0.12**	**0.04**	**0.14**	
		SFA			
0	8.39±0.17	8.42±0.04	8.45±0.04	8.33±0.01	**0.17**
12	8.30±0.01	8.41±0.01	8.37±0.03	8.32±0.02	**0.03**
LSD 5%	**0.28**	**0.07**	**0.07**	**0.03**	
		USFA			
0	91.61±0.17	91.58±0.04	91.67±0.04	91.67±0.01	**0.17**
12	91.70±0.02	91.60±0.01	91.73±0.14	91.68±0.02	**0.13**
LSD 5%	**0.28**	**0.07**	**0.23**	**0.03**	
		USFA/SFA			
0	10.93±0.25	10.88±0.06	10.83±0.05	11.01±0.01	**0.24**
12	11.05±0.02	10.90±0.01	10.97±0.02	11.02±0.03	**0.04**
LSD 5%	**0.39**	**0.09**	**0.09**	**0.05**	

The initial content (day 0) of total saturated fatty acids (SFA) and total unsaturated fatty acids (USFA) in almonds oil was 8.39 and 91.61% respectively, and the ratio between total unsaturated fatty acids and saturated ones (USFA/SFA) was 10.93 for the control almond nut

oil (Table 1). The ratio of total unsaturated over total saturated acids (TMUFA + TPUFA / TSFA) was used to report the shelf life of hazel nuts; including that the lower the ratio, the longer was product shelf life (Fokou *et al.*, 2009).

In the present study, SFA and USFA were not affected by irradiation or storing. There were moderated differences among different doses. Golge and Ova (2008) did not find significant changes in fatty acids composition of pine nuts irradiated at gamma irradiation doses up to 5 kgy. Whereas, Mexis *et al.* (2009) reported no significant ($p>0.05$) change in poly unsaturated fatty acids of almonds kernel irradiated up to a dose of 7 kGy, while monounsaturated fatty acids decreased with a respective increase in saturated fatty acids. In contrast, Afify *et al.* (2013) demonstrated that the ratios of unsaturated fatty acids to saturated total fatty acids (USFA/SFA) of seed oils were significantly altered upon irradiation.

Effect of gamma irradiation and storage period on acidity value of oil

The mean acid value of the oil extracted from non-irradiated almond was 0.47 mg KOH/ g oil (Table 2). The acid value of the almond nut oil was lower when compared with cashew nut oil (0.82 mg KOH g-1) (Aremu *et al.*, 2006) and palm oil (14.04 mg KOH g-1) (Akubugwo and Ugbogu, 2007).

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

Treatment Storage period (months)	Control	1 KGY	2 KGY	3 KGY	LSD 5%
			Acid value (mg KOH g^{-1} Oil)		
0	0.47±0.01	0.38±0.02	0.41±0.001	0.42±0.007	**0.02**
12	1.14±0.02	0.77±0.02	0.78±0.00	0.77±0.01	**0.03**
LSD 5%	**0.03**	**0.04**	**0.01**	**0.02**	
			Peroxide Value (mEqO_2 kg^{-1} Oil)		
0	5.80±0.28	4.87±0.05	3.28±0.08	3.52±0.09	**0.29**
12	5.82±0.05	4.22±0.07	4.85±0.05	5.01±0.11	**0.14**
LSD 5%	**0.46**	**0.14**	**0.16**	**0.22**	
			TBA value (mg MDA kg^{-1} oil)		
0	0.023±0.002	0.024±0.002	0.024±0.002	0.026±0.003	**0.004**
12	0.040±0.001	0.039±0.001	0.041±0.002	0.040±0.002	**0.003**
LSD 5%	**0.001**	**0.004**	**0.004**	**0.005**	
			Iodine number (g I_2 100g $^{-1}$Oil)		
0	98.49±1.20	95.14±0.61	93.79±0.83	93.67±0.03	**1.49**
12	92.83±0.03	93.16±1.93	91.79±1.92	89.02±0.39	**2.59**
LSD 5%	**1.93**	**3.24**	**3.35**	**0.63**	
			Saponification value (mg KOH g^{-1} Oil)		
0	194.84±0.37	190.92±1.42	189.09±2.19	187.07±1.01	**2.66**
12	194.50±0.21	195.42±0.12	194.54±0.65	193.84±1.06	**1.19**
LSD 5%	**0.68**	**2.29**	**3.67**	**2.35**	

