Effect of UV-C Irradiation on Fatty Acid Profile of Pistachio Kernels and Prevention of Aflatoxin Production

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Abstract

Background: Pistachio is one of the most crucial exported agricultural products of Iran. Pistachio nut is susceptible to fungal contamination and aflatoxin production.

Materials and Methods: This study examined the effect of some functional factors of the optimized rotational UV-C irradiation system on the prevention of aflatoxin production by *Aspergillus flavus*. The factors included lamp distance from the products (10, 20, and 30 cm), irradiation time (15, 30, and 45 minutes), and the number of UV lamps (3, 5, and 7).

Results: The best treatment was the lamp distance of 10 cm, 30 minutes of irradiation with three UV-C lamps. The results revealed that aflatoxin levels in the irradiated sample were significantly lower than in the control sample ($p<0.05$). Also, UV-C irradiation caused significant changes ($p<0.05$) in some fatty acid profiles, as the amount of linolenic acid in the control sample (0.85±0.07%) was higher than the value in the irradiated sample (0.15±0.07%). However, the values of other fatty acids such as palmitic acid, palmitoleic acid, stearic acid, oleic acid, and linoleic acid were not significantly different between the control and irradiated sample.

Conclusions: This irradiation system can be installed in different pistachio processing lines to disinfect products, which is very useful in reduction and prevention of the aflatoxins.

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1. Introduction

Pistachio is one of Iran's most profitable exports and has typically ranked third following oil and carpets [1]. Epidemiological or clinical tests show that the consumption of pistachio kernels has a positive impact on human health, due to its unsaturated fatty acids. Pistachio kernel contains lipids, proteins, phytosterols, xanthophyll carotenoids, some minerals (Mg and Fe), and vitamins (E, K, B_6, and B_1). In addition, pistachio has high anti-inflammatory properties and antioxidants which can be mentioned as advantages for pistachios [2]. Pistachio kernel is also used as a valuable ingredient or flavoring in food industries such ice cream or candy and cake production. Dry pistachio contains about 50% fat and 20% protein. Its main fatty acids are unsaturated fatty acids such as oleic, linoleic, and linoleic acids [3]. Pistachios must be kept in a dry condition for processing and export. Otherwise, it can absorb air humidity and due to the growth of fungus, the value of aflatoxin rises [4].

In pistachio export, the most challenging problem has been aflatoxin for many years [1]. Aflatoxins are a group of structural toxins made from specific species such as *A. flavus* and *A. parasiticus* [5]. Production of aflatoxin depends on different physical, chemical, and biological factors [6]. Aflatoxin B_1 is the most toxic type of aflatoxin known until now and is the strongest carcinogen material for the liver [7]. Food contamination with *A. flavus* has direct and indirect negative economic impacts for both exporter and importer countries [8]. Nowadays, various methods such as gamma irradiation [9] drying [10], storage temperature controlling [11], packaging [12], and edible coatings [13] are employed to maintain the quality and enhance the shelf life of edible nuts. In addition, due to less chemical and physical impacts on foods, in recent decades usage of UV irradiation has frequently been taken into consideration [14].

Different agricultural products such as peach [15], fresh pistachio [14], and lettuce [16] have been studied to examine the effect of UV-C irradiation on extending shelf life and reducing microbial count. In a related study, fresh pistachio of *'Ohadi'* and *'Akbari'* cultivars were exposed to UV-C irradiation with a dose of 0, 3, 6, and 12 kJ/m^2 for 45 days of storage. The results revealed that UV-C treatments had lower microbial infection than control samples [17]. In another research on fresh pistachio, the effect of UV irradiation was evaluated on the concentration of aflatoxin B_1 and results indicated that the aflatoxin concentration dropped from 100 to 78.189 ppb after 3 hours of exposure to UV radiation at 87.5 $\mu w/cm^2$. This value reached 42.193 ppb after 13 hours [18]. Now given significant improvements in technology, applying new methods to destroy some harmful microorganisms such as *A. flavus* without any changes in material properties, which are also affordable and eco-friendly is very necessary [19]. So according to the positive effects of UV-C irradiation on fruits and vegetables' shelf life, this study has explored the effect of continuous UV-C irradiation (in an optimized cylindrical system) on the changes in aflatoxin concentration and fatty acids of dried pistachio kernels.

