Discrimination of Sensorial Characteristics, Fungal, and Aflatoxin B_1 Contamination of Pistachio Kernels after E-beam Irradiation

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Abstract

Aims: This study was aimed to discriminate ion aflatoxin B_1(AFB_1) contamination of pistachio kernels after/E-beam irradiation. **Materials and Methods:** Pistachios were inoculated with known concentrations of *Aspergillus flavus*. Then pistachio samples were exposed to E-beam at five different doses (0, 1, 3, 5, and 7 kGy) and in various storage times (0, 30, 60, 90, and 180 days) at ambient temperature. Then, sensorial characteristics, fungal, and AFB_1 contamination of pistachio kernels were investigated. Statistical analysis was performed using the SPSS software version 22. by using descriptive statistics, Kolmogorov-Smirnov, and Kruskal–Wallis tests. **Results:** The mean percentage reduction of aflatoxin in the treated samples at doses of 1, 3, 5, and 7 kGy on different days of storage with two replicates was 38.84%, 48.79%, 53.50%, and 77.17%, respectively. The dose of 1 kGy was found to be appropriate in reducing the number of mold in pistachios without having any change in their organoleptic properties. The organoleptic properties, including color, texture, and overall palatability of pistachios, significantly changed after a dose of 5 kGy. **Conclusion:** Electron-beam (E-beam) efficiency against *A. flavus* and aflatoxin degradation increased with increasing radiation dose. Due to the sensitivity of *A. flavus* to radiation, this method can be used to improve the quality of pistachio products.

Keywords: *Aspergillus flavus,* aflatoxin B_1, E-beam, pistachio

Introduction

Tree nuts are exposed to some spoilage and pathogenic microorganisms. Pistachio nuts (*Pistacia vera* L.) are one of the most famous human consumed nuts. Iran is the first producer of pistachios, which exported to many countries.[1-3] They are mostly consumed salty, roasted, and are also used as the main ingredient of desserts such as Baklava and another excellent kind of local sweets in Iran, Syria, and Turkey.[4] Aflatoxin contamination of pistachio is one of the most significant issues related to its quality. This toxin can also be transmitted to the product through pistachio waste.[5] There is too much information about the incidence of mycotoxins, particularly aflatoxin in nuts.[6-8] Mycotoxins are metabolites produced by the fungus. Aflatoxins are mycotoxins with the highest frequency and the highest potential risk for humans and animals. Major types of aflatoxins are aflatoxin B_1 (AFB_1), AFB_2, AFG_1, and AFG_2 produced by *Aspergillus flavus,* and *Aspergillus parasiticus* particularly. Aflatoxins are carcinogenic and hepatotoxic, mutagenic, and teratogenic, and the liver is the primary target organ of AFB1.[1,9-11]

Molds in the genus *Aspergillus* often decay the kernel of pistachios. Its nuts are among the commodities with the highest risk of AF exposure. In Iran, the maximum acceptable levels for AFB_1 and total aflatoxin (AFT) in pistachio nuts are 5 and 15 ng/g, respectively.[1,12] AFB_1 is legislation in many countries and regulated by the EU, at 2 mg/kg in foods for direct human

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consumption.[13] The maximum limit of AFB_1 is 8 µg/kg for pistachios with direct human consumption and 12 µg/kg for pistachios to be subjected to other processes before human consumption.[14]

Due to the frequent requests of food consumers for safe food, processes were carried out to improve food safety and maintain its quality. The selection of suitable treatment conditions can reduce or prevent objectionable changes in food quality.[15] One of these methods is the irradiation of foods with ionizing radiation. Radiation of food has been extensively accepted in many countries as a safe method to improve food safety.[16] In addition to appropriate treatment, acceptance of the processed product is very important. The use of radiation food, up to 10 kGy increased microbiological safety and shelf life without significant change in its sensory quality. Some international organizations have mentioned that this method is effective and safe.[17]

Irradiation processing destroys the microorganisms that cause illnesses, and also the worms, parasites, and insects that spoil stored foods.[18] The objective of this survey was to estimate the effect of ionizing radiation from an E-beam to control *A. flavus* and its aflatoxin production in pistachio nut that was collected in the retail markets of Yazd.

