Journal of Food Measurement and Characterization (2021) 15:199–209
https://doi.org/10.1007/s11694-020-00620-z

ORIGINAL PAPER

Effect of gamma irradiation on the physicochemical properties of pistachio (*Pistacia vera* L.) nuts

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Received: 30 May 2020 / Accepted: 26 August 2020 / Published online: 3 September 2020

Abstract

The effects of γ-irradiation at doses of 0, 1, 1.5, 2, 4, and 6 kGy on the physicochemical and sensory properties of pistachio nuts were investigated. The results showed that the total phenol content and antioxidant activity of the pistachio samples enhanced significantly with increasing γ-irradiation dose up to 2 kGy, whereas their values significantly reduced at doses of 4 and 6 kGy. A similar trend was observed for sensory attributes of pistachios and the highest scores were recorded at 2 kGy. The irradiation reduced the soluble protein content and affected the soluble protein profiles, either in the pattern or in the intensity of the protein bands. The irradiation at each dose reduced the chlorophylls and carotenoids levels, and brightness parameters of pistachios color; higher doses caused a notable loss in pigments and the pistachio became darker. The number and concentration of volatile components isolated and identified significantly exhibited with increasing the irradiation doses. The irradiation also caused the alteration of the fatty acids composition of pistachios, which was a remarkable increment in the relative amounts of saturated fatty acids and a decrement in the unsaturated fatty acids. In conclusion, the data showed dose-dependent differences in characteristics studied. Overall, it is recommended that mild γ-irradiation (≤ 2 kGy) promised the quality of pistachios without affecting the physicochemical, structural properties, and sensory acceptance.

Keywords Pistachio · Fatty acid · SDS-PAGE · Color · SPME-GC/MS · Hardness

Introduction

Pistachio tree (*Pistacia vera* L.) is marked resistance to extreme environmental conditions and cultivated on soils that are unsuitable for other fruit crops throughout the world from south-central Asia to southern Europe countries, North Africa and the Middle East, the United States of America, and Australia [1, 2]. Pistachio kernel is a delicious and popular nut that is used in many food recipes as ingredient and recognized as a good source of health-promoting components such as antioxidants, vitamins, minerals, monounsaturated fatty acids, sterols, and polyphenols [2, 3].

The global production of pistachio has raised over the past decades and based on the Food and Agriculture Organization databases the world production of pistachio reached about 1.4 million tons in 2018, which was an increase of about 160% compared to 2008 [4]. Iran was the world's leading producer of pistachios (40% of the world's pistachios). The United States and Turkey were ranked the second and third in production of 447,700 and 240,000 tons, respectively. These three countries, as the largest pistachio producers, supply about 90% of the world's total pistachio [4]. Pistachio plays an important role in Iran's non-oil economy, and Iran earns more than 1 billion dollars yearly from the export of this valuable nut [5]. Maintaining the quality of pistachio nutrients is an important feature of pistachio to enter the global markets and export. Since many consumers prefer the raw and non-roasted pistachios and almost it is consumed after storage period, this leads to the growth of post-harvest molds that are capable of producing mycotoxins [6]; therefore, it is important to use a processing method to preserve the characteristics of pistachios and reduce possible contamination.

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Food irradiation that commonly known as "cold pasteurization" due to low-temperature processes is the physical and non-thermal method of food processing that involves subjecting foodstuffs to ionizing energy for ensuring the hygienic quality of solid or semi-solid foods [7, 8]. Three sources of radiation that are approved to use in food technology are γ-rays emitted from the ^{60}Co or ^{137}Cs radioisotopes, X-rays, and electron beam generated by machines operated below an energy level of 5 and 10 MeV, respectively [9]. Nowadays, irradiation of food is permitted in over than 60 countries for the aim of food preservation [8]. Among these three irradiation applications, γ-irradiation using ^{60}Co is currently the preferred method because of a deep penetration potential that enables the treatment of materials with less handling [7].

Gamma irradiation is used as a technique to enhance the food safety by reducing the risk of foodborne illness; inhibiting or delaying of natural losses caused by physiological processes like budding, sprouting, ripening or aging; eliminating harmful microorganisms and their toxins; reducing the allergenicity of food allergens; controlling the pests by killing and preventing the reproduction of insects and parasites[8, 10–12]. It has been also proven that irradiation processing is an appropriate approach and practical method to extend the shelf life and reduce the contaminants in various nuts [13–15]. Although, irradiation of foodstuffs doses up to 10 kGy is permitted for commercial food processing [8], but, pistachio contains high amounts of oil and their constituents are susceptible to lipid oxidation when irradiated [14].The irradiation affects the physicochemical, nutritional, and biological properties of foods [16]. It has been indicated that some nuts compounds and their biological activities maintained or increased during γ-radiation processing, while some decreased [10, 17–19]. Therefore, the levels of radiation absorbed and its effect on the characteristics of pistachios should be considered.

The aim of the present study was to investigate the effect of several doses of γ-irradiation on the antioxidant activity, pigments, soluble protein, protein binding profile, fatty acid profile, hardness, aromatic and sensorial features of pistachio nuts.

Materials and methods

Pistachio kernels

Pistachio nuts (*Pistachia vera* L.), cv. Akbari, were obtained from the Iranian Pistachio Research Centre located in Kerman Province in October of 2019. They were peeled and the defective or stained pistachios were separated, then the healthy pistachio kernels placed in sealed polyethylene bags and kept at the refrigerator until analysis for a little over a month.

Gamma irradiation

The pistachio kernels were exposed to different doses (1, 1.5, 2, 4, and 6 kGy) of γ-ray in a Cobalt 60 gamma resource with dose rate about 5.4 kGy/h using a Gammacell 220 irradiator (MDS Nordion, Ottawa, Canada) that installed at the Radiation Applications Research School, Nuclear Science and Technology Research Institute, AEOI, Tehran, Iran. The irradiation was done at 30 ± 1 °C and a relative humidity 45–55% for all samples and the absorbed dose was checked by a Red-Perspex dosimeter (Harwell Dosimeters, UK). All treatments were carried out triplicates.

Total phenolic content (TPC) and antioxidant activity

According to the previous study [20], the pistachio kernels were ground into a fine powder and 10 mL of 80% aqueous methanol was added to each one-gram sample. The suspension was stirred for 20 min and sonicated (Trans Sonic TP 690-A, Elma, Germany) twice for 15 min, and kept for 24 h at room temperature. The extracts were centrifuged at 5000×*g* for 4 min at 4 °C (Hettich Refrigerated Centrifuge Universal 320R, Germany), then supernatants were collected and used for both total phenolic content (TPC) and antioxidant activity analyses using the Folin-Ciocalteu colorimetric method and scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, respectively.

