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ORIGINAL PAPER

Effect of Co-60 gamma irradiation on *Aspergillus flavus*, Aflatoxin B_1 and qualitative characteristics of pistachio nuts (*Pistacia vera* L.)

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Abstract

In this study the effect of different doses of gamma irradiation (0, 0.5, 1, 1.5, 2, 4 and 6 kGy) on viable spore population of *Aspergillus flavus*, Aflatoxin B_1 (AFB_1) concentration and qualitative characteristics of pistachio samples were investigated. The results indicated that gamma irradiation at doses of 4 and 6 kGy reduced the viable spore population of *A. flavus* about 5 log. The maximum degradation of AFB_1 in pistachio samples was 73.26% and 83.36% that was observed at doses of 4 and 6 kGy, respectively. The total phenol content (TPC) increased at dose of 2 kGy, but it was reduced at higher doses of irradiation. The increase of irradiation doses up to 4 kGy significantly increased the antioxidant activity, while irradiation dose of 6 kGy led to a reduction in antioxidant activity. All irradiation doses increased the amount of malondialdehyde in pistachio samples and the highest amount of malondialdehyde was observed at dose of 6 kGy. The chlorophyll and carotenoid content was decreased at all absorbed doses of gamma irradiation and affected the color features and resulted to a darker color of pistachio samples. Gamma irradiation slightly decreased the solubility of proteins and altered the pattern and intensity of the protein bands. The obtained results showed that gamma irradiation at doses higher than 2 kGy is capable of controlling and reducing the contamination of pistachio samples, while lower doses ($kGy \leq 2$) of gamma irradiation caused minimum changes in pistachio samples quality.

Keywords Pistachio · *Aspergillus flavus* · AFB_1 · SDS-PAGE · Color · HPLC

Introduction

Pistachio nut (*Pistachia vera* L.) is a member of anacardiaceae family, that has been consumed or exported in different forms of raw, roasted and salted, and/or processed in confections, deserts and ice creams. Pistachio kernel with having about 20% of protein and 45% of oil is considered as a rich source of protein and oil especially unsaturated fatty acids (about 87%) [1]. Also this nut is one of the rich sources of beneficial nutrients for human health like phenolic compounds, anthocyanin, antioxidants, vitamins and minerals [2]. Pistachio nut is one of the most susceptible commodities to contamination by aflatoxin producing fungi such as *Aspergillus flavus* and *Aspergillus parasiticus*, so that pistachio contamination with these fungi during preharvest, post-harvest and storage can reduce the quality of the product and make it unsuitable for consumption and lead to critical health issues and economic losses [1].

In accordance with the Food and Agriculture Organization of United Nations (FAO) about 25% of produced nuts, especially pistachios, is lost annually due to mycotoxin contamination [3]. Recently, more than 400 compounds have been identified as mycotoxins in the world that the most important group among these mycotoxins are aflatoxins, including AFB_1, AFG_1, AFB_2 and AFG_2, and considered as a serious threat to human and animal health because of their carcinogenic, teratogenic and mutagenic nature [4]. Among aflatoxins, AFB_1, one of the four toxic secondary metabolites produced by *A. flavus* and *A. parasiticus*, has been ranked in group 1 definite carcinogen to humans by the International Agency for Research on Cancer (IARC) [5]. According to European Commission regulations the permitted maximum levels of total aflatoxins (TFAs) and AFB_1 in

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pistachio nuts is 10 µg/kg and 8 µg/kg, respectively [6]. As a result, it is necessary to use proper post-harvest preservation methods to prevent fungi growth and production of mycotoxins in this valuable product.

Irradiation is one of the most effective methods to prevent the growth of aflatoxin producing fungi during food storage. In food irradiation, the product is exposed to high energy photons, including gamma rays, electron beam, and X-rays [1, 7]. Application of gamma irradiation is preferred over other radiation methods due to its high penetration capacity, and the maximum allowable dose for commercial food irradiation in many countries is 10 kGy [7, 8]. Common applications of gamma irradiation include pest control, prevention of sprouting, delay ripening, increase storage time, decrease storage losses, and improve the food safety by means of pasteurization and sterilization [3, 9]. Research have shown that gamma irradiation can be effectively used as a decontamination method to reduce fungal and mycotoxin contamination in various food products including nuts [4, 5, 10, 11].

The aim of this study was to investigate the effect of different doses of gamma irradiation on viable spore population of *A. flavus*, reduction of AFB_1 concentration and some qualitative characteristics of pistachio nut samples.

Materials and methods

Sample preparation

Pistachio nuts (*Pistachia vera* L.), cv. Akbari, were supplied from the Iranian Pistachio Research Centre located in Kerman Province in October of 2019. Unshelled pistachios kernels were ground and packed in sterile petri dishes, then stored appropriately in dry condition at 4 ± 1 °C and prevented from exposure to direct sunlight, until later analysis. All chemicals and solutions were purchased mainly from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, US). Aflatoxin B_1 from *A. flavus* (CAS No. 1162-65-8; MW: 312.227 g/mol) was obtained from Sigma-Aldrich (Darmstadt, Germany).

