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RESEARCH ARTICLE

ASSESSING THE EFFECTS OF GAMMA IRRADIATION AND STORAGE TIME IN QUALITY PROPERTIES OF ALMOND (*PRUNUS AMYGDALUS* L.)

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Abstract: The objective of the present study was to evaluate the effect of gamma irradiation doses (1, 2 and 3 kGy), and storage period (12 moths) on the microbial load, chemical characteristics, and sensory properties of Baladi almond nuts. The present work confirmed that, irradiation resulted in reduction of the number of total bacterial and fungal load of almonds. Gamma irradiation had no effect on chemical composition of almond nut kernels with the exception of total volatile basic nitrogen (TVBN) for irradiation dose of 3 kGy. During storage, the total fat and total protein were increased, but the total sugar was decreased. The TVBN increased due to irradiation doses and storage period. A trained sensory panel found that irradiation produced no significant change in texture, color, odor, and test attributes. Present analysis revealed that gamma irradiation could be successfully employed to preserve the overall quality to improve the shelf life to ensure the safety of almond nuts.

Key words: almond nuts, Irradiation, microbial load, proximate composition storage.

Introduction

In recent years, numerous scientific studies have demonstrated the beneficial effects of the consumption of nuts for human health, these being an important component of a balanced diet due to their high nutritional value (Sanchez-Bel *et al.*, 2008). Almond nut is cultivated globally, with about 28% of its worldwide production derived from the Mediterranean region (Chen *et al.*, 2005; Wijeratne *et al.*, 2006). As other tree nuts, almond kernels are susceptible to infestation by molds, insects and larvae (Khan *et al.*, 2005) and fungi (Khosravi *et al.*, 2007). The various post-harvest procedures for control of insects and mites in stored products are chemical, biological and physical control or combination of these techniques (Johnson, 2004; Kown *et al.*, 2004).

Food irradiation has been used for treatment for disinfestations (Mansour and Al-Bachir, 1995). Compared to hydro, thermal, and chemical methods, ionizing radiation has the advantage of retaining the quality of nuts. However, radiation efficacy varies among studies due to different sample conditions during treatment (Cetinkaya *et al.*, 2006; Hallman, 2013). Many studies have been carried out on the effect of irradiation treatment to protect food commodities from oxidation (Al-Bachir, 2004; Alighourchi *et al.*, 2008), insect infestations

(Mansour and Al-Bachir, 1995; Thomas *et al*., 2008), and microbial contamination during storage and processing (Braghini *et al*., 2009; Yusof *et al*., 2007). Moreover, there are also many reports supporting the use of gamma irradiation as fungicidal agent (Aziz *et al*., 2007; Maity *et al*., 2004). However, the effect of those to qualities of almonds produced and marketed under Syrian conditions and ability to use gamma irradiation to substitute traditional methods has not been studied. Thus, this study was conducted to evaluate the ability of gamma irradiation and storage time to ensure the microbial safety and their effect on chemical quality and sensory properties of Syrian almonds nuts (*var Baladi*).

Materials and methods

Almond nuts cv. Baladi (crop year 2010/2011) were obtained from local agricultural products supplier in cotton pouches, in Damascus, Syria. Then almond nut kernels were weighed as in the sampling plan and transferred into polyethylene pouches for irradiation. Each pouch of almond nut (250 g) was considered as a replicate.

Treatments and analysis performed

Samples from almond nuts were exposed to gamma radiation at doses of 1, 2 and 3 kGy in a ^{60}CO package irradiator (dose rate 8.488 kGy h^{-1}). The irradiation was performed at room temperature (15–20 ^{o}C). The absorbed dose was determined using alcoholic chlorobenzene dosimeter (Al-Bachir, 2004). Irradiated and non-irradiated samples were stored for 12 months at room temperature 18-25 ^{o}C under relative humidity (RH) of 50-70%. Microbiological and chemical analyses were performed on controls and treated samples immediately after irradiation, and after 12 month of storage. Sensory evaluation was done within two days of irradiation.