Chlorophylls and total carotenoids

The pigments of pistachio nuts including the chlorophylls and total carotenoids were assessed [21]. 0.2 g powdered samples were added to 5 mL of 80% (v/v) aqueous acetone and kept in dark place for 15 min. The mixtures were centrifuged at 1500×*g* for 5 min, filtrated, and the absorbance of extracts then measured at 663 (A_{663}), 645 (A_{645}), and 470(A_{470}) nm by a spectrophotometer. The amount of chlorophyll a (Ca), chlorophyll b (C_b), and total carotenoids (C_t) were determined according to following equations:

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

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

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

Instrumental color

The appearance color parameters were evaluated by a Minolta Colorimeter CR-400 (Konica Minolta, Inc., Osaka, Japan) at 25 °C with a D65 illuminant and a 10 observer as references. Color data were obtained as the International Commission on Illumination (CIE) L^*, a^*, and b^* coordinate values for dentition of color in a 3 dimensional space. L^* indicates lightness with values within a range 0–100, and a^* and b^* are chromatic green-red and blue-yellow coordinates, respectively. Finally, C^* is chroma, defined as with a value of 0 at the center of a color sphere and increasing values according to the distance from the center. All color analyses were run in five replicates.

Total soluble protein

Pistachio protein extracts were obtained from the defatted and ground pistachio by stirring in Phosphate-buffered saline (PBS, pH 6.8, 10% w/v) including glycerol for 30 min. After centrifuging at 5000×*g* for 5 min, the supernatant was stored at − 20 °C for later experiment. Total soluble protein content was determined using the Bradford assay [22] against bovine serum albumin solutions as the standard at 595 nm and the result was expressed as mg of protein per g of pistachio nuts (mg/g).

Electrophoretic pattern of proteins

50 µg of protein extracts were precipitated with cold acetone and collected after centrifuging at 5000×*g* for 4 min at 4 °C. Samples were boiled in non-reducing (65 mM Tris, pH 6.8, 10% glycine, 2% SDS, 0.2% Bromophenol blue) and reducing buffer containing 5% 2-mercaptoethanol and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) following the procedure described by Laemmli method [23] on 5% stacking gel and 12.5% separating acrylamide gels using a Mini Protean II Cell system (Bio-Rad, Hercules, USA). The gels were fixed and stained with Coomassie Brilliant Blue R-250 and then decolorized after 2 h with a methanol–acetic acid–water (1:1:8 v/v) solution till the background became clear. The images were recorded with Gel Doc XR + and Quantity One 1-D Analysis software (Bio-Rad, Hercules, USA) and the relative density, molecular weight and intensity of protein bands were analyzed by Gel-Pro Analyzer (ver. 6.0).

Volatile composition

The aromatic compounds of pistachio samples were extracted and identified using a slightly modified previous method [14] by solid-phase microextraction (SPME) and gas chromatography–mass spectrometry (GC-MS), respectively. 0.5 g of each powder sample was transferred into 20-mL screw-cap glass vials containing 5 mL of ultrapure water including 0.2 g of NaCl and 10 µl of *trans*-anethole (1 g/L; Avocado Research Chemicals, Ltd., Heysham, U.K.) as an internal standard. They were sealed and placed into a water bath with temperature control and stirring. A SPME fiber 2 cm-50/30 mm DVB/CAR/PDMS fiber Supelco, Bellefonte, PA) was exposed to the sample headspace for 50 min at 55 °C while shaking the sample. The volatile compounds were desorbed from the fiber for 2 min in the injector (250 °C) of a gas chromatograph, Agilent GC model 6890A equipped with a MSD 5975 mass spectrometric detector from Agilent Technologies. Analysis was carried out using helium as the carrier gas at a flow rate of 0.8 mL/min in a split ratio of 1:20 on HP-5MS capillary column (30 m × 0.25 mm id., 0.25 µm film thickness). The temperature of oven column was set at 40 °C, held for 5 min, increased at 5 °C/min to 180 °C and held for 5 min, and then increased at 15 °C/min to 240 °C and held for 10 min. The interface temperature was 300 °C, the mass spectra were obtained by electron ionization at 70 eV and spectral range of 35–450 m/z was used. The components were identified according to GC-MS retention times (authentic chemicals), retention indices (RI) with reference to n-alkanes (C_8–C_{24}) by mass spectrum comparisons to National Institute of Standards and Technology (NIST 2017) and Wiley (Wiley Registry of Mass Spectral Data, 7th Edition) mass spectral libraries. The quantification of the volatile compounds was performed on a gas chromatograph, Agilent GC 6890A, with a flame ionization detector. The column and chromatographic conditions were the same as previously described for the GC-MS analysis.

Fatty acids profile

The fatty acids of pistachio nuts were extracted and analyzed based on the method previously described [20]. In brief, approximately 1 g of ground pistachio nuts was mixed with 3 mL of n-hexane and sonicated for 3 h at room temperature using an ultrasonic bath. Then the mixture was centrifuged, and the oil was recovered by evaporating the solvent using a nitrogen stream. Subsequently, an approximate 50 mg of extracted oil was saponified with 100 µl of dichloromethane and 1 mL of a methanolic NaOH solution by refluxing in a 15 mL sealed glass tube with screw cap at 90 °C for 10 min. Then, 1 mL of BF3-methanol (14%) was added to the solution and boiled in a water bath for 10 min. Fatty acid methyl esters (FAMEs) were extracted from a salt saturated mixture by adding 600 µL n-hexane. Finally, the organic layer was separated and injected to the GC-MS (GC-6890A and MSD 5975 Agilent) previously described for volatile compounds to identification and quantification of FAMEs.

The temperature of the injector and detector were 230 and 290 °C, respectively.

Hardness

Hardness of pistachio nuts was evaluated using a wedge probe (A/WEG) connected to a Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, UK) equipped with a load cell of 25 kg. Hardness was performed at 1 mm/s until the pistachio was fully cut. Results were expressed in N for each treatment.