Decontamination of pistachio nuts with *A. flavus* and Aflatoxin B_1

The aflatoxigenic strain of *A. flavus* R5 [12] was cultured on potato dextrose agar (PDA) medium and incubated at 28 °C for 7 days. Pistachio kernels inoculation with fungal spores was done by rolling on the culture and ground with other pistachio kernels. A suspension of 5 g of contaminated pistachio powder in 4.5 ml sterile saline solution including 0.5% Tween 80 was prepared and the spore count was performed using a hemocytometer. The population of spores in pistachio powder was adjusted to $(6.18 \pm 1.18) \times 10^5$ spore/g pistachio power. Some of the ground pistachios were spiked with AFB_1 (a final concertation of 400 ppb). The contaminated pistachio powders were stored at petri dishes in proper condition (at 4 ± 1 °C) [11].

Gamma irradiation

Pistachio powder samples packed in sterile petri dishes were exposed to different doses (0.5, 1, 1.5, 2, 4 and 6 kGy) of gamma ray in a Co^{60} gamma resource, in triplicates, using a Gammacell 220 irradiator (MDS Nordion, Ottawa, Canada) located at the Radiation Applications Research School, Nuclear Science and Technology Research Institute, AEOI, Tehran, Iran. The dose rate of gamma irradiation was 5.4 kGy/h. The temperature and relative humidity during irradiation process were 30 ± 1 °C and 45% to 55%, respectively. A Red-Perspex dosimeter (Hrwell Dosimeters, UK) was used to assess the absorbed dose.

Evaluation of *A. flavus* total count

The spore count was done by preparing a suspension of each irradiated pistachio powder samples in sterile saline (8.5 g NaCl in 1000 ml distilled water) and serially diluted a certain amount (100 µl) of each dilution was cultured in two plates containing PDA medium (Merck, Germany) (including 50 ppm chloramphenicol and 0.033 g/l of Rose-Bengal) by surface plating method in triplicates. Fungal colonies were counted after incubation at 28 °C for 3–5 days. The results were expressed as log colony forming units per gram (log CFU/g) [13].

Extraction of Aflatoxin B_1 and HPLC determination

AFB_1 was extracted from each 1 g pistachio powder samples by mixing with methanol 80% v/v and shaking properly (at 150 rpm for 24 h). The obtained extract was centrifuged (10,000×*g* for 5 min) and collected supernatant was filtered and then purified by means of an ASPEC 401 immunoaffinity column. In order to purify the extract, first 10 ml of PBS was passed through the immunoaffinity column, then loaded with sample and washed with water (10 ml), and the extract was diluted with acetonitrile (1.5 ml). 0.5 ml of eluate was collected in a glass vial then diluted with water (2 ml). 400 ml of the diluted eluate was injected to the HPLC system equipped with a Spherisorb Excel ODS1 (250 × 4.6 mm; 5 µm) column with a guard column (25 × 4.6 mm i.d.) and a Perkin Elmer LC420 fluorescence detector (364 nm excitation and 440 nm emission). The mobile phase was composed of water: methanol: acetonitrile (56:14:30, v/v/v) with the flow rate of 0.86 ml/min. A post- column derivatization was used with a zero dead volume T-piece and PTFE reaction tube (30 cm × 0.3 mm i.d.) and pyridine hydrobromide perbromide (PBPB) reagent was added at the flow rate of 0.3 ml/min. The retention time of AFB_1 was observed at 13.55 min [4].

Total phenolic content (TPC) and antioxidant activity (AOA)

Methanol solution (80%, 10 ml) was used to extract one-gram of each ground pistachio samples. The mixture was agitated (20 min) and sonicated (Sonorex Digitech DT 1028 H, Bandelin, Germany) two times for 15 min. The mixture was placed in the dark at 25 °C for 24 h. The extract was centrifuged (5000×*g* at 4 °C for 4 min) (Httich Refrigerated Centrifuge Universal 320R, Germany) and the supernatant was used to determine total phenolic compounds (TPC) and antioxidant activity (AOA) using Folin-Ciocalteu reagent method and DPPH free radical scavenging activity, respectively [14].

Determination of malondialdehyde (MDA)

Malondialdehyde determination was done by spectrophotometry method [15].About 7 g of pistachio nuts powder was mixed with 15 ml of 7.5% Trichloroacetic acid (TCA) (w/v) [containing 0.1% EDTA (w/v) and 0.1% propylgallate (w/v)] and homogenized (18,000 rpm for 1 min) and filtered. 2.5 ml of the filtrated and 2.5 ml of TCA reagent (46 mM) was transferred to a test tube and heated in boiling bath, then cooled. Absorbance of the extracts was read at 532 nm. The results were expressed as nano molar malondialdehyde per gram pistachio powder.

Chlorophylls and total carotenoids content

Chlorophylls and carotenoids of pistachio nuts were extracted by mixing about 0.2 g of ground pistachios and 5 ml of 80% (v/v) acetone solution. The extract was placed in the darkness (15 min) and centrifuged (1500×*g* for 15 min) and passed through a filter paper. The filtrated extract was quantified spectrophotometrically at 470 (A_{470}), 663 (A_{663}) and 645 (A_{645}) nm. The carotenoids (µg/g), chlorophyll a (µg/g) and chlorophyll b (µg/g) concentrations were measured using following equations [16]:

$$C_a = 12.21\left(A_{663}\right) - 2.81\left(A_{645}\right).$$

$$C_b = 21.13\left(A_{645}\right) - 5.03(A_{663}).$$

$$C_t = \left[1000\left(A_{470}\right) - 3.27C_a - 104C_b\right]/229$$

Instrumental color

The color parameters of Pistachio nuts powder samples were directly determined utilizing a Minolta Colorimeter CR-400 (Konica Minolta, Inc., Osaka, Japan) with a D65 illuminant as and an observation angle of 10 at 25 °C. The color values of $L*$, $a*$, $b*$ and $C*$ or chroma were measured based on International Commission on Illumination (CIE). $L*$ value represents lightness-darkness, and a* and b* describe redness-greenness and yellowness-blueness of color, respectively. $C*$ or chroma is 0 at the center of a color sphere and increasing according to the distance from the center. The $L*$, $a*$ and $b*$color values measurement was carried out in five replicates.