Microbiological analysis

Three replicates from each treatment, un-irradiated and irradiated, were aseptically opened, and 10 g of whole almond nut kernels were transferred to a sterilized glass bottle containing 90 ml of sterile physiological water (9 g NaCl L^{-1}). The bottle was shaken to homogenize the sample. Further dilutions were made as far as 10^{-6} (AOAC, 2010). The media used for the microbiological study were nutrient agar for the total bacterial plate counts (TBPCs), agar plate counts (APCs) (Oxoid, CM 325, UK) (48 h incubation at 30 ^{o}C). Fungi were enumerated on Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) (Merck, 1.00466, Germany) after incubation at 25 ^{o}C for 5 days. Bacteria and fungi were determined and counted as CFU g^{-1} (CFU=colony forming units) according to the methods of AOAC (2010).

Chemical analysis

Approximately 150 g of almond nut kernels were blended for 15 s in a laboratory blender, and was used in all the chemical analysis. Each sample was homogenized and analyzed in triplicates, to determine moisture and ash (drying for 6 h at 105 ^{o}C, and ashing for 4 h at 550 ^{o}C), crude fat (as extractable component in Soxhlet apparatus) and crude protein (as Kjeldahl nitrogen) using standard methods (AOAC, 2010). Total sugar was estimated using Anthrone indicator method by measuring the absorbance at 620 nm with a T70 UV/VIS Spectrophotometer, (PG Instrument Ltd). The reducing sugars were estimated by iodometric determination of the unreduced copper remaining after reaction, and the concentration of reducing sugars were expressed as g glucose/ 100 g powders (AOAC, 2010).

pH values of the solutions of almond nut kernels were determined using an HI 8521 pH meter (Hanna Instruments, Woonsocket, RI, USA). The total acidity was obtained by a direct titration with (0.1 N) NaOH and phenolphthalein as an indicator. The total acidity was calculated as ml of (0.1 N) NaOH = 0.0090 g lactic acid (AOAC, 2010). Total volatile basic nitrogen in the sample in term of mg VBN kg-1 almond nuts (ppm) was determined (Al-Bachir, 2004).

Sensory evaluation

Sensory evaluation (consumer analysis) was carried out by 30 member untrained panel. Approximately 20 g of whole almond nut kernels were placed in small glasses coded containers. Panelists were served a set of four treated samples (0, 1, 2, and 3 kGy) and they were instructed to consume the whole sample and rinse mouth with sparkling water (room temperature), in between sample evaluation. Sensory attributes evaluated included color, texture, odor and taste. Scoring was carried out on paper ballots using a 5 point hedonic scale where: 1 = extremely poor, 2 = poor, 3 = acceptable, 4 = good, 5: excellent) (Al-Bachir, 2004).

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 time on proximate composition of almond nut

Proximate composition of un-irradiated and irradiated almond nut (*var. Baladi*) kernel is given in Table 1. The almond nut analyzed contained (4.44 ± 0.09%) moisture, (17.92 ± 0.04%) crude protein, (63.74 ± 0.84%) fat, (2.92 ± 0.05%) ash, (12.32 ± 0.81%) total sugar and (2.19 ± 0.03%) reducing sugar.

The composition of the almond nuts is differ to the study based on the almond nuts reported by Bhatti *et al.* (2013) and USDA (2008) which contains 40.0% oil, 7.3% water, 22.7% protein, 5.6% fibre, 4.2% ash, and Sanchez-Bel *et al.* (2008) which contains 53.67- 58.64% oil, 3.82 – 3.93 % water, 24.16 – 28.37 protein and 4.26 – 6.10 %.

The present results indicated that the moisture, protein, lipid, ash, total sugar, and reducing sugar of almond nut were not substantially affected by gamma irradiation. Similar finding showed that gamma irradiation with doses up to 10 kGy had no real effect on moisture, protein, lipid, fibre and ash of almond (Bhatti *et al.*, 2013; Bela *et al.*, 2008; Sanchez-Bel *et al.*, 2008). Maity *et al.* (2009) reported that gamma radiation could change the seed protein quantitatively, not the qualitatively. The easy-to-digest and difficult-to-digest proteins were not significantly affected up to 4 kGy. However, the soluble free amino acids of all the seeds increased with increasing dose.

