ORIGINAL RESEARCH PAPER

MICROBIOLOGICAL, SENSORIAL AND CHEMICAL QUALITY OF GAMMA IRRADIATED PISTACHIO NUT (*PISTACIA VERA* L.)

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The present study investigated the effect of gamma irradiation and storage period on quality retention of raw pistachio nut. Var. Halebi. Kernel of the pistachio nuts were exposed to 1, 2 and 3 kGy of gamma irradiation. Irradiated and unirradiated nuts were kept at room temperature for 12 months. Used doses of irradiation significantly reduced the total bacterial plate counts (TBPCs) and total fungal counts up to undetectable level (less than 10 CFU g⁻¹). Irradiation doses of 1, 2 and 3 kGy of gamma irradiation seem to be suitable for post-harvest sanitation and decontamination treatment, without significant changes in the sensorial properties (texture, odor, color and taste), chemical quality (free fatty acids and pH value) or in contents of moisture, proteins, sugars, lipid, and ash, with respect to the control samples. The highest used dose (3kGy) slightly decreased the fatty acid content and pH value, and treatment with higher doses (2 and 3 kGy) significantly increased the total volatile nitrogen TVN.

Keywords: chemical analysis, gamma irradiation, microbial load, pistachio nuts, sensory evaluation

Introduction

The pistachio nut is grown mainly in Iran, USA, Syria, Turkey, Greece and Italy (Kucukoner and Yurt, 2003; Faruk Gamli and Hayoglu, 2007). Crude pistachio nuts are of great importance in foods worldwide and are ingredients of many recipes. They are mainly consumed salted, roasted, in confectionery and as a snack food, and are also used as the main ingredient of deserts such as baklava and other excellent kind of local sweets in Syria and Turkey (Faruk Gamli and Hayoglu, 2007).

Nuts typically have one or more pre-harvest insect pests that feed directly on the product and are capable of causing considerable damage and quality loss (Johnson, 2004). The damage caused by these insects during their feeding may also favor the entry of aflatoxin – producing molds into pistachio nuts (Campbell

et al., 2003). There are many reports about the presence of mycotoxins, especially aflatoxin in nuts (Fernance *et al.*, 2010; Reis *et al.*, 2012) For the time being, one of the most effective and prevalent methods of disinfestations is using fumigants. Some fumigants have undesirable effects on food products, especially on fruits and vegetables. Methyl bromide is one of the prevalent fumigants (Johnson, 2004). On the basis of Montreal protocol, developed countries by 2005 and developing countries by 2015 have to eliminate using it, because of its ozone depleting properties (UNEP, 2006).

Used for stored products disinfestations, irradiation as an alternative to fumigants was debated (Kwakwa and Prakash, 2006). The applicability of the irradiation process and the effects on quality characteristics in foods were investigated in many researches (Al-Bachir, 2004; Golge and Ova, 2008; Mexis and Kontominas, 2009; Sanchez-Bel et al., 2008; Smith and Pillai, 2004). Gamma irradiation can be an effective alternative technology in post-harvest pest control because of its ability to kill insects (Sirisoontaralak and Noomhorm, 2006) and inhibit mycotoxin biosynthesis during storage (Kabak et al., 2006). According to the literature, it is a practical and suitable method for nut disinfestations (Al-Bachir, 2004). However, a study of the relationship between radiation absorbed doses and possible changes in the composition of food stuffs must be carried out in order to comprehensively assess the acceptability of irradiated processed foods (Azim et al., 2009). Until now there have been no reported previous studies on irradiated pistachio nut in Syria, even though the annual pistachio production in Syria is around 48,000 tons. In this regard, the present research was undertaken to investigate the effect of medium doses of gamma irradiation on the proximate composition, chemical characteristics, microbial properties and sensorial quality of pistachio (var Halebi).