Total soluble protein

Total soluble protein determination was carried out using Bradford method [17]. The pistachio nut protein extract was prepared by mixing (30 min) pistachio powder with 10% Phosphate-buffered saline solution (pH = 6.8, W/V) with glycerol. Then the mixture was centrifuged (5000×*g* for 5 min) and the supernatant was collected and stored at − 20 °C for further use. The collected supernatant (100 µl) and Bradford reagent (5 ml) were transferred to a test tube and kept for 30 min. The absorbance was measured at 595 nm and bovine serum albumin (BSA) was used as the standard. The results were reported as mg of protein per g of pistachio nuts (mg/g).

Electrophoretic pattern of proteins

The electrophoretic pattern of pistachio proteins in SDS-PAGE was performed according to Laemmli method [18] under non-reducing and reducing conditions using a Mini Protean II Cell system (Bio-Rad, Hercules, USA). Proteins precipitation was done with cold acetone and after centrifuging (5000×*g* for 4 min at 4 °C) the precipitate was collected. For reducing condition, samples were prepared in sample buffer (65 mM Tris, pH 6.8, 10% glycin, 2 SDS, 0.2% bromophenol blue and 5% 2-mercaptoethanol) and heated in water boiling bath. For the non-reducing condition β-mercaptoethanol was excluded. 50 µg of each sample were loaded onto the gels containing of 12.5% acrylamide separating gel and 5% acrylamide stacking gel. Both gels were stained overnight with Coomassie Brilliant Blue R-250 and de-stained in a methanol-acetic acid–water (1:1:8 v/v) mixture until the background appeared clear. The gels images were captured using Gel Doc XR + and Quantity One 1-D Analysis software (Bio-Rad, Hercules, USA) and Gel-Pro Analyzer (ver.6.0) was used to analyze the molecular weight, relative density, and intensity of the bands.

Statistical analysis

Data were statically analyzed by means of a SPSS Software v.26.0 (SPSS Inc., Chicago, IL, USA) based on a one-way analysis of variance (ANOVA). Differences were significant at the $p < 0.05$ using Duncan's test. All experiments were conducted in a completely randomized design and the data were expressed as the means ± standard deviations of three replicate experiments (SD).

Results and discussion

Effect of gamma irradiation on inactivation of *A. flavus* spores

The effect of different doses of gamma irradiation (0.5, 1, 1.5, 2, 4 and 6 kGy) on *A. flavus* viable spore population is shown on Fig. 1. According to the results with the increase of gamma ray dose the spore population significantly decreased in all samples ($p < 0.05$). However, the viable spore population effectively decreased at doses of 4 and 6 kGy by 99.999% (5 log).

Iqbal et al. [4] observed that gamma irradiation at dose of 6 kGy showed a 4–7 log reduction in the viable spore population of *A. flavus* in red chilli samples. Aquino et al. [10] observed that gamma irradiation reduced the viable spore population of *A. flavus* on maize. Boonchoo et al. [19] reported 88% reduction in spore population of *A. flavus* by gamma irradiation at dose of 6 kGy in brown rice samples. Irradiation lead to microorganism inactivation through different fatal mechanisms such as DNA damage, cell membrane rupture, and/or damage to the cell wall [8]. The main inactivation mechanism by ionizing photons is the direct or indirect damage to DNA affected by free radicals generated via radiolysis of water, which lead to microorganism inactivation by inhibiting the cells replicate [20]. However, fungal spores are more resistant to ionizing radiation in compare with the bacterial spores because of their very low DNA content and melanized hyphae [8, 20]. Therefore, the appropriate irradiation dose for control and inactivation of microorganisms in food products should be selected according to the type and strain, state of microbial development and population [8].

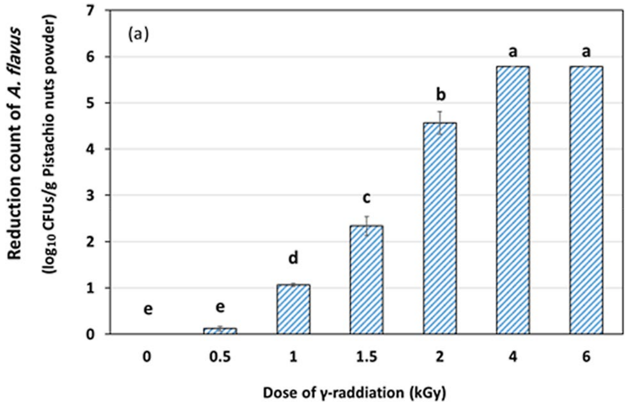

Fig. 1 Decontamination of *A. flavus* by different dose of gamma radiation on contaminated pistachio nuts. Data are means of three replicates. Different letters indicate significant differences between treatments according to Duncan's test ($p < 0.05$)

