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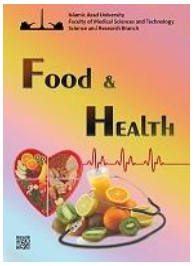

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Strategies to reduce microbial contaminations and increase the shelf life of pistachio fruit: A review

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A B S T R A C T

Pistachios are good sources of some functional compounds that are essential for human health. In addition to consuming dried pistachios (salted/roasted) or used as ingredients in a variety of confectionery and cookery products, consuming fresh pistachios is also gaining a foothold in the market. This review presents pre- and postharvest operations to prevent microbial contamination and to preserve physicochemical properties of fresh and processed pistachios for extending their shelf life. There is a potential in pistachios to be contaminated with some undesirable microbes, especially aflatoxin-producing fungi, during pre- and postharvest operations. In this regard, strategies to the prevention of aflatoxin production and the decontamination of produced aflatoxin in pistachios have been of interest to researchers. Different practices including sorting, thermal processing, biological control, ozone treatment, gamma irradiation, ultraviolet irradiation, and cold plasma have been proposed for aflatoxin decontamination. Sorting out damaged pistachios is one of the most important postharvest strategies to reduce aflatoxin levels (up to 98%) that can be done manually or electronically. The majority of pistachios (~85%) are consumed as roasted form that combining roasting with lemon juice improves the elimination of aflatoxin (up to 93%). Drying and packaging are the most important methods to maintain quality and improve the shelf life of pistachios. Laminated and metallized films with vacuum or modified atmosphere are the proper packaging for pistachios.

1. Introduction

Pistachio fruit (*Pistacia vera L.*) is an important agricultural product in worldwide, which belongs to the *Anacardiaceae* family. The pistachio fruit can be classified as a semidry drupe containing a single edible seed (kernel), encased by a thin soft coat (testa), enclosed by a creamy lignified shell (endocarp), which is surrounded by a green to yellow-red colored, depending on the degree of ripeness, fleshy hull (mesocarp and epicarp) (1). The world pistachio production in 2018 was 1,375,770 tons out of which 551,307 tons was produced in Iran (2). After harvest, the pistachios are dehulled, washed and segregated into the two categories, floaters (~15%) and sinkers (~85%). Finally, nuts are dried (<7% moisture) and sent to the market. Pistachios are consumed both raw and roasted. In addition, they are used as an ingredient in various products such as ice cream, pastries, desserts, salad, and traditional Persian foods (3). Pistachios are good sources of energy and many nutrients, minerals, proteins, and fiber that are essential for human health (Table 1). Besides nutrients, pistachios contain an array of biologically active substances, such as carotenoids, anthocyanins, flavonoids, essential oils, and vitamin C, all of which have some medical properties due to its antioxidant and antimicrobial activities (4). Pistachios are considered as perishable nuts, which susceptible to microbial contamination and chemical spoilage in the pre- and postharvest steps. Due to the high amount of lipid as well as high levels of unsaturated fatty acids, pistachios are also prone to chemical spoilage and quality decay during storage period. Unsaturated fatty acids are readily oxidized and accelerate the

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discoloration, oxidative rancidity, and the development of off-flavors (6). Examination of the microbial contamination of the nuts indicates that pistachios are more frequently invaded by molds than by bacteria (7). Mold growth in crops, especially nuts, can pose significant risks to human health through toxic metabolites called 'mycotoxins', which have mutagenic, carcinogenic, and teratogenic effects (8). Therefore, different national and international organizations have promulgated rules and guidelines concerning the handling, growth, and subsequent processing of these food commodities in an effort to contain foodborne illness. In response to these concerns, in 2000 the U.S. Food and Drug Administration (FDA) set guideline threshold levels for total aflatoxins in pistachio nuts at 20 µg/kg (9). The allowed maximum legal limits for pistachio nut are 10 µg/kg for total aflatoxin (AFT) and 8 µg/kg for AFB1 in Iran (10), and 4 µg/kg for AFT and 2 µg/kg for AFB1 in European Union (8). Economically, microbial infections of pistachios can also cause significant damage to producers' income (11). Since no comprehensive study has been done in this topic, we review recent developments in applied strategies for controlling (or degrading) aflatoxin in pistachio nuts and also, discuss common operations used to extend pistachio shelf life.

Table 1. Chemical composition of raw pistachio nut (5).

Ingredients	values
Water (g)	4.37
Energy (kcal)	560
Protein (g)	20.16
Total lipid (fat) (g)	45.32
Carbohydrate(g)	27.17
Fiber (g)	10.16
Sugars (g)	7.66
Total saturated fatty acids (g)	5.907
Total monounsaturated fatty acids (g)	23.257
Total polyunsaturated fatty acids (g)	14.38