Sensory evaluation

A group of 12-trained panelists, aged 26–48 years (7 women and 5 men, all members of the panel of the University), with wide expertise in sensory evaluation of food products was used to evaluate organoleptic properties of irradiated and non-irradiated pistachio nuts. The panelists were selected and trained following the ISO standard 8586-1 [24]. The details of the selection, training, and validation of the panelists were done according to the Vázquez-Araújo et al. [25]. Prior to the sensory analysis of samples, the panelists discussed the sensory characteristics of pistachio during two orientation sessions until they had agreed on their use of sensory features. The tests were carried out in a controlled condition by serving the nut samples in 100 mL plastic cups with lids numbered by three-digit codes. The sensory attributes including appearance color, taste, texture, and overall acceptability were scored on a scale of 0–10, where: 0 = extremely low intensity and 10 = extremely high intensity. All panelists scored the six samples on each session, the sessions were repeated three times, and the sensory scores were presented as the overall means.

Statistical analysis

All experiments were carried out using completely randomized factorial design and the data reported in this work are the mean of minimum three replicates ± standard deviation. One-way analysis of variance (ANOVA) test was taken using SPSS Software v.23.0 (SPSS Science, Chicago, IL, USA). Duncan's multiple range test was used to show significant differences of the mean values at $P \leq 0.05$.

Results and discussion

Total phenolic content (TPC) and antioxidant activity

The results of TPC and antioxidant activity of pistachio samples are given in Table 1. It was observed that the effects of irradiation on TPC were dose-dependent. The values of TPC in the non-irradiated and the irradiated samples with a dose of 1 kGy were found about 1.52 mg GAE/g and then increased with increasing the irradiation doses to 2 kGy and the highest TPC was observed in the irradiated sample with a dose of 2 kGy (1.61 mg GAE/g). However, absorbed doses of 4 and 6 kGy decreased the TPC, but at the dose of 6 kGy

Table 1 The effect of different doses of γ-irradiation on 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	1	1.5	2	4	6
TPC (mg GAE/g)	$1.52^{ab} \pm 0.05$	$1.52^{ab} \pm 0.06$	$1.59^{b} \pm 0.04$	$1.61^{a} \pm 0.06$	$1.49^{b} \pm 0.05$	$1.38^{c} \pm 0.07$
Inhibitory of DPPH activity (%)	$81.80^{d} \pm 0.19$	$85.54^{c} \pm 0.40$	$86.70^{b} \pm 0.59$	$91.29^{a} \pm 0.19$	$81.01^{d} \pm 0.48$	$77.02^{e} \pm 0.99$
TSP (mg/g)	$3.29^{a} \pm 0.08$	$3.23^{a} \pm 0.04$	$3.13^{b} \pm 0.04$	$3.07^{bc} \pm 0.02$	$3.04^{bc} \pm 0.06$	$3.00^{c} \pm 0.04$
Pigments						
Chlorophyll a	$23.33^{a} \pm 0.91$	$19.21^{b} \pm 1.10$	$17.89^{b} \pm 1.25$	$17.72^{b} \pm 1.14$	$14.94^{c} \pm 1.26$	$12.43^{d} \pm 0.72$
Chlorophyll b	$22.01^{a} \pm 1.88$	$15.39^{b} \pm 2.15$	$12.27^{c} \pm 0.83$	$12.84^{bc} \pm 1.51$	$11.32^{cd} \pm 0.50$	$9.20^{d} \pm 1.60$
Total chlorophyll	$45.34^{a} \pm 2.78$	$34.60^{b} \pm 2.98$	$30.16^{bc} \pm 1.94$	$30.56^{bc} \pm 2.49$	$26.26^{c} \pm 1.73$	$21.63^{d} \pm 2.31$
Total carotenoid	$25.66^{a} \pm 0.40$	$25.38^{a} \pm 0.52$	$26.01^{a} \pm 0.20$	$26.01^{a} \pm 0.50$	$23.32^{b} \pm 0.19$	$22.98^{b} \pm 0.24$
Instrumental color						
Lightness (*L*)	$55.01^{a} \pm 1.96$	$54.80^{a} \pm 2.03$	$54.10^{a} \pm 1.04$	$53.81^{a} \pm 2.11$	$52.87^{a} \pm 1.53$	$48.37^{b} \pm 1.12$
Redness (a^*)	$-3.97^{d} \pm 0.16$	$-3.72^{d} \pm 0.28$	$-3.51^{cd} \pm 0.13$	$-3.15^{c} \pm 0.22$	$-2.38^{b} \pm 0.33$	$-1.02^{a} \pm 0.17$
Yellowness (b^*)	$25.28^{a} \pm 1.13$	$25.07^{a} \pm 1.06$	$24.84^{a} \pm 0.68$	$24.63^{a} \pm 0.94$	$22.57^{b} \pm 1.31$	$18.95^{c} \pm 1.02$
Chroma (C^*)	$25.59^{a} \pm 0.88$	$25.34^{ab} \pm 0.77$	$25.09^{a} \pm 0.96$	$24.83^{b} \pm 0.67$	$22.70^{c} \pm 0.71$	$18.98^{d} \pm 0.75$

Data are the mean ± SD. Means with different superscripts letters in the same row are statistically different ($p < 0.05$)

the TPC significantly reduced compared to the control ($p < 0.05$). This trend of changes was also observed for antioxidant activity and a positive correlation ($R^2 = 0.88$) was seen between the antioxidant activity and TPC of pistachio nuts at different irradiation doses. Antioxidant activity of the pistachio samples exhibited significantly with increasing irradiation doses up to 2 kGy and then decreased numerically at higher doses (Table 1). Similar to TPC results, the highest and lowest antioxidant activity was observed at a dose of 2 and 6 kGy with 91.29% and 77.52% inhibitory of DPPH radical, respectively. This was in agreement with previous findings that revealed a good correlation between antioxidant capacity and phenolic compounds of various Iranian pistachio cultivars [3, 10]. In accordance with our findings, previous studies revealed a significant increase in TPC and antioxidant activity of three different Persian pistachio nuts [10] and Indian soybean genotypes [26] at the low dose of irradiation (≤ 2 kGy); while a reduction in the TPC and antioxidant activity of pistachios and soybeans reported at a higher doses (4 and 5.0 kGy), respectively. Kim et al. [27] also observed that phenolic compounds and antioxidant activity of peaches were significantly enhanced by exposure to irradiation from 0.5 to 2 kGy. They reported that many phenolic components are recognized as antioxidant agents because they have hydrogen with the activity that stimulates the hydrogen exchange reaction stabilized. An induction in the activities of key enzymes of pistachio such as phenylalanine ammonia lyase at low-dose irradiation might be attributed to an increase in phenolic compounds [9]. The increased antioxidant activity of pistachios at a low dose of γ-irradiation might be due to upregulate the pathways involved in antioxidative defenses, formation and release of free phenolic compounds because of the radiation-induced breakdown of glycosides or an increase in TPC levels. However, at a higher dose of γ-irradiation, the flux of free radicals is created causing more destructive effects and a reduced in antioxidant characteristics [10, 26].