Effect of gamma irradiation on AFB$_1$ concentration

The effect of gamma irradiation on AFB$_1$ contaminated pistachio nuts are presented in Table 1 and Fig. 2. The results indicated that by increasing gamma irradiation does, the AFB$_1$ concentration in pistachio samples decreased compared to the control. The maximum reduction in AFB$_1$ concentration was 73.27% and 86.36% and was observed at doses of 4 ≤ kGy. According to the results, there is a positive correlation between the rate of AFB$_1$ degradation and increase in doses of gamma radiation [5]. Similar results were observed by Iqbal et al. [4], gamma irradiation (2, 4 and 6 kGy) showed 86%-98% reduction in AFB$_1$ concentration in whole and ground chillies. In a study Ghanem et al. [5] investigated the effect of gamma irradiation (4, 6 and 10 kGy) on AFB$_1$ in rice, pistachio, peanut, corn and feed (corn, wheat and wheat bran) and observed that with increase in doses of irradiation the rate of AFB$_1$ degradation increased, and stated that there is a negative correlation between the oil content of food and percentage of AFB$_1$ degradation and the percentage of AFB$_1$ degradation decreases with the increase of oil percentage in food.

Table 1 Effect of different doses of gamma irradiation (0.5–6 kGy) on aflatoxin B$_1$ concentration (ppb) in contaminated pistachio nuts powder

	Dose of gamma irradiation (KGy)						
	0	0.5	1.0	1.5	2.0	4	6
	493.11^{g} ± 0.43	488.64^{f} ± 0.88	410.36^{e} ± 0.78	355.11^{d} ± 0.54	284.34^{c} ± 0.74	131.83^{b} ± 0.36	67.25^{a} ± 0.44
Reduction (%)	0	0.91	16.78	27.99	42.34	73.27	86.36

Data are the mean ± SD. Means with different superscripts letters (a, b, c, d, e, f, g) in the same row are statistically different ($p < 0.05$) according to Duncan's multiple range test

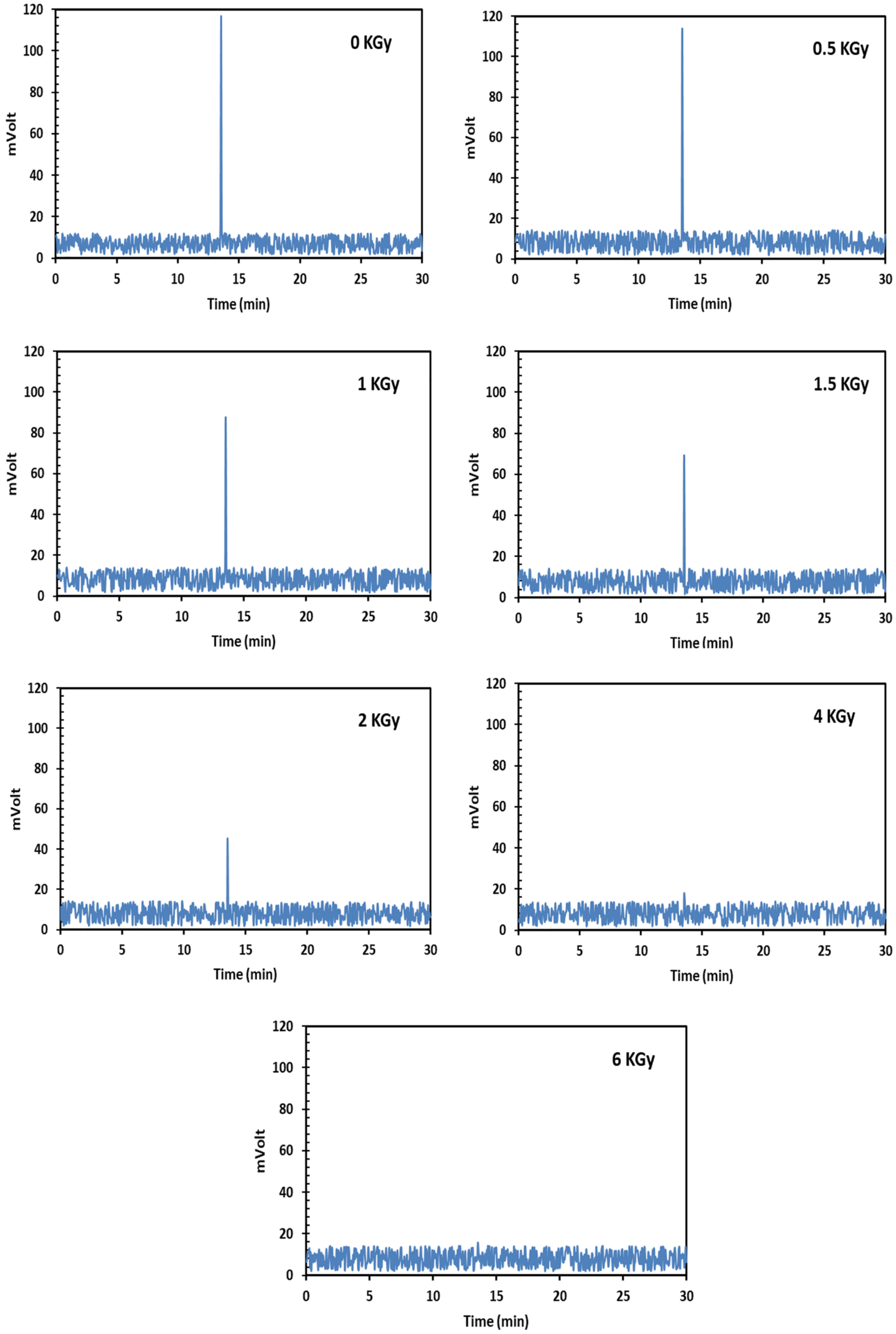

Fig. 2 Chromatograms: effect of different doses of gamma irradiation (0–6 kGy) on detoxification of contaminated pistachio nuts powder with aflatoxin B_1

In another study, Sen et al. [11] reported that the AFB_1 reduction was 47% in hazelnut samples after gamma irradiation (10 kGy, 10 min) and expressed that oil content, type of fatty acids and antioxidant compounds in the treated samples may affect the efficiency of gamma rays on the toxin. Jalili et al. [21] reported that the AFB_1 concentration showed 50.6%. Reduction in black and white peppers after gamma irradiation (30 kGy, 30% moisture).