Acid value presents free fatty acid content due to enzymatic activity and is usually indicative of spoilage. Acid value is used as an indicator for edibility of oil and suitability for use in the pain industry (Atsu Barku *et al.*, 2012). The maximum acceptable level is 4 mg KOH g-1 oil (Codex Alimentarius, 1992). Since the acid value of the almond is lower than the maximum permissible acid levels required for edible virgin fats and oils, the almond nut oil is suitable for direct consumption. As shown in Table 2. acidity values decreased ($p<0.05$) from an initial value of 0.47 to a value of 0.38, 0.41 and 0.42 mg KOH g-1 almond nut oil after irradiation at doses of 1, 2 and 3 kGy, respectively. Storage significantly ($p<0.05$) increased the acidity value for irradiated and non-irradiated samples. The acidity value determined for lipid extracted from irradiated and non-irradiated almond nut samples in the present study is in good agreement with that of Bhatti *et al.*

(2013) who reported an increase in free fatty acids from 1.11% (for control) to 1.34% for oils extracted from gamma irradiated almonds seeds, might be due to slight random hydrolysis of triglycerol molecules to free fatty acids and diacylglycerols (Al-Bachir, 2004; Anjum *et al*., 2006).

Effect of gamma irradiation and storage period on peroxide value of oil

Lipid oxidation of oil produced from irradiated and non-irradiated almonds was evaluated by measuring a) peroxide value (PV) in term of meq O_2 kg^{-1} oil and b) TBA value (Thiobarbituric acid) in term of mg MDA kg^{-1} oil sample. Changes in PV and TBA are given in Table 2. The initial PV of oil produced from non-irradiated (control) almond kernel was low (5.80 ± 0.28 meq O_2 kg^{-1} almond oil). The PV value determined for almonds oil in the present study agrees with that of Buransompob *et al.* (2003), Sanchez-Bel *et al.* (2005) and Mexis *et al.* (2009). According to Buransompob *et al.* (2003), fresh almonds had a PV less than 2.0 meq O_2 kg^{-1}, while a value of 25 meq O_2 kg^{-1} oil may be considered as the acceptable threshold for almonds (Severini *et al*., 2000).

In literature, it was mentioned that especially the hydroxyl radicals formed after irradiation of the foods that have a high water content may trigger fat oxidation and change the composition of fatty acids oppositely same reactions occur slower in dry foods (Sant Ana and Mancini-Filho, 2000; Brito *et al*., 2002).

The effect of various levels of gamma irradiation on TBA value oil extracted from irradiated and non-irradiated almond is shown in Table 2. The TBA value of oil produced from non-irradiated (control) almond kernel was 0.023 mg MDA kg^{-1} oil. There was no significant ($p > 0.05$) difference between irradiated and control groups. In the irradiated groups, no significant ($p > 0.05$) difference was found as irradiation dose level increased, while storage significantly increased the TVBN for irradiated and non-irradiated samples.

These results are somehow in agreement with those of Mexis and Kontominas (2009b) and Uthman *et al.* (1999) who reported that TBA values of irradiated almonds increased significantly ($p < 0.05$) at doses up to 6 kGy and Mexis *et al.* (2009) who reported a small, but statistically significant changes in hexanal concentration as a results of irradiation of almonds at doses up to 7 kGy

Effect of gamma irradiation and storage period on iodine value of oil

The iodine value of lipid extracted from non irradiated (control) almond samples which is useful in predicting the drying properties of oil was 98.49 ± 1.49 (g I 100 g^{-1} oil) (Table 2). The iodine value is an index for assessing the ability of oil to go rancid (Adelaja, 2006).

The iodine values of the control almonds oils (98.49 ± 1.49 g I 100 g^{-1} oil) were found to be substantially decreased to the levels as low as 95.14 ± 1.49, 93.79 ± 0.83 and 93.67 ± 0.03 g I 100 g^{-1} for samples produced from almonds exposed to 1, 2 and 3 kGy, respectively. Generally, at higher doses, the decline in the oils iodine values was more remarkable. The decreasing trend in the oil iodine value upon irradiation in this study might refer to the saturation of the oil as a result of the breakdown of double bonds due to oxidation deterioration in the fatty acids. A similar decreasing trend in iodine value has already been seen (Al-Bachir, 2004; Anjum *et al*., 2006; Bhatti *et al*., 2010; Yaqoob *et al*., 2010; Bhatti *et al*., 2013).