2. Materials and methods

1. Optimization of agro-material disinfection device

In this experiment, the disinfection device used for agricultural products included 7 ultraviolet (UV-C) lamps with a wavelength of

about 254 nm (Philips, G30T8, 30W, China) . This device, made by Karimzadeh (2020), was composed of a stainless steel cylinder with 50 cm diameter and 100 cm height [20]. By adding a blower, electromotor replacement and rebuilding of the doors, the previous system was optimized. The blower was installed next to the device and it was connected to the output door. Device compartments were rotated by a 220 V three phase electromotor with 40 rpm speed through pulley and belt assemblies (Fig. 1).

Fig. 1. Optimized apparatus for disinfection of agricultural products

The amount of irradiation intensity of UV-C lamps inside the disinfection device was determined by a UV meter (leybold didactic GMBH-666230, Germany), after which using equation 1, the dose of irradiation was calculated.

$$D = E \times t \quad (1)$$

where D is the irradiation dose (J/m^2), E denotes the irradiation intensity (W/m^2), and t represents the irradiation time (s).

2. Preparation of fungal inoculum

A. flavus PTCC5004 with a production ability of 270 µg/kg was provided by the Iranian Research Organization for Science and Technology (IROST). Fig. 2 presents the morphology of the aflatoxin culture medium type B_1. All chemicals and microbial culture medium were purchased from Merck, Germany.

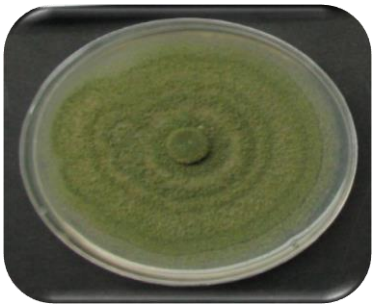

Fig. 2. Culture of *A. flavus* us ed in this experiment

3. Irradiation with UV-C disinfectant system

After the growth of *A. flavus* fungus suspension on the microbial culture media of potato dextrose agar, these fungal plates (9 cm diameter) were placed inside the continuous UV-C disinfection system. Irradiation was performed for three distances from UV-C lamps (10, 20, and 30 cm), three irradiation times (15, 30, and 45 minutes), and three lamp numbers (7, 5, and 3) in three replications. Next, treated plates were kept in an incubator (Pars Azma Company, Iran) at 27 °C for ten days, with the effect of UV-C irradiation on the *A. flavus* growth investigated daily.

4. Preparation of pistachio kernels

Dried pistachio nuts from the *Ohadi* variety were provided in May 2018 from the market in Kerman city, Iran, which were peeled manually whereby undamaged kernels were selected. Then, 150 g of pistachio kernels was weighed (Sartorius, GE412, Germany) and poured into the polyethylene packages.

Afterward, a suspension of A. *flavus* spores (10^6 cfu/ml) was meticulously prepared, adhering to all safety protocols. Subsequently, the suspension was sprayed onto the pistachio kernels within transparent films, utilizing a standard hand sprinkler, in a sterilized environment. Following the application, the packages were thoroughly mixed and securely sealed to prevent any potential re-contamination.

The pistachio packages were irradiated with the best treatment obtained from the previous experiment. All samples were kept for two months after irradiation at the normal ambient temperature with packages examined weekly. After that time, some samples were selected based on the non-presence of *A. flavus* and transferred to the Food and Drug Administration laboratory to determine the aflatoxin level in packed samples.

5. Measurement of aflatoxins of pistachio kernels

The aflatoxin contents of the prepared pistachio samples and standards were analyzed using the method 991.31 of AOAC. For this purpose, 50 g of the studied pistachio was mixed with 5 g NaCl and 300 mL methanol–water solvent (80:20) and shaken vigorously by hand for 15–30 s, and then for 30 min on a shaker (GFL, D-30938). The mixture was then filtered through a filter paper (Whatman No. 1) where the obtained extract was accordingly poured into the tubes of the Gilson-Workstation (GX-271 Aspec Gilson, USA); after dilution, it was passed through the Aflatest column at a speed of 3 ml/min. The Aflatest column was subsequently positioned in its designated location, and it was eluted with 1.25 mL of pure desorption methanol. The eluate was then transferred to a test tube for further analysis using the device. The eluted solution was subsequently diluted and homogenized with 1.75 mL of deionized water where an aliquot (100 µl) of this sample was injected into the HPLC system (Waters 1525, USA). The aflatoxins were eluted from a C18 column (5 mm, 4.6 × 250 mm × 4 µm, ODS3, Inertsil) using a mixture of methanol/acetonitrile/water (300 ml/200 ml/600 ml) containing 120 mg potassium bromide and 350 µl nitric acid 4 M as a mobile phase. The aflatoxins of B and G were detected using a fluorescence detector (2475 FLR detector). The excitation and emission wavelengths were 360 nm and 420 nm, respectively. Standard solutions of aflatoxin (0.026, 0.095, 0.25, 0.5, 1, and 2 ng/mL) were utilized to draw calibration curves to determine aflatoxin concentration of the samples [21].