Gamma irradiation breaks the seed protein and produces more amino acids (Kiong *et al.*, 2008). Moreover, the crude protein and fat in a complex matrix of foodstuffs have been reported to be more resistant to radiation than in the pure state (Sanchez-Bel *et al.*, 2008).

The radiolytic compounds that can be formed, after an irradiation treatment, from the compounds already present in the foodstuff depend directly on water content; because of this, when nuts or dehydrated materials are irradiated, the expected modifications are much less, but, the damage inflicted by irradiation on the peptide bond depends, in addition to the degree of hydration of the materials, greatly on the oxygen content, since this bond is highly stable and is not normally broken by the irradiation dose generally applied to foodstuffs (Sanches-Bel *et al.*, 2008; Krumhar and Berry, 1990).

Thus, given that the moisture content of samples in this study was very low (4.44 %), it was to be expected that protein, fat and sugar contents would remain largely unaltered throughout the assay, for all treatments applied. The level of radiation dosage, applied in this study (1, 2 and 3 kGy), did not favour the production of enough radiolytic products and ultimately did not induce significant changes in the gross composition.

However, during storage at room temperature, the total protein and total fat of almond nut also significantly ($p<0.05$) increased in both irradiated and non-irradiated samples.

Whereas, after 12 months of storage the total sugar of almond nuts significantly ($p<0.05$) decreased in both irradiated and non-irradiated samples.

Our observation is in agreement with Sanchez-Bel *et al.* (2008) who reported that all of irradiated and non-irradiated samples of almond exhibited a decline in sucrose content concentration, until the end of storage periods (6 months).

Table 1. *Effect of gamma irradiation and storage period on moisture, ash, protein, total sugar, reducing sugar and fat contents (%) of almond.*

Treatment	Control	1 KGY	2 KGY	3 KGY	LSD 5%
Storage period (Weeks)					
Moisture (%)					
0	4.44±0.09	4.33±0.12	4.43±0.12	4.37±0.06	**0.19**
12	4.05±0.79	4.24±0.12	4.06±0.32	4.06±0.32	**0.83**
LSD 5%	**1.28**	**0.28**	**0.55**	**0.26**	
Total protein (%)					
0	17.92±0.04	17.87±0.12	17.90±0.01	17.77±0.07	**0.13**
12	19.87±0.14	19.41±0.65	18.14±0.03	19.43±0.64	**0.87**
LSD 5%	**0.24**	**1.07**	**0.04**	**1.03**	
Total fat (%)					
0	63.74±0.84	63.56±1.38	61.09±0.77	60.73±0.38	**1.73**
12	57.13±4.39	62.63±0.52	62.84±0.66	62.63±0.57	**4.24**
LSD 5%	**7.16**	**2.37**	**1.63**	**1.10**	
Ash (%)					
0	2.92±0.05	2.83±0.02	2.85±0.02	2.80±0.02	**0.05**
12	2.86±0.07	2.85±0.03	2.84±0.04	2.82±0.13	**0.15**
LSD 5%	**0.13**	**0.05**	**0.07**	**0.21**	
Total sugar (%)					
0	12.32±0.81	13.04±0.48	12.64±0.37	13.24±0.35	**1.01**
12	9.31±0.74	9.74±0.69	9.94±0.42	9.56±0.50	**1.14**
LSD 5%	**1.76**	**1.35**	**0.90**	**0.98**	
Reducing sugar (%)					
0	2.19±0.03	2.20±0.03	2.17±0.04	2.14±0.06	**0.08**
12	2.23±0.02	2.25±0.03	2.23±0.01	2.22±0.03	**0.04**
LSD 5%	**0.06**	**0.06**	**0.07**	**0.10**	