2. Microbial contamination of pistachio fruit

In recent years, there has been growing concern about the prevalence of pathogens and their metabolites in food. Nuts such as pistachios have a high shelf life due to their low moisture content and they are often stored for many months. However, depending on the conditions, pathogenic microorganism can contaminate them during pre- and postharvest operations. Traditionally, pre- and postharvest practices of pistachios such as harvesting, drying, hulling, and shelling were performed with poor hygiene procedures. In addition, pathogenic microorganism have the ability to survive for long periods in relatively dry products such as pistachios (12). Molds are the predominant contaminants of nuts, especially pistachios. The presence of molds in pistachios can pose a threat to human health, because these microorganisms produce toxic metabolites called 'mycotoxins'. *Aspergillus* spp. are the most important molds found in nuts because they produce a highly toxic mycotoxin called aflatoxin, which has been proven to have potential carcinogens to humans in many studies (13). Among different commodities, pistachio is considered to be the most sensitive product to *Aspergillus* spp. growth and aflatoxin production (14). *Aspergillus flavus*, *Aspergillus nomius*, *Aspergillus pseudotamarii*, and *Aspergillus parasiticus* are the strains that play the prominent role in producing aflatoxin (15). Studies have shown that pistachios can be contaminated with aflatoxin at all stages of production and processing, from the field when the nuts were still green on the tree, till storage, and also stated that maturity is the most important stage because it was the first stage that aflatoxin was detected above permitted limits (16). The presence of aflatoxins in pistachios has been reported in different countries including Iran, Turkey, US, UK, Mexico, and Qatar. The natural occurrence of aflatoxins in pistachios in various countries is given in Table 2. These results indicate that the distribution of aflatoxins is not limited to one country or region and it is observed in almost all pistachio producing countries. In addition, contamination of crops with aflatoxins has become an important issue in world trade, and importing countries have often declared total aflatoxin action threshold levels at four µg/kg (13). As mentioned earlier, pistachio fruits and their products are mostly contaminated by molds, and studies have focused on this area. However, there were evidences that shows nuts such as pistachios can be a possible source of pathogenic bacteria as *Salmonella* spp. (17). In 2016, five different serotypes of *Salmonella* (Montevideo, Worthington, Liverpool, Enteritidis, and Senftenberg) were identified in raw California in-shell pistachios (18). The FDA also have measures to address the risk for contamination by *Salmonella* species in food containing a pistachio-derived product as an ingredient (19). The prevalence of *Salmonella* has been documented in raw pistachios in range of 0.37-2.0% (18). In the past, it was thought that low water activity environments could not be a suitable environment for survival of pathogenic bacteria. However, studies have shown that pathogenic bacteria such as *Salmonella* can survive for a long time in low water activity environments such as pistachios in many cases (20, 21). *E. coli* also was found in 1.1% of 184 pistachio kernels collected from retail markets in UK (22). Kimber, Kaur (21) investigated the viability of *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes* bacteria on pistachio at different temperatures. They reported that there was no significant decrease in bacterial counts at temperatures of -19 and 4 °C. However, at 24 °C, the total bacterial population declined significantly and initial rates of decline were 0.15, 0.35, and 0.86 log CFU/g/month on pistachios for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*, respectively. Other less harmful bacteria such as *Xanthomonas*, *Clostridium*, and *Pseudomonas* spp., as well as *Eurotium*, *Fusarium*, and *Penicillium* spp. are also occasionally found in various contaminated nuts (23). Typically, foodborne pathogens are not part of the natural epiphytic population of edible nuts that are hostile to their growth and survival (24). However, some pre- and postharvest activities, as well as damage that may be caused to nuts during the growing and processing, may be a main factor in nuts contamination. In orchards or during storage, insects can damage pistachios (hull, shell, and kernel), thereby creating pathways for exposure to microorganism contaminations (23).

Therefore, the management of pre- and postharvest operations is essential to prevent crop contamination.

3. Control of aflatoxin in pistachio nuts

Aflatoxins are secondary metabolites produced by some species of *Aspergillus*, especially *A. flavus* and *A. parasiticus*. Consumption of products containing these metabolites has detrimental effects on human and animal health. Studies have shown that these metabolites can have carcinogenic and tumorigenic effects on human health (35). *Aspergillus* spp. have four main types of aflatoxins, including B_1, B_2, G_1, and G_2, all of which are toxic for humans. B_1 and B_2 are often produced by *A. flavus*, whereas G_1 and G_2 are often produced by the *A. parasiticus* (36). In recent years, due to the deleterious effects of these toxins on the public health and economics perspectives, various strategies have been proposed and applied by researchers to prevent aflatoxins production or reduce its content. Most of these strategies are based on the two operation groups, including the prevention of aflatoxin production and the decontamination of produced aflatoxin (37).

Table 1. Chemical composition of raw pistachio nut (5).

Country	Type	Value	References
Turkey	AFB_1	3.42 μg/kg	(16)
	AFB_2	0.282 μg/kg	
	AFG_1	0.104 μg/kg	
	AFG_2	0.057 μg/kg	
	AFT	3.75 μg/kg	
Chile	AFB_1	69.9% > 0.2 μg/kg	(25)
	AFB_2	74.4% > 0.2 μg/kg	
	AFG_1	67.0% > 0.2 μg/kg	
	AFG_2	82.5% > 0.2 μg/kg	
Qatar	AFT	27.7% > 20 μg/kg	(26)
Morocco	AFT	45 %, ~163 μg/kg	(27)
	AFB_1	45 %, ~158 μg/kg	
Iran-Kerman	AFB_1	5.9 μg/kg	(28)
Iran	AFT	23.5 %, ~2.42 μg/kg	(29)
	AFB_1	23.4%, ~2.18 μg/kg	
Turkey-Ankara	AFB_1	1 to 113 μg/kg	
Greece	AFB_1	371.21 μg/kg	(16)
	AFB_2	24.7 μg/kg	
	AFG_1	218.32 μg/kg	
	AFG_2	10.65 μg/kg	
Spain-Catalonia	AFB_1	0-1037.3 μg/kg	(30)
	AFB_2	0-97.22 μg/kg	
	AFG_1	0-0.71 μg/kg	
	AFG_2	0-0.23 μg/kg	
Pakistan	AFT	2.10-6.34 μg/kg	(31)
Tunisia	AFT	21.8 μg/kg	(32)
Korea	AFT	16.22 μg/kg	(33)
Algeria	OTA	64.5% occurrence	(34)

In the prevention strategies section, different approaches including cultivation techniques, controlling the insect pests in pistachio nut orchards, and genetic engineering as pre-harvest methods, and sorting and proper storage as postharvest methods are presented (Table 3). In the following, the ability of these processes to control aflatoxins and their effects on product quality is discussed in detail.