6. Determination of fatty acid profile

Following the oil extraction from pistachio kernels using Soxhlet apparatus, the fatty acid compositions was measured by gas chromatography apparatus (Agilent 7890A, USA).

The fatty acid composition of oil samples was determined by a gas chromatograph, and it was reported in terms of relative area percentages. Fatty acid methyl esters were prepared through vigorous mixing oil and hexane (0.4g in 7 mL) with 7 mL of 2 N methanolic potassium hydroxide at 50°C for 20 min. Fatty acid esters were characterized using the gas chromatograph

(Agilent 7890A, USA) and ion flame detector. Helium was selected as the carrier with 17 ml/min flow rate. The air flow rate was 300 ml/min and hydrogen 30 ml/min. The oven, injection, and detector temperatures were maintained at 198, 250, and 280°C. The injection was carried out in split mode, with a split ratio of 20:1 [22].

7. Statistical analysis

In this study, analysis in the form of a completely randomized factorial design was performed with three factors including number of lamps (3, 5, and 7), lamp distance from samples (10, 20, and 30 cm), and irradiation times (15, 30, and 45 min), performed in three replications. The best treatment for fungal decontamination on the microbial culture was selected after which this optimal radiation treatment was evaluated on pistachio samples in three replications. Analysis of variance was performed at the 95% level of confidence using the SAS software 9.4 and Minitab 16.

3. Results

1. Effect of UV-C irradiation on the aflatoxin production

Fig. 3 indicates the growth of *A. flavus* on the culture medium for different treatments irradiated by UV-C over 10 days. It is visible that UV-C irradiation has a significant effect on the fungus growth rate and production of aflatoxin (with a 95% confidence level).

The results indicated that the growth of *A. flavus* was zero over the first three days after irradiation in all treatments, while in the control sample, the growth of *A. flavus* was immediately initiated from the first day. Although the growth of fungus in some treatments was observed from the 4th day, the plate surface in other treatments completely remained free of any fungus even until the 10th day.

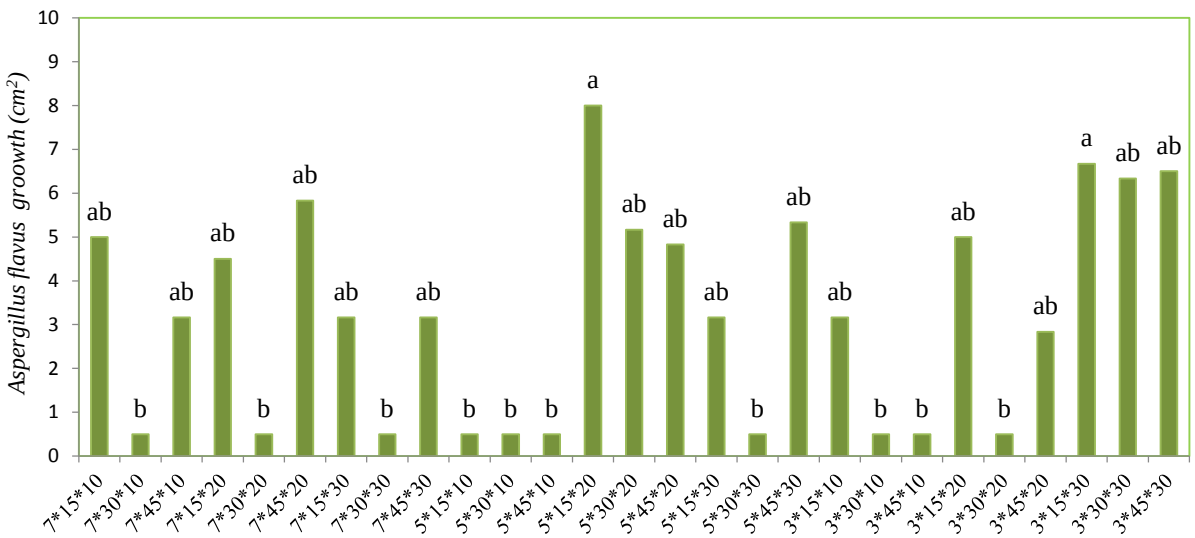

Fig. 3. Average growth of *A. flavus* fungus (cm^2) for different irradiated treatments (number of lamps, irradiation time, and lamp's distance) by UV-C irradiation for 10 days. In the treatments mentioned in the horizontal axis, the first number refers to the number of lamps, the second number is the irradiation time, and the third number denotes the distance of the lamp from the sample.