Aquino et al. [10] reported that gamma irradiation of maize samples at doses of 2 and 5 kGy reduced 68.9% and 47% of AFB_1, respectively. Radiolysis of water and production of hydroxyl radicals, ions and atoms of hydrogen following the irradiation of food and the reaction or addition of these free radicals to the double bonds in aromatic and heterocyclic rings or carbon groups of lactone and furan rings in the structure of aflatoxins can reduce the biological activity of these toxins and/or convert them into less toxic compounds [10, 21]. On the other hand, radiolysis of water and formation of free radicals increase with the increase in doses of gamma irradiation [21].

Total phenolic content (TPC) and antioxidant activity (AOA)

The results of TPC and AOA of gamma irradiated pistachio samples are provided in Table 2. All of the data were significantly different ($p < 0.05$). The increase in doses of irradiation significantly reduced the TPC of pistachio samples in compared to the control. Radiation doses of 4 and 6 kGy remarkably declined the TPC, while the highest reduction was observed at dose of 6 kGy. The highest level of TPC was observed at doses of 0.5 and 2 kGy. The TPC remained unchanged at dose of 1.5 kGy in compare to the control. Moreover, gamma irradiation improved the AOA of the pistachio samples at doses up to 4 kGy, but gamma irradiation at dose of 6 kGy reduced the AOA of the samples in compare to the control. The lowest and the highest AOA of pistachio samples were observed at doses of 2 and 6 kGy, respectively.

Similar to our results, Akbari et al. [2] reported the TPC of gamma irradiated pistachio nuts remained unchanged at dose of 1 kGy, radiation dose of 2 kGy enhanced the TPC of all pistachio samples, but the TPC of irradiated samples decreased at dose of 4 kGy and the AOA of all samples increased upon irradiation. Also in a similar study Alinezhad et al. [16] reported that the levels of TPC in gamma irradiated pistachios decreased at doses up to 2 kGy, but it reduced at doses of 4 and 6 kGy and AOA increased at doses up to 2 kGy and then diminished at higher doses of irradiation (≥ 4 kGy). The TPC and AOA of different genotypes of gamma irradiated soybeans enhanced at dose of 2 kGy and the TPC of samples decreased with the increase of irradiation doses up to 5 kGy [22]. Kim et al. [23] reported that the TPC and AOA of the irradiated peaches improved with the increase of irradiation doses up to 2 kGy (0.5, 1, 1.5 and 2 kGy). The increment in TPC of pistachio samples at low doses of irradiation can be because of the increase in the activity of key enzymes of the phenylpropanoid metabolic pathway like phenylalanine ammonia lyase [22, 23]. On the other hand, several phenolic compounds are recognized as antioxidant agents because of having hydrogen with activity which leads to the hydrogen exchange reaction with free radicals and form a resonance-stabilized structure [23].

Therefore, the increase of AOA of pistachio samples by low doses of gamma irradiation can be due to the release and increase of phenolic compounds, as well as upregulation the pathway involves in antioxidative defences, formation and release of free flavonoids due to the degradation of glycosides by radiation [2, 22]. However, increase in the formation of free radicals at higher doses of gamma irradiation causes adverse effects and reduces the antioxidant activity [22].

Table 2 The effect of different doses of γ-irradiation on the values of the total phenolic content (TPC), antioxidant activity, pigments, instrumental color parameters and total soluble protein (TSP) of pistachio nuts