Materials and Methods

Some amount of samples (1000 g) was divided into 200 g packages before the inoculation of fungal spores. Each part was placed in zippy plastic and sealed. It was then sterilized by an electron beam at a dose of 25 kg at the Radiation Processing Center (YRPC).

Sampling protocol

Three kg samples of Pistachio achieved in the markets of Yazd city were used. Samples were irradiated and placed in plastic bags approximately 200g in each bag and were sealed in plastic bags. To sterilization of samples, irradiated by 25 kGy at room temperature (25 _C) using a commercial 10 MeV E-beam system based in YRPC of Atomic Energy Organization of Iran.

Preparation of spore suspension and inoculation

A strain of *A. flavus* (*ATCC 5004* obtained from Department of Mycology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran) producing AFB_1 was grown on potato dextrose agar (PDA) (Liofilchem Diagnostic 610102, Italy) slant for 10 days at 25°C.[19,20] Samples (200 g) of pistachio inoculated with 10^6 spore suspension (5 cc) of the *A. flavus*. Spore concentration was determined by a hemocytometer. Following inoculation with the fungus each sample was mixed quite to homogenization. Pistachio samples were irradiated by 1, 3, 5, and 7 kGy E-beam system. Nontreated pistachio served as the controls (0 kGy).

The experiments were done on duplicates for each radiation dose and each sample. To calculate the amount of fungal sporulation on different days, irradiated and nontreated pistachio were stored in the room temperature on different days (0, 30, 60, 90, and 180 days) and evaluated.

Fungi cultivation and colony morphology

Ten grams of each pistachio sample was mixed with 90 ml sterile Ringer's solution and taken to dilution, then 100 µl of it were cultured on the surface of PDA culture plates. Plates were incubated for 7 days at 25°C ± 1°C in the darkness, and the average colonies in triplicate were daily calculated. After 2 days on Potato dextrose medium at 25°C, the colony of low, white, cottony, becoming green with sporulation was grown.

Spore-counting

Twenty grams of pistachio samples with sterile distilled water in a 100 ml Erlenmeyer flask were mixed. Then, Erlenmeyer was placed on a shaker for 6 h. Spore concentration in 100 ml of suspensions was evaluated by using a Neubauer's chamber (hemocytometer). Neubauer slide was counted, and the spores produced by the fungus growth were estimated at 20 g of pistachios.[21]

Sensorial test

About 200 g of pistachios in form of single layer was packed in a small plastic bag and properly sealed for irradiation by E-beam (1, 3, 5, and 7 kGy). Each bag of pistachios was considered as a replicate. The absorbed dose was determined using a dosimeter. Irradiated and un-irradiated samples were stored at the room temperature 18°C–25°C for 3 months. Sensorial analyses were performed on controls and treated samples after irradiation during 3 months of storage. Evaluation (consumer analysis) was carried out by 30 taste panel team. They were chosen using the following criteria: ages between 25 and 30, non-smokers, without reported cases of food allergies, and not regular consumers of dried nut products**.** Approximately 30 g of pistachio nuts were placed in small glasses coded with random numbers of sensory containers. They were served a set of five treated samples (0, 1, 3, 5, and 7 kGy), and they were trained to consume the samples. Sensory attributes evaluated included color, texture, flavor, and overall. The samples were evaluated using an acceptance test based on a nine-point hedonic scale (9 = like extremely and 1 = dislike extremely).[22]

Statistical analysis

Statistical analysis was performed using IBM SPSS statistics for windows, version 22.0." Armonk, NY: IBM Corp (2013) by using descriptive statistics, Kolmogorov–Smirnov, and Kruskal–Wallis tests. $P > 0.05$ was considered to be not significant.

Results

Reduction of *Aspergillus flavus* growth by E-beam in pistachio kernels

In this study, E-beam had significant effects on the decreasing *A. flavus* growth in pistachio (1, 3, 5, and 7 kGy). In the current study, the vegetative spores in PDA culture were decreased by a dose of 1 kGy of E-beam to 71%–97.95% and in 3, 5, and 7 kGy to 0 after irradiation. There were significant differences between the control group and different doses in decreasing of fungal growth ($P < 0.05$).