Chlorophylls and carotenoids

The pigments content of pistachio nuts including chlorophylls and total carotenoids were influenced with different γ-irradiation doses (Table 1). It was found that the chlorophyll *a* and *b* concentrations decreased remarkably with increasing the irradiation dosage ($p < 0.05$). Although the amount of chlorophyll *b* was slightly lower than chlorophyll *a* in the control sample, but the rate of reduction in concentration of chlorophyll *b* was greater at different doses of irradiation. For example, the reduction in chlorophyll *b* in doses of 1, 1.5, 2, 4, and 6 kGy was 30, 44.2, 41.6, 49 and 58%, while the rate of reduction in chlorophyll *a* at the same doses was 17.6, 23.3, 24, 36, and 47%. The reduction in chlorophyll *b* may due to more selective demolition of chlorophyll *b* biosynthesis or degradation of chlorophyll *b* precursors [28]. The total chlorophyll content also showed a similar downward trend in irradiated samples and there was a decrease of 52% of total chlorophyll content in pistachio irradiated at 6 kGy as compared to the non-irradiated sample. As depicted in Table 1, total carotenoid content of irradiated pistachios did not change up to 2 kGy. However, the amount of carotenoids was significantly reduced in pistachios irradiated with 4 and 6 kGy and decreased by 9.11 and 10.4% compared to the control sample, respectively. Similarly, Ramamurthy et al. [29] also showed a 5–10% decrement in the amounts of carotenoids in the irradiated (1–3 kGy) capsicum.

Instrumental color

The instrumental color results showed that there was a reduction in the brightness parameters of nuts with increasing radiation doses and pistachio became darker (Table 1). The L^* was significantly decreased at a radiation dose of 6 kGy, while it remained unchanged at doses up to 4 kGy ($p < 0.05$), and the b^* index also considerably reduced at doses ≥ 2 kGy. On the contrary, a^* values were remarkably increased after irradiation at doses ≥ 4 kGy; this increase mainly explained that during irradiating of pistachios, the intensity of the green color decreased whereas the intensity of the red color increased [2]. The highest and lowest chroma were observed in the control and 6 kGy-irradiated samples, respectively. The results showed as dose increased chroma decreased, and a remarkable decline in chroma was observed in pistachios irradiated at the doses ≥ 2 kGy. In general, changes in pistachio color parameters during irradiation indicated that the nuts got darker at higher doses of irradiation because of the browning reactions. This could be implied by the breakdown of the glycosidic and peptidic linkages enhanced during irradiation. Compounds such as carbonyls and amino groups are the results of the breakdown, which leads to the Maillard reaction and finally the production of colored components [30]. Similar results were observed by authors who found a statistically decrease in L^* and b^* values of irradiated pine nuts and pistachio at a higher dose of radiation (≥ 5 kGy) which led to a darker color of nuts [14, 31]. Sánchez-Bel et al. [32] showed a gradual darkening of almonds by increasing the irradiation doses from 1 to 10 kGy.

Total soluble protein

The results showed that total soluble protein content of pistachio nuts decreased at different doses of γ-irradiation (Table 1). The total soluble protein in non-irradiated pistachios was 3.28 mg/g, the amount of which did not change significantly at the dose of 1 kGy ($p < 0.05$), but with increasing the dose of irradiation, the soluble protein level reduced significantly and reached to the maximum decrease using irradiation dose of 6 KGy (3.00 mg/g). Some food processes can change the nature and solubility of proteins depending on the intensity and time of the process [33]. Noorbakhsh et al. [34]observed a considerable decrease in soluble protein level of steam-roasted pistachio extract compare to raw pistachio because of the irreversible modifications in the structural and chemical of proteins [34]. The present result was in agreement with Afify et al. [35]who reported that irradiation (0, 0.5, 1, 2, 3, 5, and 7.5 KGy) decreased the soluble protein content of almond, peanut and sesame. It is known that γ-radiation is generally attributed to directly decompose proteins or propagate the proteolytic enzyme activity, which helps to rearrangement of the small molecular weight protein to a high molecular weight and aggregate the protein and leads to decrease its solubility. In addition, the secondary and tertiary structures of a protein are unfolded by irradiation and thus the hydrophobic groups interact and reduce water binding [35]. On the other hand, when secondary and tertiary structures of a protein are unfolded, the hydrophobic groups interact and reduce water binding.

Electrophoretic pattern of soluble proteins

The effect of different doses of γ-radiation on pistachio soluble protein patterns are illustrated in Figs. 1 and 2. As shown in Fig. 1, several protein bands were identified in a molecular weight range from 6 to 106 kDa and 6 to 73 kDa in reducing and non-reducing conditions for all samples, respectively. The results showed that the interactions between the polypeptides were broken and the protein subunits were separated in reducing condition. However, these interactions were maintained in non-reducing condition. Therefore, by comparing the two conditions, it is possible to observe the effect of irradiation on protein-protein interactions. The results of SDS-PAGE of protein extracts obtained from samples prepared in reducing and non-reducing conditions showed that both gels had a difference in the pattern of protein bands and irradiation changed their intensity. Therefore, that different dose of irradiation sometimes increased or decreased the intensity of a protein band. Naei et al. [11] reported irradiation (1-100 kGy) caused main alters in the pattern and intensity of the protein extracts of pistachio and in the 100 kGy γ-irradiated sample, the intensity of the protein bands was significantly decreased, while some disappeared entirely. The densitometry analysis of protein bonds in non-reducing conditions showed that with increasing irradiation dose (except for protein bands in the molecular weight of 7 kDa at a dose of 4 kGy), the