Effect of gamma irradiation and storage period on saponification value of oil

The oil extracted from non-irradiated almond had a high saponification values (194.84 mg KOH g-1 oil) (Table 2). There is an inverse relationship between saponification number and weight of fatty acids in the oil. Dosunmu and Ochu (1995) reported that the almond oils contain a great number of fatty acids of low molecular weight, and can thus be employed in the soap industry and in manufacture of lather shaving creams (Eka, 1980). As shown in Table 2, saponification values decreased ($p < 0.05$) from an initial value of 194.84 to a value of 190.92, 189.09 and 187.07 mg KOH g-1 almond nut oil after irradiation at doses of 1, 2 and 3 kGy respectively.

The present findings were in contrast with previous studies, where it were found that saponification values of oil extracted from irradiated almond was increased upon irradiation (an increase from 185-187 to 204-231 mg KOH g-1) (Bhatti *et al*., 2013) which indicated that large original molecules of oils containing long-chain fatty acids degraded to smaller molecules as a results of oxidation and cleavage of bonds (Agatemor, 2006).

Effect of gamma irradiation and storage period on color of oil

The effects of gamma irradiation and storage on color are shown in Table 3. Almond oil color varies from light yellow to deep red, and most pigments in plants, especially red, purple and blue, belong to the flavonoid class of anthocyanins, with other flavonoid compounds acting as co-pigments (Chukwumah *et al*, 2009). Color can be affected by gamma radiation (Mexis and Kontominas, 2009 Mexis *et al*., 2009). In the present study, some color change due to irradiation was found in almonds oil samples at time zero and after 12 months of storage. In almonds oil, their L* (lightness) increased at 1, 2 and 3 kGy (time zero), and at 1 kGy (twelfth month). An increase in a* (redness) in almonds oil samples was observed at 1, 2 and 3 kGy (time zero and twelfth month). Also an increase in b* (yellowness) in almonds oil samples was observed at 1 and 2 kGy (time zero and twelfth month).

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

Treatment Storage period (months)	Control	1 KGY	2 KGY	3 KGY	LSD 5%
		L			
0	55.08±0.07	55.12±0.13	57.21±0.32	59.63±0.84	**0.86**
12	51.38±0.09	52.62±0.13	51.70±0.42	49.64±0.16	**0.45**
LSD 5%	**0.17**	**0.25**	**0.85**	**1.37**	
		a			
0	25.45±0.49	28.53±0.18	28.31±0.19	28.29±0.90	**0.99**
12	30.20±0.11	32.68±0.13	34.90±0.10	35.04±0.11	**0.22**
LSD 5%	**0.81**	**0.35**	**0.35**	**1.45**	
		b			
0	29.85±1.19	35.25±0.59	31.57±0.53	26.83±0.29	**1.38**
12	28.01±0.40	32.58±0.45	31.76±0.38	27.88±0.67	**0.92**
LSD 5%	**2.01**	**1.19**	**1.05**	**1.17**	
		ΔE			
0	68.59±0.92	66.40±0.48	67.22±0.28	68.93±0.20	**1.03**
12	74.06±0.23	71.50±0.24	73.62±0.15	77.36±0.35	**0.48**
LSD 5%	**1.53**	**0.86**	**0.50**	**0.65**	

Mexis and Kontominas (2009a) reported that color parameters b* of hazelnuts increased ($p<0.05$) after irradiation at dose of > 5 kGy, while color parameter L* and a* remained unchanged by irradiation. Mexis et al. (2009) reported a decreased in L* parameter ($p<0.05$) at dose > 3 kGy, while color parameters a* and b* remained unaffected after irradiation of almonds kernels at doses up to 7 kGy.

Golge and Ova (2008) reported statistically significant change in L* and b* values for pine nuts at doses between 0.5 and 5 kGy. Mexis and Kontominas (2009a) reported statistically significant change in a* values for cashew nuts at doses 1.0, 1.5 and 3 kGy.

Conclusion

The physicochemical properties of oil extracted from irradiated and non-irradiated Baladi almond nuts have been studied. The result obtained shows that the almond oil is good for consumption when freshly produced, since the physico-chemical characteristics are within the stipulated limits

recommended by Codex. High saponification value guarantees the use of the oils in cosmetics industry. However, the oil recorded high iodine values suggesting almond oil is highly unsaturated and may be susceptible to rancidity. The results of this study showed that gamma irradiation up to 3 kGy did not significantly affect the oils quality, as judged from fatty acid profiles, peroxide value, iodine value, saponification number and TBA value, and with little change in acidity value, iodine value and color parameters L*, a* and b* due to irradiation.

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