Materials and methods

Samples of peeled kernels cv. Halebi pistachio nuts (crop year 2010/2011) were purchased from local shops in Damascus, Syria. About 250 g of pistachio kernels in form of single layer was packed in small polyethylene bag for irradiation (film thickness of the polyethylene bag 0.087 mm properly sealed). Each bag of pistachio kernels was considered as a replicate. Irradiated and unirradiated samples were stored at room temperature 18 to 25 °C under a relative humidity (RH) of 50 to 70%.

Treatments and analysis performed

Samples from pistachio kernels were exposed to gamma radiation at doses of 1, 2 and 3 kGy in a 60CO package irradiator (ROBO, Russa). The source strength was approximately 55 kCi with a dose rate of 8.488 kGy h⁻¹. The irradiation was performed at room temperature (15-20 °C). Each replicate was irradiated in box from both sides. The absorbed dose was determined using alcoholic chlorobenzene dosimeter (Al-Bachir, 2004). For each treatment, 12 bags of pistachio kernels were allocated and all were stored at room temperature. Microbiological and chemical analyses were performed on controls and on treated samples immediately after irradiation and after 12 months of storage. Sensory evaluation was done within two days of irradiation.

Microbiological evaluation

Three replicates from each treatment, un-irradiated and irradiated, were aseptically opened, and 10 g of whole pistachio kernels were transferred to a sterilized glass bottle containing 90 ml of sterile physiological water (9 g kg⁻¹ NaCl) and homogenized. Decimal dilutions (AOAC, 2010) were used for microbial counting. 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°C). Fungi were enumerated on Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) (Merck, 1.00466, Germany) after incubation at 25°C for 5 days.

Chemical analysis

Approximately 150 g of pistachio nut kernels were blended for 15 s in a laboratory blender and were used for all the chemical analyses. Each sample was homogenized and analyzed in triplicate to determine moisture and ash (drying for 6 h at 105° C, and ashing for 4h at 550° C), crude fat (as extractable component in Soxhlet apparatus) and crude protein (as Kjeldahl nitrogen) using standard methods (AOAC, 2010). pH values of the solutions of pistachio nut kernels were determined using a 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 used as an indicator. The total acidity was calculated as ml of 0.1 N NaOH = 0.0090g lactic acid. Total volatile basic nitrogen in the sample in terms of mg VBN kg⁻¹ pistachio kernels was also determined (Al-Bachir, 2004).

Sensory evaluation

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

Statistical analysis

Sensorial analysis results 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) method at 95% confidence level (Snedecor and Cochran, 1988).

Results and discussion

The effects of gamma irradiation (1, 2 and 3kGy) and storage time (12 months) were assessed by evaluating change in microbial load, nutritional composition and sensorial properties of the selected pistachio nut samples. Considering both effects together, it is possible to understand the influence of irradiation dose independently of storage time and vice versa, an essential requirement to consider gamma irradiation as a feasible conservation technique.

Proximate composition

The proximate chemical composition of dried pistachio nut kernels is shown in Table 1. The contents of moisture, protein, lipid, ash, total sugar, and reducing sugar were: 42.0 ± 0.9 , 214.7 ± 3.2 , 554.3 ± 4.1 , 22.4 ± 0.1 , 100.6 ± 6.7 , and 26.6 \pm 0.1 g kg⁻¹, respectively. However, most of those components were in accordance with those reported for pistachio kernels by many investigators (Kucukoner and Yyrt, 2001; 2003; Pala et al., 1994). The whole Halebi pistachio nut kernel moisture content was quite low compared to other kind of nuts (Jeong et al., 2012; Bhatti et al., 2013), or oil seeds (Kimbohguila et al., 2010). The low moisture level of product samples could store for longer time without spoilage, since higher moisture content could lead to food spoilage through increasing microbial action (Kimbohguila et al., 2010). The Halebi pistachio kernels are a good source of protein (214.7 g kg⁻¹), oils (554.3 g kg⁻¹) and sugar (100.6 g kg⁻¹). Similar values for protein and oils contents of pistachio and other nut kernels were reported (Jubeen et al., 2012; Sanchez-Bel et al., 2008; Miraliakbari and Shahidi, 2008). The high oil and protein contents make the seed a potential source of commercial vegetable oil and protein. The concentrations of protein in the seeds analyzed suggest that pistachio nut contribute to the daily protein need of 23.6 g for adults, as recommended by the National Research council (1975). Total carbohydrate of pistachio was generally low, due to the high levels of crude fat and crude protein. The irradiation with 1, 2, 3kGy did not show any significant effect on the proximate composition (moisture, protein, lipid, ash, total sugar, and reducing sugar) of the pistachio nut (var. Halabi) kernels (Table 1). The total protein and total sugar contents were found to be depleted by storage in all pistachio nut samples (irradiated and unirradiated), whereas the fat content was increased due to the decreasing of protein and carbohydrate.