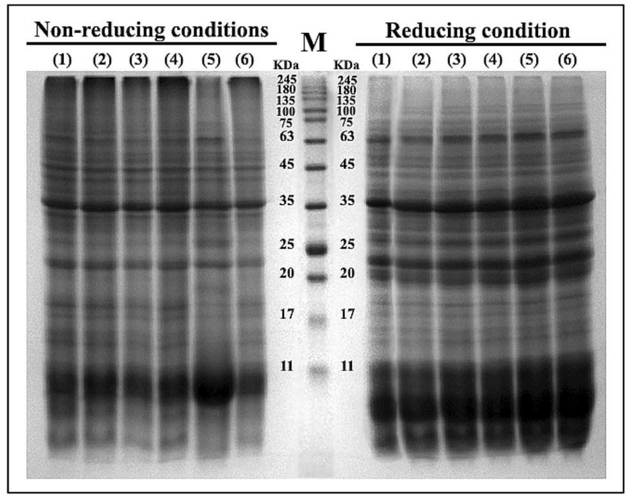

Fig. 1 Profiles of proteins extracted from non-irradiated (lane 1) and γ-irradiated pistachios (Lane numbers of 2, 3, 4, 5 and 6 for 1, 1.5, 2, 4, and 6 kGy, respectively) under non-reducing and reducing conditions. "M" indicates a molecular weight marker (SinaClon BioScience, Prestained protein ladder,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

intensity of all protein bonds decreased and the lowest intensity of protein bands was observed in all samples at a dose of 6 kGy (Fig. 2). This could indicate the interaction of peptide subunits and the formation of protein aggregates with higher molecular weights that correspond to the results of reduction of total soluble proteins in Table 1. Shokraii and Esen [36] reported that globulin (66%) is a major fraction of pistachio protein, followed by albumins (25%), glutelins (7%), and prolamins (2%), respectively. In contrast to other tree nuts, there is little information about allergenic proteins in pistachios. However, it has been reported that a number of these protein bonds identified in pistachio kernel, storage protein are among the allergic proteins. The main allergens are characterized as Pis v1 (a 2S albumin, 7 kDa MW), Pis v2 (an 11S globulin, 32 kDa MW), Pis v3 (a 7S vicilin, 55 kDa MW), Pis v4 (a manganese superoxide dismutase, 25.7 kDa MW), and Pis v5 (an 11S globulin, 36 kDa MW) [34, 37–39] The present results showed that by increasing the irradiation dose, the solubility of proteins and the intensity of peptide bonds in different molecular weights decreased, but the intensity of Pis v1 peptide bands at 7 kDa range increased at dose of 4 kGy and subsequently with increasing the irradiation dose to 6 kGy the intensity of protein bands decreased. This could be due to the effect of γ-irradiation on the cross-linking between peptide subunits and the accumulation of proteins in dimers and tetramers due to the action of free radicals. Ahn et al. [38] did not show a change in the electrophoretic pattern of the two major pistachio allergens (Pis v1 and Pis v2) by irradiating pistachios up to a dose of 4 kGy, but they observed a decrease in the production of allergic peptides associated with reduced antioxidant activity and

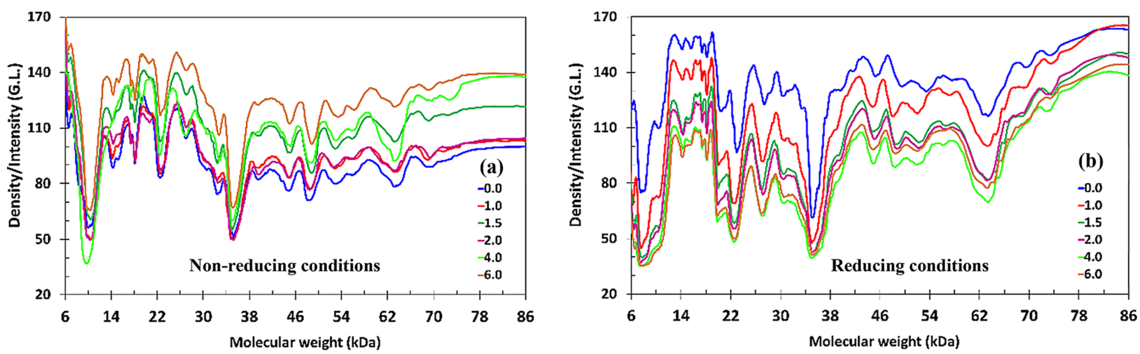

Fig. 2 Densitometry analysis of the proteins present in SDS-PAGE profile of soluble proteins extracted from nonirradiated (0.0 KGy) and irradiated (1.0–6.0 kGy) pistachio nuts in non-reducing (**a**) and reducing conditions (**b**)

phenolic compounds at ≥ 6 kGy. It was also reported that γ-irradiation alone was unable to modify the allergenicity of food and when used in combination with other food processing, it had a significant effect on the allergenic ability of food [11, 33]. However, changes in the production of allergic peptides due to pistachio irradiation require further studies, and in general, irradiation at doses less than 2 kGy has no effect on the subunits of pistachio proteins, but higher doses may lead to degrade of disulfide bonds and protein-protein interactions.

Volatile compounds

The volatile compounds found in pistachio nuts using SPME-GC/MS and their quantitative changes during irradiation processing are shown in Table 2. A total number of 14 volatile compounds were isolated, identified and quantified in non-irradiated pistachio nuts. These consisted of 5 aldehydes, 5 terpenes, 3 alcohols, 1 hydrocarbon, 1 ester, 1 pyrrole, and an additional compound (Octamethyl cyclotetrasiloxane) found in all samples. As seen in Table 2, the number of aromatic compounds detected in irradiated samples was higher than non-irradiated nuts. It was observed that with increasing the irradiation dose, more compounds were detected. A total number of 15, 18, and 20 aromatic compounds were detected in irradiated nuts at doses 1, 1.5, and 2 kGy, respectively. Besides, 4 aldehydes and 3 alcoholic compounds were identified more in the irradiated nuts at the doses of 4 and 6 kGy than the non-irradiated sample; so that 21 volatile components were found in irradiated pistachios at doses ≥ 4 kGy. Furthermore, the total content of volatile compounds in non-irradiated and irradiated nuts were distinctly different ($p < 0.05$). Values ranged from 5.95 mg/kg for non-irradiated nuts to 14.97 mg/kg for irradiated pistachios at dose of 6 kGy. On the other hand, the volatile content of pistachio nuts showed an upward trend by increasing the irradiation doses, therefore, both the number and concentration of volatile compounds extended by increasing the irradiation dose. The present results revealed that the terpenes were the main compounds in non-irradiated and irradiated pistachio nuts up to 1.5 kGy, followed by aldehyde components and 1-methy-1 h-lpyrrole. In addition, the irradiated samples at doses ≥ 2 kGy contained higher amounts of aldehyde compounds, so that the aldehydes comprised about half of the volatile compounds in the irradiated sample at 6 kGy. However, -pinene was the most abundant volatile compound in all samples. Previous studies have also shown that terpenes and aldehydes were major aromatic compounds in fresh and roasted pistachios in different regions and α-pinene was the predominant compound in the characteristics of pistachio nuts [2, 20, 40, 41]It has been stated that the lipid peroxidation is raised by the attack of free radicals produced during irradiation on unsaturated fatty acids and this leads to the formation of secondary oxidation products of fat peroxidation such as carbonyl, alcohol, and ketone compounds [15]. The authors reported that the oxidation of unsaturated lipids like unsaturated fatty acids because of processing such as irradiation could produce a variety of volatile compounds including aldehydes, ketones, alcohols, and esters that can significantly affect the organoleptic properties of foods in extremely small quantities [14, 42].