4. Sorting

Sorting is one of the most important postharvest operations of pistachios that increases their marketing. In Iran, as the biggest producer of pistachios in the world, sorting and segregating of undesirable pistachios is mainly done by human operators, which is affected by several factors, such as age of operators, fatigue and visual acuity, motivation, and environment conditions, for these reasons, automated systems are especially welcome (54, 55). In general, pistachio sorting has several stages including separation of trash, water flotation to segregate empty-shell and immature nuts, hull removal, sorting to remove closed-shell nuts, and electronic sorting based on both physical (size, density) and optical (UV, infrared) parameters to segregate and remove stained shell (13, 56). If needed, manual sorting is performed to complete the electronic process and segregate pistachios with insect damage. Finally, to increase marketability, pistachios are sorted by size. Sorting out damaged pistachios is one of the most important postharvest strategies to reduce aflatoxin levels that can be done manually or electronically. Most aflatoxin contaminations occur in the orchards and are associated with damage caused to the fruit's hull, mainly early-split and insect-damaged, prior to harvesting (57). Studies, in recent years, have shown that sorting has a significant effect on the rate of aflatoxin contamination and can reduce it by up to 98% (43).

Table 2. The occurrence of aflatoxins in pistachios sampled in various countries markets or orchards.

	Strategy	Treatment conditions	Observations	Reference
Prevention Strategy	Pre-harvest; Biological control	Spraying the pistachio tree with aqueous suspension of *P. anomala* at 3×10^7 cells/ml	22% reduction in colonization of *A. flavus* 77% reduction in spore number of *A. flavus*	(38)
	Pre-harvest; Biological control	*B. subtilis* UTB3 at 5 and 8 dpi	>2 logs reduction in *A. parasiticus*	(39)
	Pre-harvest; Biological control	*B. subtilis* M419; inoculation 1 ml at 10^8 CFU/mL	~ 56% inhibition in the sporulation of *A. flavus*	(40)
	Postharvest; Chemical control	Applying the salicylic acid at 0-11 mmol/L	100% inhibition of *A. flavus* growth at 9 mmol/L 100% reduction of AFB_1 at 7 mmol/L	(41)
	Postharvest; Wet sorting		90.86% reduction of AFB_1	(42)
	Postharvest; sorting	Separation of all stained pistachios	98.8% reduction of AFB_1	(43)
Decontamination Strategy	Pre-harvest; Biological control	*B. subtilis* M419; inoculation 1 ml at 10^8 CFU/mL	87% reduction of AFT	(40)
	Pre-harvest; Biological control	*B. subtilis* UTB1; inoculation 1 ml at 10^4 CFU/mL	~64% reduction of AFT, 12% reduction of sporulation (*A. flavus*)	(44)
	Pre-harvest; Biological control	Essential oils of clove, cinnamon and celak diluted at 9%	100% reduction of AFT	(45)
	Decontamination; Ozonation	Ozonation at 9.0 mg/L for 420 min	24% reduction of AFT 23% reduction of AFB_1	(46)
	Decontamination; Gamma irradiation	Radiation at 10 kGy	68.8% reduction of AFB_1 in peeled pistachio 84.6% reduction of AFB_1 in unpeeled pistachio	(47)
	Decontamination; Gamma irradiation	Radiation at 3 kGy	Total fungal counts: less than 10 CFU/g	(48)
	Decontamination; E-beam irradiation	Radiation at 7 kGy	77.17% reduction of AFB_1	(49)
	Decontamination; microwave radiation	10 min at 100% power of high frequency output of 2,450 MHz and 1.0 kW	72.5% reduction of AFB_1	(50)
	Decontamination; Cold plasma	600 W, 15.56 MHz RF power generator; argon and oxygen (10:1 v/v); 5 min	5.4 log reduction of *A. brasiliensis*	(51)
	Decontamination; UV-C light	At 265 nm for 45 min	~91% reduction of AFB_1 ~99% reduction of AFB_2 ~96% reduction of AFT	(52)
	Decontamination; Roasting	Roasting at 150 ºC for 120 min	~95% reduction of AFB_1 ~87% reduction of AFB_2	(53)
	Decontamination; Roasting	Roasting with 30 ml water, 15 ml lemon juice and 2.25 g of citric acid at 120 ºC for 1 h	49.2% reduction of AFB_1	(35)