Properties	Dose of gamma irradiation (kGy)						
	0	0.5	1	1.5	2	4	6
TPC (mg GAE/g)	$1.52^{ab} \pm 0.05$	$1.60^{a} \pm 0.06$	$1.52^{ab} \pm 0.06$	$1.49^{b} \pm 0.04$	$1.61^{a} \pm 0.06$	$1.49^{b} \pm 0.05$	$1.38^{c} \pm 0.07$
Inhibitory of DPPH activity (%)	$81.80^{d} \pm 0.19$	$90.45^{a} \pm 0.45$	$86.70^{b} \pm 0.40$	$85.54^{c} \pm 0.59$	$91.29^{a} \pm 0.19$	$85.57^{c} \pm 0.48$	$77.02^{e} \pm 0.99$
MDA (n mol/g)	$8.23^{a} \pm 0.16$	$8.61^{a} \pm 0.16$	$8.61^{a} \pm 0.16$	$10.26^{a} \pm 0.13$	$10.06^{a} \pm 0.29$	$12.52^{a} \pm 0.39$	$12.71^{a} \pm 0.26$
TSP (mg/g)	$2.92^{d} \pm 0.06$	$3.29^{a} \pm 0.08$	$3.23^{a} \pm 0.05$	$3.05^{b} \pm 0.06$	$3.13^{bc} \pm 0.02$	$3.00^{cd} \pm 0.04$	$3.07^{bc} \pm 0.02$
Pigments							
Chlorophyll a	$23.33^{a} \pm 0.90$	$21.24^{b} \pm 1.58$	$19.21^{bc} \pm 1.10$	$17.89^{c} \pm 1.25$	$17.72^{c} \pm 1.14$	$14.94^{d} \pm 1.26$	$12.43^{e} \pm 0.72$
Chlorophyll b	$22.01^{a} \pm 1.87$	$18.21^{b} \pm 2.35$	$15.39^{bc} \pm 2.15$	$12.27^{d} \pm 0.83$	$12.84^{cd} \pm 1.51$	$11.32^{de} \pm 0.50$	$9.19^{e} \pm 1.59$
Total chlorophyll	$45.34^{a} \pm 2.78$	$39.45^{b} \pm 3.92$	$34.60^{c} \pm 2.98$	$30.16^{cd} \pm 1.94$	$30.56^{cd} \pm 2.48$	$26.26^{de} \pm 1.73$	$21.63^{e} \pm 2.31$
Total carotenoid	$25.66^{b} \pm 0.40$	$26.76^{a} \pm 1.09$	$25.38^{b} \pm 0.51$	$26.01^{ab} \pm 0.20$	$26.01^{ab} \pm 0.50$	$23.31^{c} \pm 0.18$	$22.98^{c} \pm 0.24$
Instrumental color							
Lightness (*L*)	$61.88^{a} \pm 0.83$	$62.05^{a} \pm 0.44$	$61.55^{a} \pm 0.32$	$61.53^{a} \pm 0.43$	$61.02^{a} \pm 1.50$	$60.66^{a} \pm 0.66$	$60.89^{a} \pm 0.53$
Redness (a^*)	$-6.44^{d} \pm 0.25$	$-6.28^{cd} \pm 0.22$	$-5.85^{bc} \pm 0.15$	$-6.07^{cd} \pm 0.40$	$-6.17^{cd} \pm 0.22$	$-5.24^{a} \pm 0.28$	$-5.40^{ab} \pm 0.21$
Yellowness (b^*)	$32.81^{a} \pm 0.31$	$32.52^{ab} \pm 0.12$	$31.42^{bc} \pm 0.48$	$32.38^{ab} \pm 0.43$	$31.59^{bc} \pm 0.69$	$30.77^{cd} \pm 0.34$	$30.18^{d} \pm 1.21$
Chroma (C^*)	$33.44^{a} \pm 0.28$	$-33.12^{ab} \pm 0.13$	$31.97^{bc} \pm 0.50$	$32.94^{ab} \pm 0.49$	$32.19^{bc} \pm 0.69$	$31.21^{cd} \pm 0.37$	$30.66^{d} \pm 1.22$

Data are the mean ± SD. Means with different superscripts letters (a, b, c, d) in the same row are statistically different ($p < 0.05$) according to Duncan's multiple range test

Detemination of malondialdehyde

The results of malondialdehyde content of gamma irradiated pistachio nuts are set out in Table 2. According to the results levels of the malondialdehyde in irradiated pistachio samples significantly increased with the increase in doses of irradiation ($p < 0.05$). Malondialdehyde in gamma irradiated pistachios at doses of 0.5 and 1.5 kGy remained unaffected compared to the control and the highest levels of malondialdehyde was observed in gamma irradiated samples at doses of 4 and 6 kGy. However, irradiation at dose of 6 kGy showed the highest increase in malondialdehyde in compare with the control.

Mexis and Kontominas [1] reported that the peroxide value of pistachio nuts and peanuts increased with the increase in doses of gamma irradiation (1, 1.5, 3, 5 and kGy). Also in another study increase in thiobarbituric acid value (TBA) with increasing gamma ray doses was observed in rice samples [24]. In two separate studies, a significant increase in peroxide value with increasing gamma irradiation doses in pine nuts (0.5, 1, 3 and 5 kGy) [25] and pistachios, hazelnuts, almonds and walnuts (1, 3, 5 and 7 kGy) [7] was reported. Boonchoo et al. [19] observed that TBA value of gamma irradiated brown rice samples increased at dose of 6 kGy. Gamma rays cause lipid oxidation via oxidation, dehydration, decarboxylation and polymerization reactions of fat molecules, on the other hand, hydroxyl radicals generated in the irradiated food can alter the chemical nature of fatty acids by initiating lipid oxidation [25]. The oxidation of unsaturated fatty acids of pistachio nuts such as linoleic acid and oleic acid to peroxides and convert to carbonyl compounds such as acetaldehyde, pentanal, propanol and hexanal can result in a color reaction between these compounds and TBA [24].