As seen in Table 2, in addition to increasing the total concentration of volatile compounds due to irradiation, aldehyde components including Heptanal, Octanal, Nonanal, and 2-Nonenal and alcohols comprising 1-Hexanol, Octanol, and 1-Nonanol were also formed during irradiation and their content increased by increasing the irradiation doses. Those compounds did not exist in non-irradiated nuts. Among them, Heptanal with green sweet flavor is one of the major components of irradiated nuts that may be derived from linoleic acid [42]. Similarly, other aldehyde and alcohol compounds, which often are the lipid oxidation products maybe created in this way, too. Frankel [42] observed that irradiation leads to the formation of primary and secondary volatile lipid oxidation substances. Hexanal, known as an

Table 2 Volatile compounds identified (mg/kg) in non-irradiated and various doses irradiated pistachio nuts using SPME/GC-MS

Compounds	Chemical group	RT (min)	Kovats index	Gamma irradiation dose (kGy)					
				0	1	1.5	2	4	6
Pentanal	Aldehyde	3.023	679	0.05 ± 0.01	0.06 ± 0.01	0.11 ± 0.03	0.17 ± 0.01	0.19 ± 0.03	0.19 ± 0.01
1-Methyl-1 h-pyrrole	Pyrrole	3.472	715	0.88 ± 0.12	0.72 ± 0.05	0.83 ± 0.21	0.81 ± 0.17	0.87 ± 0.18	0.87 ± 0.32
Hexanal	Aldehyde	5.651	800	0.75 ± 0.09	0.98 ± 0.29	1.01 ± 0.36	1.38 ± 0.45	1.67 ± 0.40	1.99 ± 0.64
1-Hexanol	Alcohol	8.004	866	nd	0.08 ± 0.01	0.13 ± 0.01	0.37 ± 0.16	0.53 ± 0.11	0.58 ± 0.05
Heptanal	Aldehyde	8.721	900	nd	nd	0.12 ± 0.04	0.59 ± 0.09	1.48 ± 0.39	2.13 ± 0.65
α-Thujene	Terpene	9.377	920	0.08 ± 0.01	0.10 ± 0.02	0.13 ± 0.02	0.24 ± 0.04	0.40 ± 0.14	0.51 ± 0.02
α-Pinene	Terpene	9.495	928	1.44 ± 0.26	1.47 ± 0.32	1.39 ± 0.34	1.45 ± 0.27	1.81 ± 0.38	2.37 ± 0.49
Benzaldehyde	Aldehyde	10.291	959	0.22 ± 0.02	0.28 ± 0.11	0.25 ± 0.05	0.33 ± 0.06	0.40 ± 0.05	0.61 ± 0.08
β-Pinene	Terpene	11.484	981	0.24 ± 0.03	0.28 ± 0.06	0.29 ± 0.03	0.36 ± 0.04	0.44 ± 0.09	0.53 ± 0.15
Octamethylcyclotetrasiloxane	Other	11.703	994	0.12 ± 0.04	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.02	0.09 ± 0.01	0.10 ± 0.01
1,2,4-trimethylbenzene	Hydrocarbon	13.811	996	0.56 ± 0.13	0.51 ± 0.13	0.46 ± 0.13	0.38 ± 0.01	0.35 ± 0.11	0.31 ± 0.07
Octanal	Aldehyde	13.927	1003	nd	nd	0.07 ± 0.01	0.15 ± 0.01	0.18 ± 0.02	0.22 ± 0.03
Limonene	Terpene	14.739	1034	0.44 ± 0.06	0.43 ± 0.09	0.46 ± 0.08	0.55 ± 0.14	0.57 ± 0.13	0.58 ± 0.21
Octanol	Alcohol	15.631	1070	nd	nd	nd	0.09 ± 0.01	0.17 ± 0.04	0.28 ± 0.02
α-terpinolene	Terpene	17.005	1087	0.39 ± 0.03	0.44 ± 0.16	0.41 ± 0.12	0.44 ± 0.07	0.55 ± 0.06	0.60 ± 0.13
Nonanal	Aldehyde	17.944	1100	nd	nd	0.19 ± 0.02	0.33 ± 0.09	0.37 ± 0.02	0.46 ± 0.06
1-Nonanol	Alcohol	18.266	1155	nd	nd	nd	nd	0.25 ± 0.02	0.56 ± 0.04
2-(E)-nonenal	Aldehyde	18.609	1165	nd	nd	nd	0.25 ± 0.02	0.38 ± 0.03	0.51 ± 0.10
Benzyl acetate	Ester	19.043	1178	0.38 ± 0.06	0.36 ± 0.05	0.37 ± 0.04	0.41 ± 0.05	0.52 ± 0.12	0.57 ± 0.18
Decanal	Aldehyde	20.276	1204	0.33 ± 0.04	0.41 ± 0.06	0.58 ± 0.21	0.29 ± 0.05	0.39 ± 0.08	0.64 ± 0.10
2-(E)-decenal	Aldehyde	22.193	1262	0.07 ± 0.02	0.09 ± 0.01	0.09 ± 0.03	0.21 ± 0.01	0.27 ± 0.02	0.36 ± 0.01
Total				5.95 ± 0.92^{f}	6.33 ± 1.38^{e}	7.01 ± 1.74^{d}	8.91 ± 1.86^{c}	11.88 ± 2.3^{b}	14.97 ± 2.47^{a}

RT Retention time, *nd* non-detected. Data represent the mean of three repetitions. Means with different superscripts letters in the same row are statistically different ($p < 0.05$)

indicator of oxidation and quality of fatty foods, is also the results of the decompose of linoleic acid [14]. In this study, its concentration increased with increasing radiation dose, which is a sign of the development of fat oxidation during irradiation. The present result agreed with Mexis and Kontominas [14]. who found that volatile compounds including secondary oxidation of pistachio and almond nuts markedly increased after irradiation.