Effect of irradiation and storage time on chemical properties of almond

Total acidity and pH: Change in total acidity and pH value of irradiated and non-irradiated almond nut samples during storage at room temperature are shown in Table 2. The mean acid value (lactic acid %) of the almond nut were 0.33, 0.28, 0.27 and 0.31% for sample treated with 0, 1, 2 and 3 kGy, respectively. Total acidity values increased during storage time. Comparison of data in Table 2, leads to conclusion that storing time had more pronounced effect on total acidity than irradiation. Total acidity values of irradiated and non-irradiated almonds samples are very small between 0.27 and 0.49% and lies within desirable limits 0.0–3.0% (Kimbonguila *et al.*, 2010). Given the very low moisture content of almond nut kernels and the relatively low irradiation doses applied, it is most probable that no free fatty acids were produced through triglycerides hydrolyses. According to the literature, irradiation of high moisture content foods results in high hydroxyl radicals concentrations which trigger fat oxidation, leading to changes in fatty acid composition of fatty foodstuffs. Such reactions are expected to be slower in dry foodstuffs such as nuts (Brito *et al.*, 2002; Santana and Mancini, 2000). Increases in irradiation doses up to 10 kGy resulted in an increase in free fatty acids (FFA) levels. A dose-dependent decrease in the triacylglycerol content and concomitant increase in free fatty acids was observed after gamma irradiation of nutmeg (Niyas *et al.*, 2003). This suggested a breakdown of acylglycerol during radiation processing resulting in the release of free fatty acids (Niyas *et al.*, 2003). The pH values of almond samples ranged between 6.26 - 6.50. However, there was no significant ($p>0.05$) changes in pH value of almond nut after the irradiation treatment nor after storage (Table 2).

Total volatile basic nitrogen (TVBN): The effect of various levels of gamma irradiation (1, 2 and 3 kGy) and storage time (12 months) on TVBN contents of almond nut is shown in table 2. The data present in Table 2 illustrated that the amount of the TVBN in non-irradiated (control) almond nuts were 288.13 g TVBN kg^{-1} almond nut part per million (ppm), whereas the gamma irradiation with doses levels of 1, 2 and 3 kGy increased significantly (p>0.05) the TVBN value. The TVBN values of almonds nuts were 325.41, 322.28 and 353.08 g TVBN kg^{-1} almond nut (ppm) at irradiation dose level of 1, 2 and 3 kGy, respectively. During storage, the TVBN decreased in irradiated almond kernels. The increase in TVBN in stored and irradiated almonds nuts (immediately after irradiation) may be attributed to the formation of oxidation or radiation-induced degradation products. Adamo *et al.* (2004) have opined that the destructive processes of gamma irradiation are capable of breaking the chemical bonds of poly-sup-products, thereby releasing soluble sup-products of low molecular weights, leading to an increase of such products. It is obvious that as irradiation doses increased, high energy is absorbed by the food substrate given rise to generation of new volatile compounds through oxidation (Lee and Ahn, 2003).

Table 2. *Effect of gamma irradiation and storage period on total acidity (%Lactic acid), PH value and volatile basic nitrogen (VBN)(P.P.M) of almond.*

Treatment	Control	1 KGY	2 KGY	3 KGY	LSD 5%
Storage period (Weeks)					
Total acidity (Lactic acid %)					
0	0.33±0.03	0.28±0.01	0.27±0.01	0.31±0.01	**0.04**
12	0.49±0.03	0.48±0.02	0.48±0.03	0.49±0.02	**0.04**
LSD 5%	**0.06**	**0.03**	**0.06**	**0.03**	
pH value					
0	6.31±0.02	6.27±0.02	6.27±0.03	6.26±0.02	**0.04**
12	6.50±0.14	6.42±0.01	6.42±0.01	6.42±0.02	**0.13**
LSD 5%	**0.23**	**0.03**	**0.05**	**0.04**	
Volatile basic nitrogen (P.P.M)					
0	288.13±21.21	325.41±19.18	322.28±16.38	353.08±3.63	**31.21**
12	297.44±10.33	281.64±41.92	283.55±11.77	281.66±12.12	**43.65**
LSD 5%	**37.81**	**73.90**	**32.34**	**20.27**	

Effect of irradiation and storage time on microbial load of almond

The control sample of almond nuts exhibited rather low microbiological contamination; the initial contamination bacterial load of almond kernel sample was of 2.44 log CFU g^{-1}, and the initial fungal viable count was 3.37 log CFU g^{-1}. The results given in Table 3 showed that this total bacterial and fungal load decreased due to irradiation. Although all almond samples contained no living bacteria or fungus after irradiation at doses of 1, 2 or 3 kGy, therefore 1 kGy was enough for complete decontamination of almond nuts. The results suggested that Baladi almond kernels should be treated with a dose of 1 kGy or more to keep them microbiologically acceptable for 12 months. No information in the literature is available on the effect of gamma irradiation on the microbial load of Baladi or other varieties of Syrian almond, but decrease in microbial load of other variety of almonds (Bhatti *et al.*, 2013; Jeong *et al.*, 2012), seeds (Aziz *et al.*, 2007), and walnuts (Al-Bachir, 2004) following gamma irradiation is reported by several researchers.