Chlorophylls and carotenoids

Table 2 shows the effect of gamma irradiation on chlorophylls and total carotenoids of pistachio nuts. All data were statistically significant ($p < 0.05$). The results indicated that the chlorophyll a and b in pistachio samples decreased with the increase in doses of gamma irradiation. Nevertheless, the degradation rate of chlorophyll b was higher than chlorophyll a. The highest reduction of chlorophyll a was observed at doses of 4 and 6 kGy, which reduced 35.96% and 46.72% of chlorophyll a, respectively. The lowest amount of chlorophyll a was observed at dose of 0.5 kGy, which was 96.8% compared to the control sample. The chlorophyll b considerably decreased with increasing the dose of gamma irradiation and this reduction was 48.57% and 58.25% at doses of 4 and 6 kGy, respectively, that the highest reduction of chlorophyll b was at dose of 6 kGy compare to the control. Gamma irradiation at dose of 0.5 kGy with 17.2% reduction in chlorophyll b showed the lowest reduction compare to the control. Total chlorophyll also decreased significantly with increasing gamma irradiation dose (Table 2). Gamma irradiation of pistachio nuts at doses of 4 and 6 kGy reduced total chlorophyll by 42.08% and 52.29% compared to the control sample. The highest amount of total chlorophyll was at dose of 0.5 kGy and was 12.99% compare to the control. In accordance with Table 2 the increase in doses of gamma irradiation declined the amount of total carotenoids compared to the control sample. The highest reduction in total carotenoids was 9.16% and 10.44% compare to the control, which was observed in irradiated pistachio samples at doses of 4 and 6 kGy, respectively. The carotenoid content in irradiated pistachio samples was not significant at doses up to 2 kGy compare to the control. Ramamurthy et al. [26] observed that increasing gamma irradiation dose (1, 2 and 3 kGy) reduced the chlorophyll content in capsicum samples. Byun et al. [27] reported that gamma irradiation (20 kGy) can degrade the chlorophyll b and reduce its content. In a study Kyung et al. [28] reported that the reduction in chlorophyll b is because of the selective devastation of chlorophyll b biosynthesis or the degradation of its precursors.

Instrumental color

The L^*, a^* and b^*color parameters of irradiated and non-irradiated pistachio nuts are presented in Table 2. All of the data were statistically significant ($p < 0.05$). According to the results, gamma irradiation at doses up to 6 kGy had no effect on L^* parameter or lightness of pistachio nut samples and did not changed the L^* parameter values compare to the control sample. On the other hand, a^* parameter that represent the red color increased slightly after irradiation at doses of 4 and 6 kGy and tended to move towards the red spectrum. The b^* parameter decreased at doses higher than 4 kGy compared to the control sample and tended to move towards blue spectrum. The C^* parameter or chroma decreased at gamma irradiation doses higher than 1 kGy, so that the highest reduction was observed at dose of 6 kGy compare to the control. These changes in color parameters after gamma irradiation resulted in darkening of the color of pistachio samples. The reason for the darkening of pistachios can be explain by the increase in the destruction of glycoside and peptidic bonds during irradiation, which leads to maillard reaction and formation of color compounds by producing products such as carbonyl and amino compounds and their reactions [1]. Similar to the results, Alinezhad et al. [16] reported darkening of pistachio samples with increasing the dose of gamma irradiation (1, 1.5, 2, 4 and 6 kGy). Mexis and Kontominas [1] observed that after gamma irradiation the L^* and b^* parameters decreased at irradiation doses higher than 5 kGy and a^* parameter increased and led to darker pistachio nuts. Sánchez-bel et al. [3] reported that the darkness of pistachio samples gradually increased with increasing gamma radiation dose (1–10 kGy). Gölge and Ova [25] reported that increase in gamma irradiation dose led to darker color of pine nuts. Boonchoo et al. [19] reported gamma irradiation at dose of 6 kGy resulted in yellowing of brown rice samples due to a non-enzymatic browning reaction.

Soluble protein

The effect of gamma irradiation on total soluble protein of pistachio nuts is summarized on Table 2. Results suggested that gamma irradiation slightly enhanced the protein solubility of pistachio samples ($p < 0.05$), so that the highest amount of solubility was observed at dose of 0.5 kGy and the lowest amount of solubility was at dose of 4 kGy. In general, there were no significant differences between gamma irradiated samples. In contrast to our findings, in a similar study Alinezhad et al. [16] observed that total protein solubility of pistachio nut reached to its maximum reduction with increasing the dose of gamma irradiation (1 < kGy). Afify et al. [29] reported that the protein solubility of peanut, soybean, and sesame was affected by gamma irradiation and decreased. They also stated that radiation, as a factor in the degradation and aggregation of proteins could be the reason for the decrease in solubility of the protein. Gamma irradiation reduces the solubility via direct the disintegration of proteins, improving the activity of lysosomal enzymes or increasing disulfide bonds and rearrangement of the low molecular weight proteins into high molecular weight proteins and aggregate protein [29]. Noorbakhsh et al. [30] observed that the solubility of proteins in steam-roasted pistachio samples decrease and explained that structural and chemical protein modifications can reduce the solubility of protein. On the other hand, gamma irradiation increases hydrophobic interactions and decreases protein solubility by cross-linking and aggregating protein chains [31].

Electrophoretic pattern of proteins

The effect of different doses of gamma irradiation on pistachio soluble protein pattern are illustrated in Figs. 3 and 4. Protein bands in non-reducing and reducing conditions were in the molecular weight range from 3 to 272 and 1 to 132 kDa respectively. According to the results the reducing condition (containing beta-mercaptoethanol) caused breakdown of disulfide bonds and separation of protein subunits and formation of low molecular weight proteins. While the proteins remained unchanged under non-reducing condition. The results obtained from both gels in non-reducing and reducing conditions showed a conversion in the pattern of the pistachio kernel proteins