Fatty acid profile

The fatty acids composition of total lipids extracted from non-irradiated and irradiated pistachio nuts at different doses are listed in Table 3. A total number of 13 fatty acids including 7 saturated and 6 unsaturated fatty acids were identified in raw pistachios. Oleic acid (61.21%), linoleic acid (22.41%), and palmitic acid (11.83%) were the most abundant fatty acids in raw pistachio nuts. Oleic, linoleic, and palmitic acids have been identified as the predominant fatty acids in pistachio oil in the literatures [14, 16, 17, 19, 20]. Although the types and amounts of some fatty acids in the present study were slightly different because of the cultivars, environments and ecological conditions [16]. Table 3 indicated that the percentage of total saturated fatty acids increased (from 14.36 to 23.14) with the irradiation doses up to 6 kGy, whereas the percentage of total monounsaturated fatty acids (from 63.22 to 57.01) and polyunsaturated fatty acids (from 23.72 to 17.14) decreased ($p < 0.05$). As can be seen from Table 3, palmitoleic and oleic acids as monounsaturated fatty acids and linoleic and linolenic acids as polyunsaturated fatty acids were drastically reduced after irradiation, wherase other unsaturated fatty acids remained unaffected by irradiation. The results also showed an insignificant difference ($p < 0.05$) in reducing the amount of unsaturated fatty acids between 4 and 6 kGy doses of irradiation. Table 3 illustrated that in 18-carbon fatty acids, which are important in nutrition and health, oleic acid was considered to be the most affected by irradiation which decreased by about 6%, followed by linoleic and linolenic acids. On the other hand, the saturated fatty acids were proportionally influenced by irradiation dose and they had a sharp increase after the irradiation. Although the amounts of pentadecanoic, palmitic, margaric, stearic, arachidic, and behenic acids did not change ($p < 0.05$),

Table 3 The effect of different doses of γ-irradiation on fatty acid composition (%) of pistachio nuts

Compounds	Gamma irradiation dose (kGy)					
	0	1	1.5	2	4	6
Myristic acid (14:0)	0.17 ± 0.02^{f}	0.44 ± 0.09^{e}	0.85 ± 0.03^{d}	1.14 ± 0.11^{c}	1.43 ± 0.11^{b}	1.88 ± 0.04^{a}
Myristoleic acid (14:1)	0.03 ± 0.02^{a}	0.01 ± 0.00^{b}	nd	nd	nd	nd
Pentadecanoic acid (15:0)	0.02 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.08 ± 0.01^{a}	0.10 ± 0.01^{a}	0.11 ± 0.01^{a}
Palmitic acid (16:0)	11.83 ± 0.85^{e}	12.87 ± 0.14^{d}	14.82 ± 0.16^{c}	15.32 ± 0.17^{bc}	15.79 ± 0.19^{b}	16.63 ± 0.33^{a}
Palmitoleic acid (16:1)	1.88 ± 0.11^{a}	1.37 ± 0.08^{b}	1.35 ± 0.03^{b}	1.36 ± 0.09^{b}	1.32 ± 0.01^{b}	1.34 ± 0.07^{b}
Margaric acid (17:0)	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}
Stearic acid (18:0)	1.91 ± 0.34^{e}	2.11 ± 0.07^{de}	2.34 ± 0.15^{cd}	2.65 ± 0.25^{c}	3.52 ± 0.28^{b}	4.01 ± 0.12^{a}
Oleic acid (18:1)	61.21 ± 1.52^{a}	60.44 ± 0.38^{ab}	59.14 ± 0.18^{bc}	58.33 ± 0.58^{c}	56.71 ± 0.58^{d}	55.57 ± 0.30^{d}
Linoleic acid (18:2)	22.41 ± 1.63^{a}	20.72 ± 0.56^{b}	19.78 ± 0.29^{b}	18.16 ± 0.66^{c}	17.35 ± 0.47^{cd}	16.45 ± 0.47^{d}
Linolenic acid (18:3)	0.54 ± 0.03^{a}	0.45 ± 0.01^{b}	0.39 ± 0.04^{c}	0.36 ± 0.03^{c}	0.30 ± 0.02^{d}	0.26 ± 0.03^{d}
Arachidic acid (20:0)	0.30 ± 0.04^{a}	0.33 ± 0.01^{a}	0.34 ± 0.04^{a}	0.36 ± 0.04^{a}	0.34 ± 0.01^{a}	0.36 ± 0.02^{a}
Gondoic acid (20:1)	0.86 ± 0.07^{a}	0.86 ± 0.03^{a}	0.94 ± 0.14^{a}	0.85 ± 0.10^{a}	0.87 ± 0.03^{a}	0.90 ± 0.10^{a}
Behenic acid (22:0)	0.09 ± 0.02^{a}	0.09 ± 0.02^{a}	0.10 ± 0.01^{a}	0.12 ± 0.02^{a}	0.11 ± 0.01^{a}	0.10 ± 0.01^{a}
SFA	14.36 ± 1.12^{f}	16.04 ± 0.19^{e}	18.55 ± 0.03^{d}	19.72 ± 0.57^{c}	21.33 ± 0.49^{b}	23.14 ± 0.25^{a}
UFA	86.94 ± 2.14^{a}	83.86 ± 0.19^{b}	81.62 ± 0.41^{c}	79.07 ± 1.16^{d}	76.56 ± 0.92^{e}	74.53 ± 0.47^{f}
MUFA	63.99 ± 1.41^{a}	62.69 ± 0.47^{b}	61.44 ± 0.12^{c}	60.54 ± 0.57^{c}	58.91 ± 0.55^{d}	57.82 ± 0.20^{d}
PUFA	22.95 ± 1.62^{a}	21.17 ± 0.57^{b}	20.17 ± 0.33^{b}	18.53 ± 0.64^{c}	17.65 ± 0.50^{cd}	16.71 ± 0.44^{d}
UFA/SFA	6.08 ± 0.54^{a}	5.22 ± 0.07^{b}	4.40 ± 0.02^{c}	4.01 ± 0.17^{cd}	3.58 ± 0.06^{de}	3.22 ± 0.05^{e}

nd non-detected. Data are the mean ± SD. Means with different superscripts letters in the same row are statistically different ($p < 0.05$)

the amounts of myristic, palmitic, and stearic acids significantly increased after the irradiation up to 6 kGy.