5. Biological control of aflatoxin

Biological control is one of the successful strategies to manage aflatoxins in nuts considered as an alternative to chemical treatments. In recent years, various microorganisms including nontoxigenic strains of the *A. flavus* and *A. parasiticus*, bacteria and yeasts have been studied for this purpose in the pre- and postharvest stages. Control or reduction of aflatoxins by microorganisms is carried out through different mechanisms. The most important mechanism is the use of antagonists that reduce aflatoxin production by inhibiting the growth of fungi. Hence, researchers have been investigating the effect of various antagonistic microorganisms, especially from the genus *Bacillus* (39, 40, 58), *Acinetobacter*, *Agrobacterium*, *Pseudomonas*, *Achromobacter*, and *Streptomyces* (59, 60) on the rate of food infection by aflatoxin. Siahmoshteh, Siciliano (39) reported that *Bacillus subtilis* and *Bacillus amyloliquefaciens* were able to reduce the mycelial growth of *A. parasiticus* in pistachio until 5 days post inoculation. Researchers have attributed the possible mechanisms of antagonistic activity of bacterial strains to the competition for nutrition and space and production of antifungal metabolites (61). Lipopeptides are the most prominent antifungal compounds produced by *Bacillus* and include the surfactin, iturin, and fengycin families (40, 62). Cytoplasmic membranes of fungi contain sterol derivatives which could be the target of antifungal metabolites (63). In addition to antagonistic activity, bacteria such as *Bacillus* spp.

have been shown to have significant ability to degrade aflatoxins. Farzaneh, Shi (58) reported that *B. subtilis* UTBSP1 could considerably remediate AFB1 from nutrient broth culture and pistachio nut by 85.66% and 95%, respectively. In another detoxification strategy, some organisms (*Lactobacillus rhamnosus* strains and *Saccharomyces cerevisiae*) show the ability to adsorb aflatoxins to their binding sites (64). In this regard, many studies have been conducted in recent years to identify and isolate atoxigenic strains from pistachio orchards that could be used as biocontrol agents in the future (65-67).

Table 3. Summary of applied strategies for production control or degradation of aflatoxin in pistachio nuts.

	Strategy	Treatment conditions	Observations	Reference
Prevention Strategy	Pre-harvest; Biological control	Spraying the pistachio tree with aqueous suspension of *P. anomala* at 3×10^7 cells/ml	22% reduction in colonization of *A. flavus* 77% reduction in spore number of *A. flavus*	(38)
	Pre-harvest; Biological control	*B. subtilis* UTB3 at 5 and 8 dpi	>2 logs reduction in *A. parasiticus*	(39)
	Pre-harvest; Biological control	*B. subtilis* M419; inoculation 1 ml at 10^8 CFU/mL	~ 56% inhibition in the sporulation of *A. flavus*	(40)
	Postharvest; Chemical control	Applying the salicylic acid at 0-11 mmol/L	100% inhibition of *A. flavus* growth at 9 mmol/L 100% reduction of AFB_1 at 7 mmol/L	(41)
	Postharvest; Wet sorting	-	90.86% reduction of AFB_1	(42)
	Postharvest; sorting	Separation of all stained pistachios	98.8% reduction of AFB_1	(43)
Decontamination Strategy	Pre-harvest; Biological control	*B. subtilis* M419; inoculation 1 ml at 10^8 CFU/mL	87% reduction of AFT	(40)
	Pre-harvest; Biological control	*B. subtilis* UTB1; inoculation 1 ml at 10^4 CFU/mL	~64% reduction of AFT, 12% reduction of sporulation (*A. flavus*)	(44)
	Pre-harvest; Biological control	Essential oils of clove, cinnamon and celak diluted at 9%	100% reduction of AFT	(45)
	Decontamination; Ozonation	Ozonation at 9.0 mg/L for 420 min	24% reduction of AFT 23% reduction of AFB_1	(46)
	Decontamination; Gamma irradiation	Radiation at 10 kGy	68.8% reduction of AFB_1 in peeled pistachio 84.6% reduction of AFB_1 in unpeeled pistachio	(47)
	Decontamination; Gamma irradiation	Radiation at 3 kGy	Total fungal counts: less than 10 CFU/g	(48)
	Decontamination; E-beam irradiation	Radiation at 7 kGy	77.17% reduction of AFB_1	(49)
	Decontamination; microwave radiation	10 min at 100% power of high frequency output of 2,450 MHz and 1.0 kW	72.5% reduction of AFB_1	(50)
	Decontamination; Cold plasma	600 W, 15.56 MHz RF power generator; argon and oxygen (10:1 v/v); 5 min	5.4 log reduction of *A. brasiliensis*	(51)
	Decontamination; UV-C light	At 265 nm for 45 min	~91% reduction of AFB_1 ~99% reduction of AFB_2 ~96% reduction of AFT	(52)
	Decontamination; Roasting	Roasting at 150 ºC for 120 min	~95% reduction of AFB_1 ~87% reduction of AFB_2	(53)
	Decontamination; Roasting	Roasting with 30 ml water, 15 ml lemon juice and 2.25 g of citric acid at 120 ºC for 1 h	49.2% reduction of AFB_1	(35)