Fig. 4 displays The *A. flavus* growth in the control sample and treatment 3-30-10, which are completely different. Hosseini *et al.* reported that UV-C irradiation reduced the growth of fungi on fresh pistachio fruit, where the appropriate distance for fruits from the UV-C source was reported 15 cm [23].

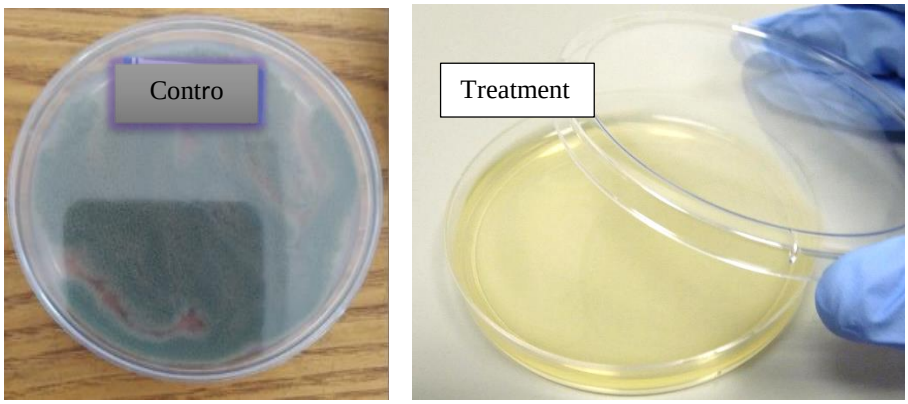

Fig. 4. Growth of *A. flavus* spores on control and irradiated culture medium of 3-30-10 treatment

2. Effect of the optimal UV-C irradiation on the reduction of aflatoxin level in dried pistachio kernels

The results of statistical analysis revealed that there was a significant difference between aflatoxin B_1 concentration in control and UV-treated pistachios ($p<0.05$). The amount of aflatoxin B_1 in the control sample was 9.35±0.001 ng/g and in the treated sample (with the 3 UV-C lamps, from 10 cm distance, and the duration of 30 minutes) was 0.0727±0.014 ng/g (Fig. 5). Based on the Iran's national standard, the maximum level of aflatoxin in pistachio kernels should be less than 1 ng/g. Thus, the level of aflatoxin in the control sample has been higher than the Iranian standard level. On the other hand, the results indicated that UV-C irradiation had a good performance in the prevention from *A. flavus* growth and aflatoxin production.

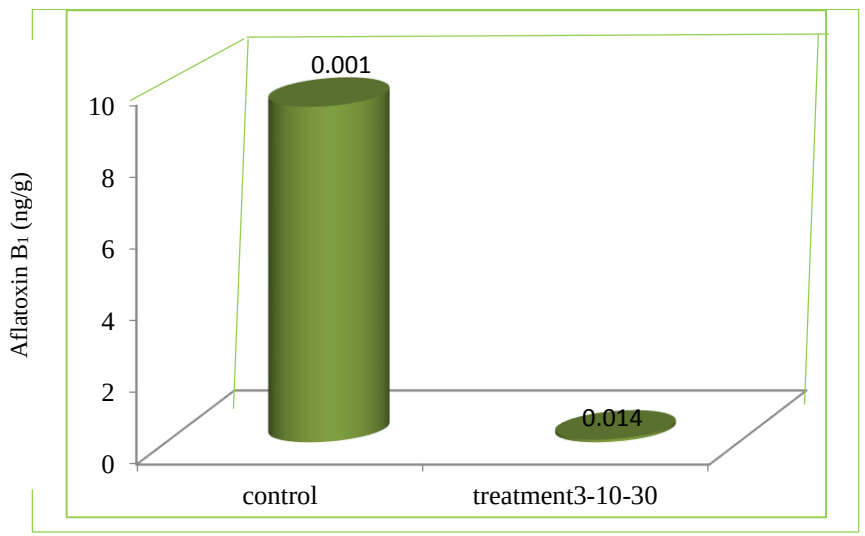

Fig. 5. Comparison of the aflatoxin B_1 (ng/g) between the control and irradiated sample.