Fungal contamination of pistachio kernels detected by spore count methods was found to decrease significantly ($P < 0.05$) with increasing irradiation dose, too. In samples irradiated at 1 kGy dose, spore count was reduced but not completely inactivated. With 3, 5, and 7 kGy, these fungi were undetected (>1 log or 10 CFU/g) immediately after irradiation and after 6 months of storage. Inactivating effect of E-beam on vegetative A. flavus spores in pistachios in days (0, 90, 60, 30, and 180) are presented in Table 1.

The highest yield of E-beam on fungal spore survival was obtained at doses of 3, 5, and 7 kGy on days 60, 90, and 180, which showed a 100% decrease in the life of the irradiated fungus [Table 2].

The highest rate of E-beam yield on fungal growth (germination) was related to 3, 5, and 7 kGy doses on days (0, 30, 60, 90, and 180), which resulted in 100% sterilization of spores [Table 3].

The highest rate of E-beam efficiency on inactivation of spore survival (reduction percentage) was related to doses of 3, 5, and 7 kGy on days (60, 90, and 180), which resulted in 100% inactivation of spores [Table 4].

As shown in Figure 1, the highest reduction in *A. flavus* (CFU/ml) was related to doses of 3, 5, and 7 kGy on days (0, 30, 60, 90, and 180), which resulted in 100% inactivation.

The highest reduction in *A. flavus* Spore (Spore/ml) was related to doses of 3, 5, and 7 kGy on days (60, 90, and 180), which resulted in 100% inactivation, too [Figure 2].

The mean percent reduction in aflatoxin levels on different days of storage and doses of 1, 2, 3, and 2 were 0.8, 0.9, 0.8, and 0.5, respectively [Figure 3].

Sensorial test

Pistachio samples observing the parameters of texture, color, flavor, and overall in the irradiated samples (1, 3, 5, and 7 kGy). for color and texture, in all irradiated samples with 1, 3, 5, and 7 kGy not found a significant difference in relation to the control in a significance level of ($p > 0.05$) but in 3, 5, 7 kGy was showed a difference for the parameters flavor and overall with the control group ($P < 0.05$) [Figure 4].

Discussion

The efficiency of E-beam on the viability of *A. flavus* performance in some studies. The obtained results with this technique, in spores growth, determined the effective effect of E-beam radiation, was showed in the decrease of viable spores

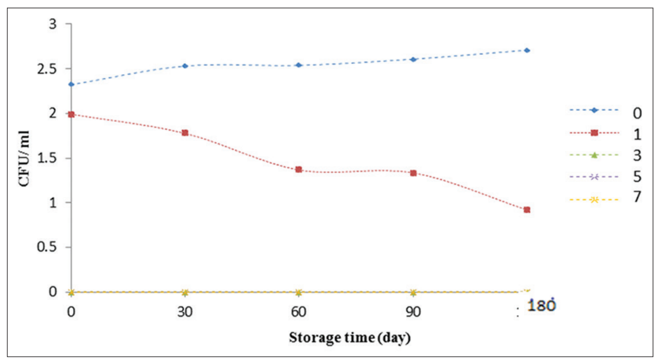

Figure 1: *Aspergillus flavus* (CFU/ml) changes at different doses and times of storage

Table 1: Inactivating effect of E-beam on vegetative *Aspergillus flavus* spores on pistachios in different days

Irradiation	Storage times (day)					*P*
	0	30	60	90	180	
Control	210±26.45	340 53.94	346±80.2	406±56.9	513±11.54	<0.05
1	98.33±53.44	60±55.67	23.33±15.27	21.66±8.33	8.33±4.08	>0.05
3	ND	ND	ND	ND	ND	>0.05
5	ND	ND	ND	ND	ND	>0.05
7	ND	ND	ND	ND	ND	>0.05
P	<0.05	<0.05	<0.05	<0.05	<0.05	-

ND: Not done

Table 2: Inactivating effect of E-beam on spore load of *Aspergillus flavus* in pistachios in different days