Our results, related to the effect of gamma irradiation on proximate analysis, are consistent with previous reports which also reveal non significant differences in moisture, fat, ash and protein contents between irradiated and unirradiated walnuts (Al-Bachir, 2004). Similarly, Bela et al. (2008) also reported that the protein and crude fiber contents of almonds did not change after irradiation. In agreement with our present results, Bhatti et al. (2010), and Yaqoob et al. (2010) also determined that gamma irradiation (2-10kGy) did not affect significantly (p<0.05) the lipids, protein, fiber and ash content of either sunflower nor maize seeds.

Treatment	Control	1 KGY	2 KGY	3 KGY	*LSD 5%			
Storage peri	od (months)							
Moisture (%)								
0	4.20±0.09	4.36±0.25	4.41±.0.20	4.42±0.17	0.35			
12	4.17±0.35	3.90±0.9	4.07 ± 0.8	4.02±0.12	0.36			
LSD 5%	5.8	4.2	3.5	3.4				
Total protein (g kg ⁻¹)								
0	214.7±2.3	211.5±3.3	208.9±7.0	215.1±3.0	8.1			
12	168.9±0.5	193.4±6.5	181.4±0.9	193.3±.6.5	8.7			
LSD 5%	3.8	11.7	11.3	11.5				
Total fat (g kg ⁻¹)								
0	554.3±4.1		553.5±4.0	555.1±6.0	9.3			
12	602.6 ± 56.8	636.3±12.5	646.6±10.9	634.0±7.9	56.2			
LSD 5%	95.1	22.5	19.2	16.3				
Ash $(g kg^{-1})$								
0	22.4±0.1	21.4±0.5	22.3±0.4	21.9±0.3	0.7			
12	22.0±0.1	22.6±0.8	22.3±2.5	22.4 ± 1.4	2.8			
LSD 5%	.0.2	1.6	4.0	2.3				
Total sugar (g kg ⁻¹)								
0	100.6±6.7	98.1±3.9	105.2 ± 3.7	108.8±4.6	9.1			
12	92.9±1.1	93.2±0.9	95.4±1.6	95.3±0.9	2.2			
LSD 5%	10.9	6.3	6.4	7.5				
Reducing sugar (g kg ⁻¹)								
0	26.6±0.1	26.3±0.3	26.4±0.3	29.7 ± 2.4	2.3			
12	11.8±0.1	12.2±0.2	12.3±0.1	12.3±0.1	0.3			
LSD 5%	0.2	0.5	0.5	4.8				

 Table.1 Effect of gamma irradiation and storage period on moisture, ash, protein, total sugar, reducing sugar and fat contents (g kg-1) of pistachio

*LSD=Least Significant Difference

Chemical properties

Total acidity and pH value: Total acidity (as lactic acid g kg⁻¹) and pH value for unirradiated and irradiated pistachio nuts are shown in Figures 1 and 2. The total acidity of unirradiated (control) samples of Halebi pistachio was 4.3 ± 0.1 g kg⁻¹. The pistachio nut kernels present low values of free fatty acids. This product could be stored for a longer time without being deteriorated. Similar findings showed that the selected Congo oil seeds also present low values of free fatty acids, between 20 and 30 as g kg⁻¹ acid oleic (Kimbohguila et al., 2010).