6. Ozone treatment of pistachios

Ozone is a very strong oxidizing agent widely used to control pests and to prolong the shelf life of crops. It can be used in both gaseous and aqueous states. One of the most important benefits of using ozone is that no residual toxins leave after the process, because it decomposes into oxygen quickly after use (68). Hence, the direct use of ozone in food processing has been approved by Food and Drug Administration (FDA) and World Health Organization (WHO) (69, 70). In recent years, several studies have been conducted on the control of some microorganisms (including mold and bacteria) in pistachio with ozonation, after harvest. The high fungicidal efficacy of gaseous ozone for the reduction of *A. flavus* was reported previously by Savi, Piacentini (71). They illustrated that the growth of *A. flavus* completely inhibited at 60 μmol/mol ozone after 180 min. In addition to its fungicidal properties, ozone has a high ability to decompose mycotoxins, especially aflatoxins (72). In this regard, after ozonation of pistachio kernels at 9.0 mg/L for 420 min, a 24% and 23% decrease in AFT and ATB_1 has been reported, respectively (46). The aflatoxin degradation mechanism with ozone has been attributed to the reaction of the ozone with C8-C9 double bond of the furan ring of aflatoxin through electrophilic attack based on the Criegee mechanism, causing the formation of monozonide derivatives such as ketones, aldehydes, and organic acids (73). By eliminating the double bond between C8 and C9 in the furan ring, aflatoxin toxicity is significantly reduced or completely disappeared (71). It was also showed that aqueous ozone solution could reduce aflatoxin in pistachios (74). The results of the former study illustrated that after treatment with 8 ppm ozone solution, aflatoxin B_1, B_2, G_1, G_2 and AFT levels decreased by 47.9%, 12.5%, 45.8%, 43.9%, and 44.4%, respectively. Ozone is more effective for reducing aflatoxin on pistachios with hard skin than pistachios with soft shell (74). Ozone was also found to be effective in decreasing the level of *Salmonella serovars*, *Escherichia coli*, and *Bacillus cereus*, which developed on pistachio nuts after harvest and during storage (75). The effectiveness of ozone against pathogenic bacteria depends on treatment conditions and increased with increasing exposure time and ozone concentration. One of the important issues in food processing is the influence of process conditions on its physicochemical and organoleptic properties that should remain as constant as possible and not degrade nutritionally. Studies have shown that ozone treatment at moderate condition has no significant effect on physicochemical (pH, moisture content, color, and free fatty acid values) and organoleptic (appearance, flavor, sweetness, rancidity, and overall palatability) properties of pistachio and thus, it could be appropriate strategy for reducing pathogens on pistachios (46, 75).

7. Gamma irradiation treatment of pistachios

Gamma irradiation is a non-thermal processing technique, that has high potential for the inactivation of pathogenic microorganisms and extending the shelf life with maintaining quality attributes (76). According to the literature, the use of gamma irradiation has many advantages over the conventional methods, including less damage to health promoting compounds, high penetration power, proper processing time, and high performance in low moisture food (77). In recent years, gamma irradiation, due to its potential, has received considerable attention for pest control in fruits and nuts, like pistachios. Studies have shown that irradiation of pistachio nuts at 1-3 kGy seem to be sufficient for decontamination in the postharvest period, without significant changes in sensory and chemical properties (48). Song, Kim (77) used gamma irradiation to inactivation of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* in pistachios. The population of pathogens decreased with increasing irradiation dose, and after irradiation at a dose of 5 kGy, the population of pathogens decreased to under the detection limit (10 log CFU/g), without effecting color change. Based on the previous findings, gamma radiation may damage microbial DNA and, to a lesser extent, denature proteins or remove hydrogen atoms from the bases of the DNA strands by hydroxyl radicals generated from water (76). In addition to the bactericidal property, it has also been reported gamma irradiation can be effective in controlling hazardous pistachio molds, so that a dose of 3 kGy could be sufficient for elimination of *A. flavus* and their toxins production ability in the pistachio nuts (78). Moreover, studies have shown the ability of gamma irradiation to degradation of aflatoxin (37, 47, 79). It was illustrated that the rate of aflatoxin B_1 degradation was positively correlated with the increase in irradiation dose and reached 68.8 % at 10 kGy in pistachio (47). The efficiency of aflatoxin degradation by gamma irradiation depends on the type of food material and its characteristics such as moisture, density, and etc. (79). The mechanism of aflatoxin degradation by gamma radiation can be the result of radiolysis of water or other compounds (80). The resulted free radicals attack the terminal furan ring in aflatoxin B_1 and reduce its biological activity. There are always concerns that processing treatments like gamma irradiation may have a detrimental effect on the health benefits of the pistachio compounds. Lipids (especially unsaturated fatty acids) are the major constituents of pistachios and they are very susceptible to oxidation during processing. Oxidation of unsaturated fatty acids leads to the formation of peroxides which responsible for the development of rancid off-flavors in irradiated foods. It was shown that irradiation caused partial decomposition of triglycerides, and also, illustrated that the radiolysis of fatty acids causes the formation of two groups of long chain hydrocarbons (81). Besides to the lipids, Akbari, Farajpour (82) investigated the effect of gamma irradiation on the bioactive compounds and their properties in three different Persian pistachio nuts. They reported that irradiation up to 2 kGy could increase anthocyanin, phenolic content, and antioxidant activity of pistachios. Sensory evaluation of irradiated pistachios also revealed that gamma irradiation at low levels did not significantly change sensory properties (odor, color, texture and taste) (74). However, there are some results show that irradiation at doses above 5 kGy makes pistachios unacceptable for consumption (83).