Irradiation	Storage times (day)					*P*
	0	30	60	90	180	
Control	$7.25\times10^6\pm0$	$7.50\times10^6\pm0$	$1.25\times10^6\pm0$	$1.25\times10^6\pm0$	$2\times10^6\pm0$	0.05<
1	$1.75\times10^6\pm2.82$	$3.25\times10^6\pm2.82$	$1.125\times10^5\pm0.70$	$2.50\times10^5\pm0$	$2.50\times10^5\pm0$	>0.05
3	$1.37\times10^6\pm2.12$	$1\times10^6\pm0$	0	0	0	0.05<
5	$1.125\times10^6\pm0.70$	$1\times10^6\pm0$	0	0	0	>0.05
7	$6.25\times10^5\pm0.70$	$6.25\times10^5\pm0.70$	0	0	0	>0.05
P	>0.05	>0.05	>0.05	>0.05	>0.05	

Table 3: Efficiency of E-beam treatment on *Aspergillus flavus* growth in pistachio nut in storage time

Dose (kGy)	Storage time				
	0	30	60	90	180
Control	0	0	0	0	0
1	94.67	71	93.75	97.95	88.88
3	100	100	100	100	100
5	100	100	100	100	100
7	100	100	100	100	100

Table 4: Efficiency of E-beam treatment on viability *Aspergillus flavus* in pistachio nut

Dose (kGy)	Storage time				
	0	30	60	90	180
Control	0	0	0	0	0
1	84.47	78.33	80	80	87.50
3	72.41	86.66	100	100	100
5	84.47	93.33	100	100	100
7	91.37	93.33	100	100	100

with the increase of irradiation doses.[23] Gamma radiations doses of 2, 4, and 6 kGy were applied on fungi and chilli samples. The results have found that the dose of 6 kGy reduced the fungal load by 5 logs, so reduced the level of AFB_1 and AFs in the ground and whole chillies by 1–2 logs.[24] Nemţanu *et al*. found at an irradiation dose of 1.7 kGy *Aspergillus* spp. were only 45% inactivated when infected maize seeds were exposed to E-beam treatment. Also, it was noted that total inactivation occurred at a higher dose of 4.8 kGy for *Aspergillus* spp.[25]

The results of Aziz. findings showed that irradiation with gamma rays at a dose of 4 kGy reduced the level of fungal growth greatly relative to the un-irradiated control and a dose of 5 kGy eliminated viable fungi for maize, chick-peas, and groundnut seeds. Aziz *et al.* also reported that gamma irradiation at 5 kGy killed the total number of fungi in maize and other cereal grains such as wheat, barley, and sorghum.[26] Silva *et al*. found that fungal contamination frequency decrease was observed to be proportional to the increase of radiation dose. The irradiated samples with 8 kGy reduced the fungal frequency in comparison to the control samples by 75%.[27]

Silva *et al*. found an effective reduction with peanut by ionizing radiation with a 7 kGy dose. Decreasing by 90% the number of fungi, the reduction of the fungi in comparison with controls was 82.6% and 81.9%, respectively, demonstrating an effective reduction of the initial contamination.[18]

Prado *et al*. when studying peanuts irradiated and disinfected observed a reduction of fungi infection at 5 kGy, and total fungi reduction at 10 kGy, after 180 days in storage. Irradiated and nondisinfected nuts reduction with 5 kGy doses and total destruction with 10 kGy doses. Gamma irradiation in 10 kGy or higher displayed to be an efficient process to reduce the

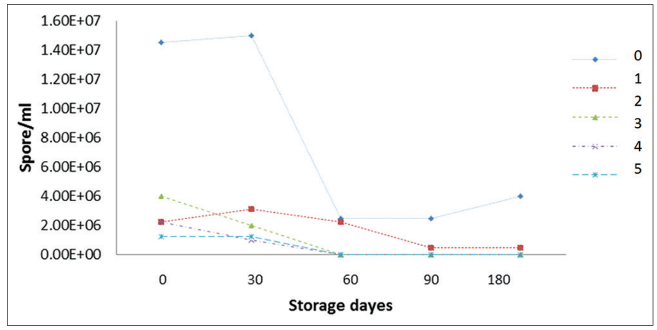

Figure 2: *Aspergillus flavus* spore (spore/ml) changes at different doses and times of storage