Total acidity increases significantly (p<0.05) from an initial value of 4.3 to 5.1 g kg⁻¹ after irradiation at lower used dose (1 kGy), while the higher dose of 3 kGy decreases significantly (p<0.05) the total acidity to 3.9 g kg⁻¹. Throughout the storage period of 12 months, the total acidity of both unirradiated and irradiated with 1 and 2 kGy pistachio nut samples decreased significantly (p>0.05). Previous

work by our group on this parameter in walnuts also showed that the free fatty acid increases as irradiation dose and storage time increases (Al-Bachir, 2004). The increasing free fatty acid in nuts after irradiation may be attributed to the degradation of large lipid molecules producing smaller molecules including free fatty acids.

The pH value of unirradiated pistachio samples was 6.43, and only the higher dose of 3 kGy significantly (p>0.05) decreased the pH value to 5.69 (Figure 3).

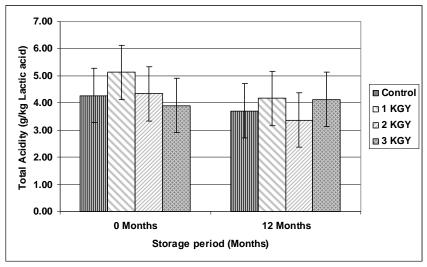


Figure 1. Effect of gamma irradiation and storage period on total acidity (g kg⁻¹ Lactic acid) of pistachio

Prior to irradiation, nine compounds were identified in cashew nuts belonging to six chemical classes of aldehydes, ketons, alkanes, alchohols, and aromatic, increasing in concentration especially at high irradiation doses (Mexis and Kontominas, 2009).

Total volatile basic nitrogen (TVBN): The results show that there was an interaction between treatment and storage time on the total volatile basic nitrogen (TVBN). As seen in Figure 3, the gamma irradiation and storage period affected the TVBN of pistachio nut significantly (p<0.05). Immediately after irradiation, TVBN values of unirradiated control samples of pistachio nuts (403 mg kg⁻¹) were higher than those of the irradiated ones (358 and 393 mg kg⁻¹ for sample irradiated with 2 and 3 kGy respectively), and also higher than those of control stored for 12 months (393 mg kg⁻¹). Cardeal et al. (2005), and Carasek and Pawliszyn (2006) showed differences in volatile and non-volatile compounds which may be related to (a) different SPME fibers used for analysis or (b) different nut cultivars analyzed. Hydroperoxides are highly reactive compounds that decompose rapidly, yielding a complex mixture of volatile and non-volatile compounds such as aldehydes, ketons, hydrocarbons, etc., which may affect the overall quality of products (Gracia-Liatas et al., 2006). It is well known that vegetable oils undergo

oxidative deterioration during processing and storage resulting in the formation of hydroperoxides, aldehydes, ketons and carboxylic acids, which decreases the nutritive and organoleptic value of the products (Bhatti et al., 2010; Richardsa et al., 2005).

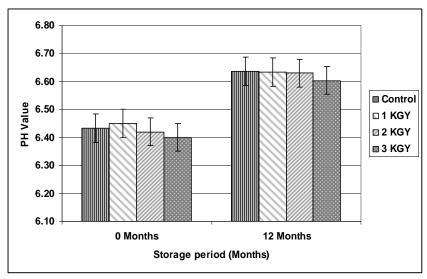


Figure 2. Effect of gamma irradiation and storage period on pH value of pistachio