8. Ultraviolet (UV) treatment of pistachios

UV irradiation process is a non-thermal technique performed at wavelengths between 100 to 400 nm. UV rays are divided into three sub-sections including UV-A (315-400 nm), UV-B (280-315 nm), and UV-C (100-280 nm). In recent decades, UV radiation has received much attention due to its advantages for application in food disinfection and decontamination. Studies have shown that UV rays, as a postharvest practice, have a high ability to prevent the growth of *Aspergillus* and to degrade the produced aflatoxin (79). Irradiation of contaminated pistachios by *Aspergillus* spp. also indicated that UV-A and UV-B can reduce significantly the percentage of fungus germination and secondary metabolites production (84). However, UV-B had more deleterious effects on AFB1 than UV-A, which could be due to the higher energy of UV-B rays (84). The reduction of aflatoxin in pistachio nuts depends on the thickness of the irradiated sample layer. Experiments have indicated that decreasing the thickness of the specimens from 10 mm to 2 mm resulted in an increase in the percentage of aflatoxin reduction from 18.9% to 49.3% (79). UV-C is another section of UV rays with high energy that has some uses in postharvest practices such as reduction of the respiration rate and browning index, delay fruit ripening and lethal effect on the molds and bacteria (85). The fungicidal activity of UV-C radiation is more pronounced in nuts adjusted at high moisture level (52). Jubeen, Bhatti (52) reported that UV-C irradiation at 265 nm for 45 minutes reduces the aflatoxins B_1, B_2 and G_1 in fresh pistachio (moisture 16%) by 95.27, 96.03 and 100%, respectively. UV radiation can inactivate mycotoxins (aflatoxins) by altering their structure. The terminal furan ring in structure of aflatoxins is affected by UV radiation, thus the active site for binding is eliminated (86). Nutritional value and sensory properties are the most important factors affecting consumer acceptance and must be maintained unchanged during processing. Hosseini, Akhavan (85) used a rotational UV-C irradiation system for extending the shelf life of fresh pistachio. They recommended a UV-C irradiation treatment at a dose of 2.1 kJ/m^2 for fresh pistachio preservation based on the physicochemical, microbial, and sensory parameters. It has been found that high energy UV rays produce free radicals and subsequently lipid radicals, which then leads to cross linking in protein and carbohydrate, and peroxidation of unsaturated fatty acid (87). Therefore, due to the possibility of nutritional value degradation, it is advisable to avoid the processing of nuts at high doses of UV rays (85, 88).

9. Cold plasma treatment of pistachios

Cold plasma, as non-thermal technique, is a novel processing that carried out at around the ambient temperatures and does not have the negative effects of heat treatments. It is a partially or wholly ionized gaseous mixture that consider as the fourth state of matter (89). Cold plasma has gained much attention in food processing to inactivate the pathogenic microorganism and prolong the shelf life of food. The use of cold plasma has been less commonly applied for pistachio processing, and few studies have been performed to date. Microbial studies have shown that cold plasma has the potential to decontamination of pistachios. Pignata, D'Angelo (51) showed that treatment of pistachios with argon/oxygen (10:1 v/v) plasma for 1 min results in two-log reductions of fungal population. The inactivation mechanism of fungi with plasma has not yet been fully understood. However, several hypotheses have been put forward by researchers for fungal inactivation, such as the cell wall destruction, cell apoptosis, cellular protein destruction, fragmentation and release of DNA, and deformation of mycelial tip (89). The decontamination effects of cold plasma application on pistachio nuts that were contaminated with *A. flavus* and *A. parasiticus* are also studied (90, 91). Treatment of pistachios with cold plasma (18–20 min), resulted in a decrease of ~3.5-4.5 log in the *Aspergillus* spp. population. The effectiveness of the cold plasma method depends on various factors, such as treatment time, type of gas, product type, target microorganism, initial concentration of microorganisms, and so on (51, 89). Investigation of the effect of cold plasma on aflatoxin degradation has revealed that this technique has the potential to eliminate aflatoxin. However, there is no study on the effect of plasma on aflatoxin degradation of infected pistachios. In other nuts, however, this ability has been proven (92). The mechanism of aflatoxin degradation with cold plasma has been completely reviewed by Pankaj, Shi (37). During cold plasma treatment, ions (O^-, O^+, H^-, H^+, H_3O^+), free radicals (H·, OH·, O·, NO·), and molecular species (O_2, O_3, N_2, H_2O_2) are formed, which are considered responsible for aflatoxin degradation (37, 93). Investigation of the organoleptic properties of plasma treated pistachio has shown that plasma treatment does not cause any apparent changes in the product structure. Also, no significant differences were observed between the sensory properties (texture, odor, and general acceptance) of the samples (treated vs control) by trained panelists (91).