As observed in Table 2, the amount of aflatoxin B_2 in the control sample was 0.567, and it was not detectable for the treated sample (3-10-30), suggesting the significant reduction of the aflatoxin B_2 in the treated pistachio kernels. The amounts of both aflatoxin G_1 and G_2 in

control and treated samples were not detectable either, since *A. flavus* is active in the production of aflatoxin B_1 and B_2. Fig. 6 indicates the fungus growth in both the control and 3-10-30 UV-treated samples which were infected by *A. flavus*.

Table 2. The concentration of three types of aflatoxins measured in control and UV-treated samples

Aflatoxin type	*Average concentration (ng/g)*	
	Treatment	*Control*
B_2	*Not detectable*	0.567
G_1	*Not detectable*	*Not detectable*
G_2	*Not detectable*	*Not detectable*

Fig. 6. Appearance characteristics of controlled (left side) and UV-C irradiated (right side) pistachios inoculated with *A. flavus* at the end of the storage period

3. Fatty acid profile of pistachio kernel

Fig. 7 depicts the comparison of the fatty acid analysis (6 fatty acids in pistachio oil) in control and irradiated samples ($p<0.05$). Among these fatty acids, oleic acid (58%) and palmitic acid (9%) are the most abundant unsaturated and saturated fatty acids in pistachio kernels, respectively. The fatty acid profiles of palmitic acid (9.65 and 9.85), palmitoleic acid (0.35 and 0.55), stearic acid (0.95 and 1.25), linolenic acid (58.4 and 58.2), oleic acid (29.3 and 29.1), and linoleic acid (0.85 and 0.15) were obtained in the control and irradiated samples (3-10-30 treatment), respectively. There was no significant difference between control and treated samples ($p<0.05$) in terms of palmitic, palmitoleic, stearic, oleic, and linoleic acids. This has been observed possibly due to the low penetration of UV-C irradiation and its surficial effect [24]. The significant difference between control and treated samples was only observed for linolenic acid, which was higher in the control.

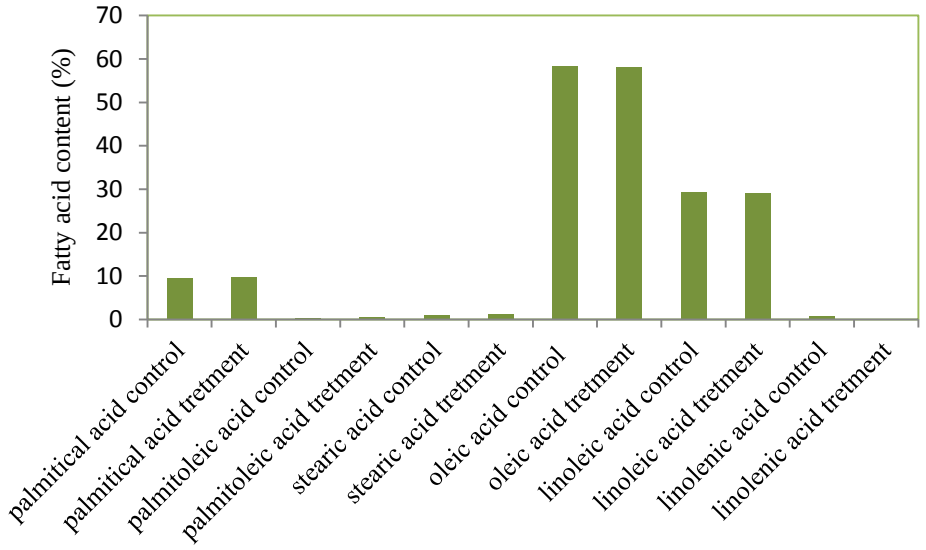

Fig. 7. The fatty acid profile of pistachio kernel in control and UV-treated sample (3-10-30)

4. Discussion

Based on the results obtained from investigating the impact of UV-C irradiation on aflatoxin production, the 3-30-10 and 3-30-20 treatments were selected as the two best treatments for prevention from aflatoxin production in pistachio kernels due to the following reasons. The 30-minute irradiation time (compared to the 45-minute) has the advantage of higher speed on the processing line. The lower the distance between the lamps and the samples, the more irradiation can penetrate the product and it would have a more efficient impact (especially in the samples with higher density). In addition, use of fewer lamps for UV-C decontamination is economically more affordable.