Microbial load

The microbiological profile of the treated and non-treated pistachio nut samples is shown in Table 2. The bacterial population in unirradiated pistachio nut samples was 2.50 \log_{10} CFU g⁻¹ while for the fungal spores it was 2.54 \log_{10} CFU g⁻¹. The microbial load of used Halebi pistachio nuts was found to be comparatively low, which is in accordance with the Syrian microbial food standards (nuts included) that include less than 4.7 \log_{10} CFU g⁻¹ in total aerobic bacteria (SASMO, 2007). Irradiation significantly (p<0.05) improved the microbiological quality of the pistachio nuts by the reduction/elimination of the bacterial load and total fungal spores. In samples irradiated at 1, 2 and 3kGy, these organisms were undetected (less than one log₁₀ CFU g⁻¹) immediately after irradiation and after 12 months of storage. The decrease in bacterial and fungal count as a result of irradiation was in agreement with other studies on almonds (Bhatti et al., 2013), cereal (Braghini et al., 2009), pine nut (Golge and Ova, 2008), crop seeds (Aziz and Mahrous, 2004) and cereal grain (Mahrous et al., 2003). Silva et al. (1987) suggested that the use of 3kGy dose of gamma irradiation to increase inhibition of post-harvest fungi of pecan kernels. Ionizing radiation can directly (damage DNA) or indirectly (free radicals) kill the microorganism (Kwakwa and Prakash, 2006; Smith and Pillai, 2004).

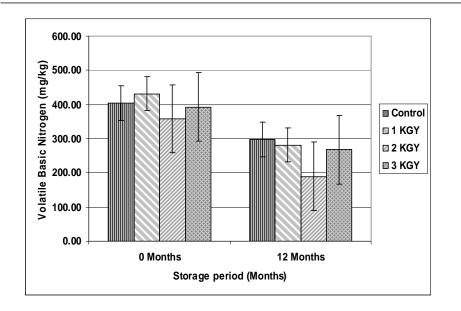


Figure 3. Effect of gamma irradiation and storage period on volatile basic nitrogen (VBN)(mg kg⁻¹) of pistachio

Treatment	Control	1 KGY	2 KGY	3 KGY	*LSD 5%			
Storage period (mo	onths)							
	Total bacterial count (log10 cfu g ⁻¹)							
0	2.50±0.04	<1	<1	<1	0.03			
12	3.10 ± 0.06	<1	<1	<1	0.06			
LSD 5%	0.12							
	F	ungal count (log ₁₀ spores g	-1)				
0	2.45±0.04	<1	<1	<1	0.04			
12	2.70±0.03	<1	<1	<1	0.03			
LSD 5%	0.08							
*I SD-I goot Signi	figent Difference							

Table 2. Total bacterial (\log_{10} cfu g⁻¹) and fungal (\log_{10} cfu g⁻¹) count of pistachio

*LSD=Least Significant Difference

Sensory evaluation

Figure 4 displays the sensory evaluation of irradiated pistachio nut kernels. Sensory panelists found no significant differences (p<0.05) between the unirradiated and 1, 2 and 3 kGy irradiated samples for taste, flavor, odor and texture. In their study on Iranian pistachios, Kashani and Valadon (1984) concluded that there was a slight increase in peroxide values, but no off- flavoring occurred at 1 kGy irradiation dose. The data obtained from the consumers

indicated that immediately after irradiation no significant differences (p<0.05) in flavor and aroma were observed between irradiated (0.5, 1.0 and 1.5 kGy) and control walnuts (Al-Bachir, 2004). Other studies indicated that cashew nut remained organoleptically acceptable at doses less than 3 kGy (Mexis and Kontominas, 2009).

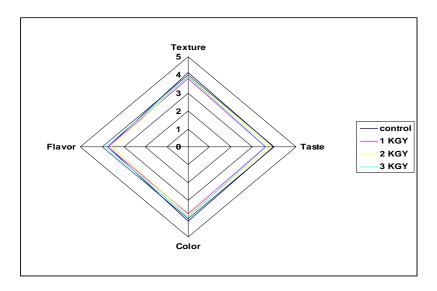


Figure 4. Effect of gamma irradiation on the taste, texture, color and flavor of pistachio

Conclusion

Medium doses of gamma irradiation of 1, 2 and 3 kGy could arrest microorganism contamination of pistachio nuts, an important item of commerce, while maintaining the quality, as judged from proximate constituents, chemical properties and sensory evaluation. Hence medium dose gamma irradiation could be recommended as a viable alternative to chemical methods for arresting microorganism growth.

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