10. Roasting treatment of pistachio nuts

Pistachio nuts are usually thermally processed before consumption in order to improve sensorial properties. Pistachio aroma after thermal processing has been identified as determinant for consumer acceptance (94). Roasting, as a postharvest practice, is the most common thermal treatment applied to pistachio nuts at temperatures above 110 °C. According to literature, 85% of all pistachio is consumed as roasted form (95). In previous studies, the effect of roasting process on physical, chemical, and biological parameters of pistachios has been extensively investigated. Roasting alters and substantially improves the flavor, texture, color, and appearance of nuts (96). During the roasting, moisture decreases as the temperature increases. Structurally, roasting makes the texture more brittle and crumble (97). During roasting process, pistachio microstructures change dramatically and the communicating porous system extends considerably, as cells start to detach from each other so that the porosity and pore volume of nuts increases. Protein bodies also are distorted and the endoplasmatic network is destroyed completely (98). Roasting treatment causes the chlorophylls (bright green color) change to pheophytins and pyropheophytins (yellow-brown olive color). Roasting also reduced total starches and dextrins, total available carbohydrates, and total free sugars of pistachios (96). The Millard non-enzymatic reaction is one of the possible reactions in the formation of brown-to-black compounds during roasting in a severe condition, in which reducing sugars and free amino acids are take part and change the color of pistachio to a golden appearance on the surface. Maillard reaction and Strecker degradation of amino acids are known to be responsible for creating flavor and color in roasted pistachios (98, 99). In terms of health, the amount and profile of pistachio lipids is very desirable. In previous studies, different and contradictory results have been reported about the effect of roasting process on pistachio fatty acid profile. Hojjati, Noguera-Artiaga (100) indicated that roasting of pistachios at 135 °C for 20 min causes a significant decrease in the content of unsaturated fatty acids (74.7%), compared with raw pistachios (85.8%). While, Rodríguez-Bencomo, Kelebek (101) and Ghazzawi and Al-Ismail (102) reported a slight increase in the content of unsaturated fatty acids. As a result of the roasting process, one can expect that a close relationship exists between increasing microstructure size (or increasing oxygen penetration) and lipid oxidation rate (98). In addition, pistachios contain many natural bioactive compounds that act as antioxidants in various ways, and are important for human health. Researchers have shown that roasting increases phenolic compounds and antioxidant activity in pistachios (100-102). Moreover, roasting is considered as an effective physical method to degrade of aflatoxins in nuts. It was observed that roasting at 150 °C for 30 min eliminated 19-66% of the AFB1 from pistachio nut (53). Rastegar, Shoeibi (35) also reported that combining roasting with lemon juice improves process efficiency and further eliminates aflatoxin (up to 93%). According to studies, the efficiency of the heat treatment process for aflatoxin degradation is influenced by several factors including process temperature, time of exposure, moisture content, initial level of aflatoxin, and type of food (53, 103). In addition, thermal processing extends the shelf life of pistachios by control of the microbial contamination. Inactivation of microorganisms during roasting is affected by various factors (12). As Casulli, Garces-Vega (104) showed, with increasing pistachio humidity, the roasting efficiency for *Salmonella* inactivation significantly increased. For example, the time to achieve a 4 log reduction decreased 50 to 80% when humidity increased from ~3 to 30%. When sampling nuts from the market, it was also reported that *Aspergillus* spp. contamination is significantly higher in raw samples than in roasted samples (105).

11. Drying process of pistachio nuts

Drying is a very important operation in pistachio processing which increases its shelf life. After harvesting, the pistachio contains 40-50% moisture (wet basis), which should be reduced to below 7% to protect its nutritional and organoleptic values during storage up to a year or longer under normal conditions (106). In Iran, pistachio drying is carried out both in traditional and industrial methods. In the traditional method, pistachios are dried on concrete floor at ambient temperature (in sunlight or shade). Since in this method, the drying rate is very slow (2-3 days), the moisture content of the final product and air temperature cannot be controlled, and the potential for contamination (dust, animals, insects and microorganisms) is high, therefore the final product is of poor quality (106, 107). Therefore, in the last two decades, the design and development of dryers to accelerate the drying process and improve the quality of pistachio crops have attracted the attention of artisans and researchers. In this regard, various types of dryers such as bin dryer, vertical cylindrical dryer, vertical funnel dryer, and vertical continuous flow dryer were manufactured (107). In a comparative study among dryers, Kashani Nejad, Tabil (107) found that bin dryer is the best commercial dryer for dehydration of pistachios and produces nuts with good quality. While, Shakerardekani, Karim (108) have recommended a combination of bin dryer and sun drying or combination of bin dryer and solar dryer, as effective drying techniques to prevent adverse quality effect on pistachio nut. In the drying process, reactions may occur that affect the quality of the nuts and their marketability. Autoxidation is the most common type of rancidity in nuts caused by the reaction between unsaturated fatty acids with oxygen. Heat and light are the most important stimuli of this reaction that must be considered in the drying process. During these reactions, volatile carbonyl compounds are formed that give the product an unpleasant odor and taste (107). Gazor and Minaei (109) observed that changes in air temperature and velocity in a bin dryer did not have a significant effect on the amount of pistachio nuts fat and protein, but if the temperature reached 90 °C, the peroxide content would increase. In recent years, there have also been many efforts to evaluate and develop pistachio nuts dryers, such as modeling the infrared-convective drying of pistachios under fixed and fluidized bed conditions (110), modeling the microwave-convective drying of pistachios (111), performances evaluation of the solar drying of pistachios with air recycling system (112), and evaluation the drying kinetics of pistachio kernels in a fixed bed drying system (113). All of these studies have tried to improve the thermal efficiency of the dryer and the product quality using new technologies.