The present findings were in agreement with others who observed that irradiation up to 7 kGy increased saturated fatty acids and decreased unsaturated fatty acids in pistachio nuts [14, 19]. In contrary, Gölge and Ova [31] reported that the level of palmitic, stearic, oleic, and linoleic acids in pine nuts were unchanged by the irradiation doses up to 5 kGy. The pistachio nuts had high level of unsaturated fatty acids (86.94%), which is ready to lipid oxidation by irradiation [19]. Irradiation is known to cause the breakdown of the unsaturation site in the unsaturated fatty acids of oil and the autoxidation occurs on the unsaturation site of fatty acids similar to oleic, linoleic, and linolenic acids that have unsaturation structures [43]. Gamma irradiation interacts with fat molecules and accelerates interactions like oxidation, decarboxylation, dehydration, and polymerization resulting in the autoxidation process in unsaturated fatty acids in the presence of oxygen and finally changes the chemical constituents of the lipid fractions [31, 43] Likewise, the ratio between unsaturated fatty acids and saturated fatty acids (USFA/SFA) was changed. This ratio showed a marked decrease by increasing the irradiation doses; while the ratio for non-irradiated pistachio was 6.08 and it reached 3.20 for sample irradiated at a dose of 6 kGy.

Hardness

The effect of irradiation on the hardness of pistachio nuts is depicted in Fig. 3. The result showed the hardness of nuts unaffected by irradiation at doses up to 2 kGy ($p > 0.05$) while it affected significantly at higher irradiation doses. The cutting force was inversely correlated with nut crunchiness [20]. According to the present results, irradiated pistachio nuts with 4 and 6 kGy doses were more crunchy. In line with our results, [27] found no significant difference in the softness of irradiated peaches up to 1 kGy, while the hardness of the peaches irradiated at 1.5 and 2 kGy doses were statistically reduced over the controls. A significantly softer texture induced by irradiation has also been observed in cooked rice [30].

Sensory evaluation

The scores obtained for the sensory attributes of pistachio nuts are illustrated in Fig. 4. The panelists observed no significant difference in the appearance color of the samples after irradiating up to 2 kGy ($p > 0.05$), while the samples became darker and had significantly lower scores in higher

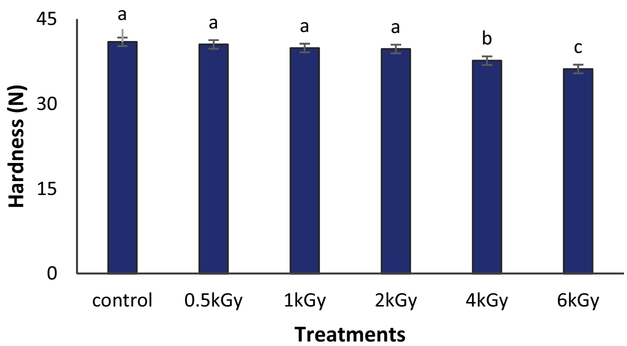

Fig. 3 The effect of different doses of γ-irradiation on the hardness (N) of pistachio nut kernels

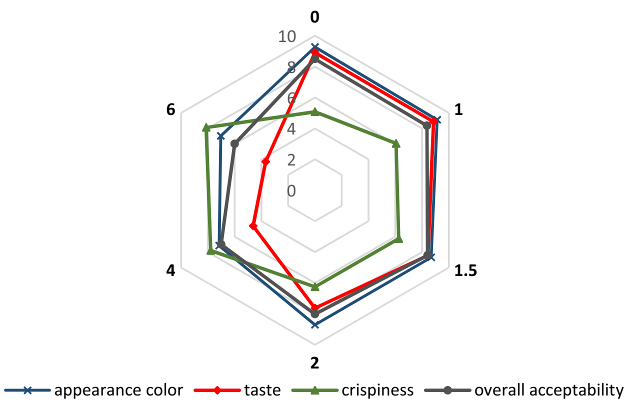

Fig. 4 The effect of different doses of γ-irradiation on the sensory attributes of pistachio nuts

doses of irradiation ($p < 0.05$). Positive correlations were found among the brightness parameters, L^* and b^*, and appearance color with the coefficient of determination (R^2) taking values of 0.71 and 0.80, respectively. It was also revealed that irradiation at doses < 4 kGy did not affect the texture of the samples ($p > 0.05$), but a significantly more fragile tissue in the samples were produced by irradiation at a dose of 6 kGy. The taste of samples was affected ($p < 0.05$) at doses ≥ 2 kGy, so that it had the lowest score at a dose of 6 kGy, which was a sign that it was not consumable. Concerning the sensorial evaluation, the irradiation at doses up to 2 kGy had no significant effect on the overall acceptability of pistachio nuts, while the quality features of pistachio nuts were reduced at this higher irradiation dose ($p < 0.05$). Mexis and Kontominas [14]observed that the sensorial attributes of pistachio nuts including color, texture, taste, and odor remained acceptable when irradiated at doses up to 3 kGy. Sánchez-Bel et al. [32] reported that the sweetness, texture, and color of almonds was unaffected by irradiation but the overall quality of samples treated by 10 kGy was statistically decreased. Gölge and Ova [31] reported that irradiation (< 5 kGy) unaffected the texture, flavor and general impression of pine nuts.

Conclusions

Our findings from the investigating the impact of different γ-irradiation doses (0, 1, 1.5, 2, 4, and 6 kGy) on the properties of pistachio nuts revealed that the TPC and antioxidant activity of the pistachios enhanced significantly with increasing irradiation dose from 0 to 2 kGy. The maximum levels of TPC and antioxidant ability were observed at 2 kGy and then reduced significantly at doses of 4 and 6 kGy. A similar trend was observed for sensory properties of pistachios and the taste and overall acceptability scores increased with increasing the irradiation doses up to 2 kGy. So that irradiated pistachios with a dose of 2 kGy were the most desirable among the panelists and irradiated samples with higher doses were significantly less acceptable. All irradiation doses were effective on pistachio pigments. The levels of chlorophyll *a*, *b* and carotenoids decreased considerably with increasing the irradiation doses. In addition, the color parameter of pistachio affected by irradiation and it became darker at higher doses. The irradiation decreased the soluble proteins and changed the pattern and intensity of proteins. The fatty acids composition of pistachios modified by irradiation and when the irradiation doses were increased the saturated fatty acids increased. In addition, with increasing the irradiation doses the number and concentration of volatile components remarkably enhanced. The hardness of nuts unaffected by irradiation at doses up to 2 kGy whereas a significantly softer texture induced by irradiation observed at higher irradiation doses. The data obtained from the experiments effectively characterized the dose-dependent effect of irradiation on the quality parameters studied of pistachios. Irradiation at 2 kGy was most effective for the extension of pistachio quality with added advantages.

Acknowledgements This research was supported by Agricultural Sciences and Natural Resources University of Khuzestan.

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