There are several reasons to prove the effectiveness of UV-C irradiation for preventing microbial growth. The nature of the UV-C beam is such that if it encounters with DNA in a living system, it leads to mutations in genes thus causing disorder in the stability of cells; eventually this disorder leads to the death of the cells or disables them [25]. Ultraviolet irradiation causes a series of phytoalexins to accumulate in the tissues of living organisms. Phytoalexins are antimicrobial and often antioxidative substances which are extremely effective in tissue resistance against fungus attacks [26]. The results of UV-C radiation on the growth of *A. flavus* and aflatoxin production in hazelnuts indicated that the irradiation for 6 hours reduced aflatoxins B_1 and G_1 by about 25%, but it did not affect aflatoxin B_2 and G_2 [27]. The irradiation of wheat grains at different times (0, 5, 10, 20, 40, 80, and 160 minutes) showed that the irradiation within 5 minutes had the best performance in the reduction of aflatoxin level, while more irradiation time made the fungus regrow [28]. The pistachio cluster was irradiated with UV-A and UV-B for 6 and 16 hours with two UV lamps (15 watts) and was kept for 7-21 days. The results revealed that UV irradiation had a significant effect on *A. flavus* colonies as well as the mycotoxin population [29].

The concentrations of unsaturated fatty acids vary among certain pistachio cultivars; however, oleic acid stands out as the predominant unsaturated fatty acid. Within the polyunsaturated fatty acids, linoleic acid accounted for the highest percentage, while the content of linolenic acid was comparatively lower. No significant differences were observed among pistachio cultivars concerning fatty acids, except for linoleic acid. levels of unsaturated fatty acids vary among different cultivars, with oleic acid being identified as the most significant unsaturated fatty acid [30].

Tsantili et al. (2010) confirmed the presence of fatty acids including myristic acid, palmitic acid, palmitoleic acid, margaric acid, stearic acid, oleic acid, vaccenic acid, linoleic acid, linolenic acid, arachidic acid, gondoic acid, and benzoic acid in some local pistachio varieties. Among saturated, monounsaturated, and polyunsaturated fatty acids, palmitic acid, oleic acid, and linoleic acid have the highest amount, respectively [31]. Note that the presence of unsaturated fatty acids in the pistachio kernel enhance the quality of pistachio [30]. Some researchers have considered the high percentage of oleic acid to boost the shelf life and stability of pistachio oil, and its consumption also reduces human blood cholesterol [32]. The high percentage of oleic and linoleic acids to palmitoleic and linolenic acids may enhance the stability and long-term shelf life of pistachios [33]. Al-Bachir (2015) in the investigation of pistachios purchased from local Syrian supermarkets concluded that the fatty acids of palmitoleic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid are present in the profile of pistachio fatty acids. The most unsaturated fatty acids were oleic followed by linoleic acid[34].

Fallah et al. (2018) researched to investigate the impact of gamma irradiation on the fatty acid profile in different cultivars of pistachios (Ohadi, Kaleh Qouchi, Ahmad Aghaei, and Akbari). After using gamma irradiation, some fatty acids including linoleic and oleic acids became isomers that are nutritionally valuable. Oleic and linoleic fatty acids as well as some other derived fatty acids such as α- and γ-linoleic acid are essential fatty acids for the body and should be supplied by food. In addition, use of gamma irradiation did not affect the organoleptic properties of pistachio cultivars [35].

5. Conclusion

The results of this study indicated that the use of three UV-C irradiation lamps for 30 minutes at a 10 cm distance from samples can prevent from the growth of *A. flavus* spores. Also, in the irradiated sample, the concentration of aflatoxin B_1, which is the most dangerous type of aflatoxin, was reduced considerably. Characterization of the profile of fatty acids in the treatment sample indicated that palmitic acid, palmitoleic acid, stearic acid, oleic acid, and linoleic acid had no significant difference with the amounts of these fatty acids in control, where the only significant difference was observed for the linolenic acid in the control and treated sample. Furthermore, in another paper presented by the authors of this research [25], it was reported that the ultraviolet irradiation method did not have a negative effect on some chemical properties of pistachio (Ohadi) including phenolic compounds or peroxide value as well as some organoleptic properties such as taste, odor, texture, and color. This irradiation system can be installed in different pistachio processing lines to disinfect products, which is very useful in reducing and preventing aflatoxin contamination.

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