Regarding fungi, Calado *et al.* (2011) studied the effect of gamma radiation (doses of 0.25 to 10 kGy) on the survival of yeast and *Aspergillus parasiticus*, on the most ubiquitous and toxigenic fungi. The authors reported that both yeast and *A. parasiticus* load decreased with at least 3 kGy, and did not survive at all when irradiated with a dose of 10 kGy. The sensitivity of fungi to gamma irradiation has been established by many investigators (Aziz *et al.*, 2007; Mahrous *et al.*, 2003) who recorded that the dose required for complete inhibition of fungi in different food products ranged from 4 to 6 kGy. Aziz and Moussa (2004) found that maize, check-peans and groundnut seeds collected from the Egyptian marked were heavily contaminated by moulds and irradiation at a dose 4.0 kGy reduced mould growth greatly relative to unirradiated controls and there was no growth of the toxigenic moulds and mycotoxins in seeds using a dose of 5.0 kGy.

Table 3. *Total bacterial (log_{10} CFU/g) and fungal (log10 spores /g) count of almond.*

Treatment	Control	1 KGY	2 KGY	3 KGY	LSD 5%
Storage period(Weeks)					
Total bacterial count (log_{10} CFU/g)					
0	2.44±0.07	>1	>1	>1	**0.06**
12	3.23±0.05	>1	>1	>1	**0.04**
LSD 5%	**0.13**				
Fungal count (log_{10} spores /g)					
0	2.37±0.10	>1	>1	>1	**0.13**
12	2.74±0.03	>1	>1	>1	**0.02**
LSD 5%	**0.16**				

Effect of irradiation and storage time on sensory quality of almond

Taste, flavor, color and texture for almond nut samples are presented in Table 4. There were no significant differences between control samples and irradiated almond samples. Previous work by other groups on those parameters in other kinds of nut also showed no effect of irradiation treatment; Golge and Ova (2008) reported that irradiation did not affect sensory attributed of pine nuts. Likewise, Al-Bachir (2004) reported no substantial changes in volatiles and odor between non-irradiated and irradiated walnuts at doses of 0.5, 1.0 and 1.5 kGy. Mexis *et al.*, (2009) reported that almonds remained organoleptically acceptable after irradiation up to a dose of 3 kGy. Mexis and Kontominas, (2009) reported that sensory evaluation showed that texture and color of cashew nuts were not affected by irradiation. O'Mahony (1985) reported no change in flavor and mouth feeling of irradiated almonds were detected by the panellists. However, deterioration in odor and taste is related mainly to lipid oxidation, but also to amino acid and/or carbohydrate breakdown (Siegmund and Murkovic, 2004).

Table.4 *Effect of gamma irradiation and storage period on the taste, texture, color and flavor of almond.*

Treatment	Control	1 KGY	2 KGY	3 KGY	LSD 5%
Taste	3.88±0.99[1]	3.67±0.87	3.54±1.10	3.79±1.25	**0.61**
Flavor	3.92±1.06	3.71±0.86	3.58±1.21	3.58±1.25	**0.63**
Color	4.00±0.83	3.96±0.86	3.67±1.09	3.71±1.33	**0.60**
Texture	4.25±0.68	4.00±0.78	4.00±0.83	3.83±1.17	**0.51**

[1] Data represent a 5 point scale ranging from 1 (very bad) to 5 (very good); n = 30 person

Conclusions

The present study showed that gamma irradiation could be used as a decontaminating tool on Baladi almond nut. A dose of 1 kGy is sufficient to eliminate bacterial and fungal load of almond kernels. The obtained results seem to indicate that the irradiation treatment did not affect the chemical quality and sensory properties of almond nuts fruits.

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