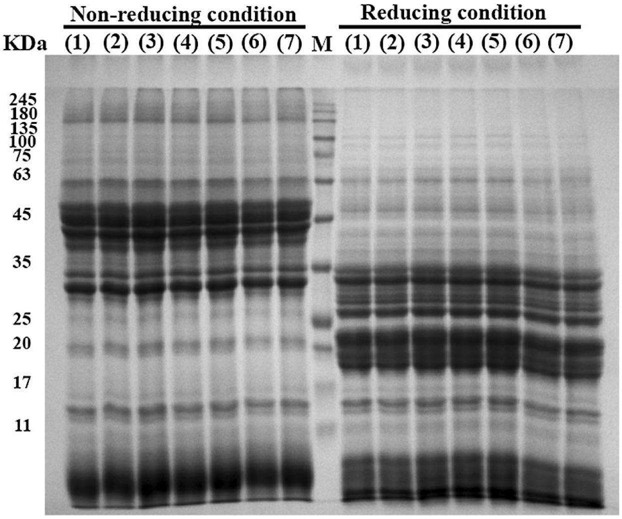

Fig. 3 Profiles of proteins extracted from non-irradiated (lane 1) and γ-irradiated pistachios (lane numbers of 2, 3, 4, 5, 6 and 7 for 0.5, 1, 1.5, 2, 4, and 6 kGy, respectively) under non-reducing and reducing conditions. "M" indicates a molecular weight marker (SinaClon BioScience, Prestained protein ladder, PR901641)

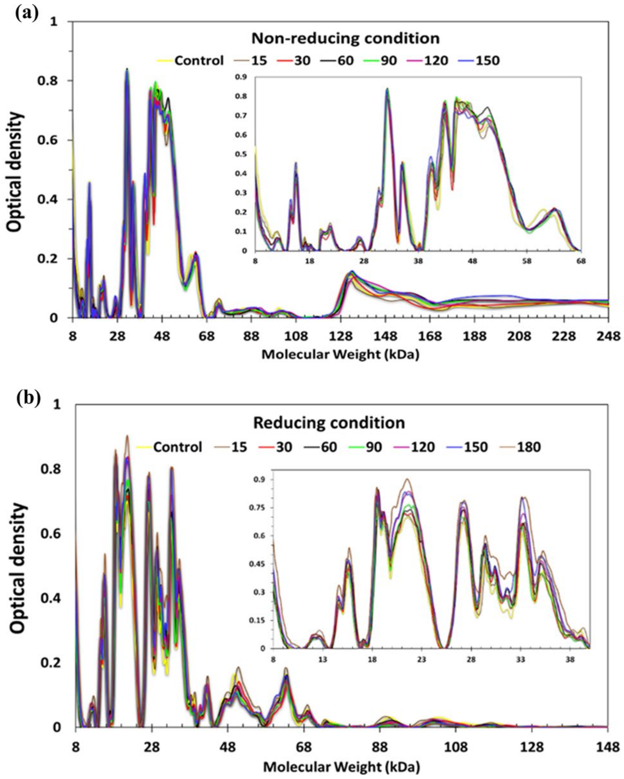

Fig. 4 Densitometry analysis of the proteins present in SDS-PAGE profile of soluble proteins extracted from non-irradiated (0.0 kGy) and irradiated (1.0–6.0 kGy) pistachio nuts in non-reducing (**a**) and reducing conditions (**b**)

under the influence of different doses of gamma irradiation. Therefore, the changes in pattern of the proteins or protein–protein interactions due to gamma irradiation can be understood by comparing the results. According to the analysis of the densitometry in non-reducing condition, the intensity of protein bands increased with increasing the gamma irradiation doses up to 2 kGy, but irradiation at doses of 4 and 6 kGy reduced the intensity of protein bands in the molecular weight of 43 to 50 kDa, so that the lowest intensity was achieved at dose of 4 kGy (Fig. 4).

Alinezhad et al. [16] reported that gamma irradiation altered the electrophoretic pattern of soluble proteins of pistachio nuts, so that gamma irradiation doses up to 6 kGy reduced the intensity of protein bands in all samples, while gamma irradiation at dose of 4 kGy led to a reduction in protein bands in molecular weight of 7 kDa. In another study Naei et al. [32] reported that gamma irradiation (1, 10 and 100 kGy) drastically changed the pattern and intensity of proteins extracted from pistachio samples and caused reduction or absence of some protein bands. Krishnan et al. [31] observed the formation of new bands and disappearance of some of the protein bands related to two varieties of soybeans after gamma irradiation. The appearance or disappearance of these bands could be due to the modification of the physicochemical characteristics of proteins such as oxidation, which causes condensation, polymerization or aggregation of proteins and reduces the solubility of the proteins.

Conclusion

The results of this study showed that gamma irradiation at doses of 4 and 6 kGy significantly reduced the viable spore population of *A. flavus* (5 log) in pistachio samples and the AFB_1 reduction in these absorbed doses was 73.27% and 86.36%, respectively. The TPC was increased at doses up to 2 kGy and decreased with the increase in irradiation dose ($4 \leq$kGy). Increasing gamma irradiation doses up to 4 kGy enhanced the AOA of the samples, but it declined at dose of 6 kGy. Lipid oxidation remarkably increased with increasing gamma irradiation dose and the highest levels of malondialdehyde was observed at dose of 6 kGy. The increase in irradiation dose decreased the chlorophyll a and b content and by affecting the color parameters resulted in darker color of pistachios. Solubility of proteins slightly increased with the increase in irradiation dose and changed the pattern and intensity of proteins bands. In accordance with the obtained results changes in spore population, AFB_1 concentration and quality characteristics of pistachio nuts were dose dependent. Gamma irradiation at doses of 4 and 6 considerably reduced the microbial contamination and toxin concentration and lower doses effectively maintained the quality characteristics of pistachio nuts.

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