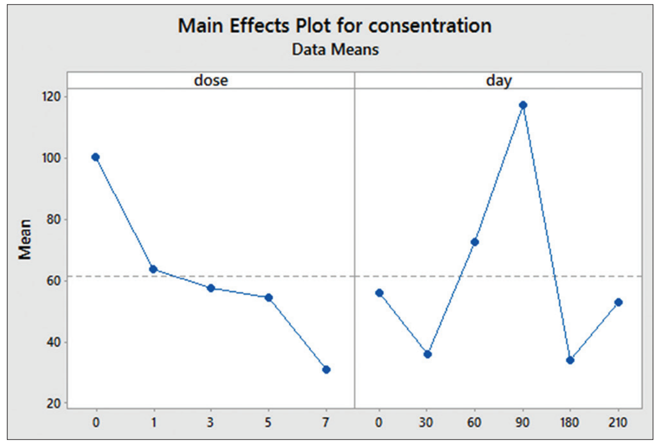

Figure 3: Mean change in aflatoxin levels at different doses and times of storage

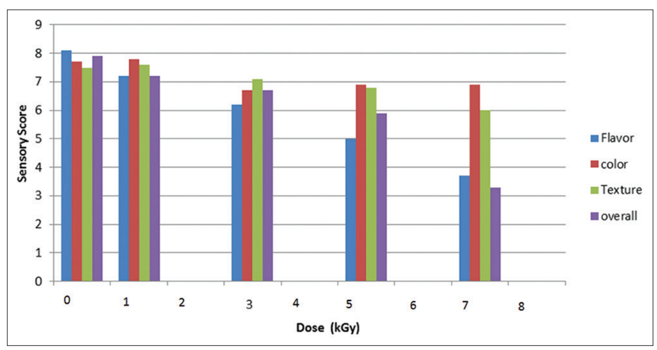

Figure 4: Sensory evaluation of pistachio nuts: texture; flavor; color and overall. Values are the mean of 30 determinations, scoring scale: 1–9 (9 like extremely, 1 dislike extremely) for flavor, color, texture, and overall

mycoflora of peanuts.[28] Maity *et al.* observed that 100% growth inhibition of *A. flavus* occurred at 3 kGy ($P < 0.05$) when fungus-infected rice was exposed to gamma radiation.[29] Aziz *et al*. showed that the population of *A. flavus* in inoculated maize was reduced significantly by increasing irradiation dose, with a dose of 4 kGy eliminating viable fungi.[26]

Sensorial test

Sánchez-Bel *et al*. studied the deterioration of almonds quality after treatment with E-beam at doses of 3, 7, and 10 kGy, during a storage period of 5 months. in the sensory analysis, in which the tasters did not find sensory differences between the controls and those irradiated at doses of 3 or 7 kGy, whereas almonds irradiated at 10 kGy exhibited a rancid flavor and a significant decrease in general quality.[30] Ma *et al*. examined the effect of the Co-60 irradiation 0.1, 0.5, 1.0, and 5.0 KGy on the sensory properties of fresh walnuts. The radiation of 0.1, 1.0, and 5.0 KGy doses increased oil oxidation and decreased sensory quality in a dose of 0.5 KGy reduced oil oxidation and were remained sensory properties.[31] Mexis and Kontominas indicated that cashew nut remained organoleptically acceptable at doses <3 kGy.[32] Al-Bachir found out the sensory evaluation of irradiated pistachio nuts. This study sensory testers found no significant differences between the unirradiated and 1, 2, and 3 kGy irradiated samples for taste, flavor, odor, and texture.[22] Silva *et al*. showed the acceptance of evaluated test for sensorial attributes in peanut observing the parameters of appearance, odor, and flavor in the irradiated samples (5 and 7 kGy), and the control shows that no difference was found.[18] We conclude in this study that a dose of 1 kGy is the best dose was applied for the preservation of fresh pistachio nut over a 90-day storage time.

Conclusion

The effectiveness of E-beam against *A. flavus,* increased with increasing the dose of irradiation. Due to the high observed sensitivity of the *A. flavus* to the E-beam irradiation, this method can be used to improve the safety of pistachio and nut products in future.

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Conflicts of interest

There are no conflicts of interest.

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