12. Packaging and storage of dried pistachio nuts

As mentioned earlier, pistachios contain high amounts of oil, the major part of which is unsaturated fatty acids. This has made pistachios susceptible to oxidative changes during the storage, resulting undesired flavors and odors as well as decreasing the nutritional value. Moreover, if environmental conditions are suitable for the growth of fungi and bacteria, it can cause microbial contamination, which is both safety and quality problematic. Therefore, it is necessary to protect the pistachios from environmental changes by proper storage and packaging. Studies have shown that the penetration of moisture, light, and gases into the packaging causes undesirable appearance changes and accelerates chemical reactions, and also, the presence of oxygen inside the packaging accelerates the oxidation reactions. Therefore, the packaging material and inside atmosphere are two determinants of pistachio shelf life. Shakerardekani and Karim (114) investigated the effect of different types of plastic packaging films on the moisture and aflatoxin contents of pistachios during 10 months storage at ambient temperature. They showed that all the plastic packaging materials (food-grade polyvinyl chloride, polyethylene terephthalate, polyamide/polypropylene, and nylon) except LDPE (low density polyethylene) delayed the moisture absorption and aflatoxin formation. During storage, dried pistachios act as hygroscopic materials and that their moisture content increase depending on the type of packaging films. It is noticeable that the presence of moisture increases the possibility of the fungi growing and consequently aflatoxin formation (115). It was also observed that cellophane films was unsuitable for packaging of pistachios due to their high moisture permeability, low strength, low sealing ability, and high cost of production (116). In study conducted by Raei, Mortazavi (117), the effect of different packaging materials including five-layer film (two-layer polypropylene, two-layer polyamide, and one-layer glue), modified polypropylene, and metallized plastic (polypropylene coated with a layer of aluminum) on roasted pistachio quality was investigated. The results showed that the metallized and five-layer films were more effective in maintaining the pistachio quality than the modified polypropylene film. Vacuum and modified packaging systems also have a significant impact on extending the shelf life of pistachios. As we know, oxygen concentration is an important parameter affecting pistachio oxidation process. Consequently, to control the oxidation reactions and prevent aerobic microbial growth, reducing O_2 concentration in the storage atmosphere over the food through vacuum or adding gases such as nitrogen, carbon dioxide, and ozone could be an appropriate way (118). Researchers have illustrated that storage of raw pistachios by vacuum or modified packaging system (30% CO_2, 70% N_2, and 4 ppm O_2 gas) reduce the formation of hydroperoxides as an indicator of lipid oxidation (119, 120). In addition, there are evidences showing that storage the pistachios under 100% CO_2 and low temperatures has the best results among the different treatments in terms of maintaining physical properties and sensory attributes and extending the shelf life (120, 121). Carbon dioxide effectively prevents the growth of bacteria (aerobic strains) and molds, which need oxygen to survive (122). Generally, the concentration of filling gases (O_2, CO_2, and N_2) varies depending on the nature of the products. As a result, further studies are needed to accurately determine the gases mixture in the modified packaging system for pistachios.

13. Shelf-life extension of fresh pistachio fruit

Due to the importance of fresh products and their nutritional properties, the consumption of raw pistachios has received more attention in recent years. Fresh consumption of pistachio in Iran has been reported to be about 10%, in 2019 (85). Harvested pistachios are very perishable and can cause significant economic damage if not processed properly. Various postharvest changes such as moisture evaporation, discoloration, softening of tissues, increasing the microbial growth, and enzymatic browning can occur for fresh pistachios and reduce its marketing (85). Therefore, it is necessary to take appropriate techniques to prevent these changes. Given the importance of the issue, researchers have proposed a variety of strategies to prevent fresh pistachios from spoiling during storage. Sheikhi, Mirdehghan (123) applied a new organic-based postharvest sanitizer (a combination of H_2O_2, Na_2CO_3, K_2CO_3, citric acid, and acetic acid) for fresh pistachios under modified atmosphere packaging. They determined the best combination of GRAS sanitizers (3.3% H_2O_2, 4.1% Na_2CO_3, 3.9% K_2CO_3, 8114.5 mg/L citric acid, and 7574.0 mg/L acetic acid) to control the enzymatic browning and total viable count and to maintain the sensory quality. Moreover, the sodium nitroprusside spraying can be considered as a multifunctional signaling molecule to reduce the browning of the shell and maintain the quality of freshly harvested pistachio fruit (124). In another strategy, researchers used edible coatings in combination with plant extracts or antioxidant compounds to extend the shelf life of fresh pistachios. In this regard, various polysaccharides such as gum Arabic, alginate, chitosan and carboxymethyl cellulose have been used to coating the fresh pistachios, and in most cases satisfactory results have been reported (125-127). Edible coatings act as barriers (semi-permeable layers), which reduce the transfer of moisture and gases diffusion through the fruit surface (126). In addition, bioactive compounds and organic acids such as thyme essential oil, *Zataria multiflora* essential oil and salicylic acid are used to enhance the effectiveness of edible coatings (128). These compounds prolong the storage life of fresh pistachios by having biological activities such as antimicrobial and antioxidant. Packaging type and systems are other strategies used to extend the shelf life of fresh pistachios. Unlike the conventional packaging, fresh pistachios can be packaged appropriately using modified atmosphere packaging (MAP), controlled atmosphere (CA) or vacuum packaging (VP) to control the deterioration reaction and microbial growth by reducing O_2/or enhancing CO_2 concentration in the storage atmosphere (129). Studies on raw pistachio have also shown that packaging systems, especially the modified atmosphere, have high ability to delay quality losses and extend its shelf life (119, 129, 130). Modified atmosphere packaging can well control the ripening process of fruits and vegetables by reducing respiration rate and minimizing metabolic activity, thereby increasing postharvest life (131).

14. Conclusion

Aspergillus spp. are the most important pathogenic microorganisms in the pistachio industry that produce a carcinogenic mycotoxin called aflatoxin. Various strategies for the control and elimination of aflatoxin in pistachio nuts have been presented in recent years, which are discussed in detail here. In general, none of these strategies can completely eliminate aflatoxin, so it is better to combine these strategies. Moreover, the effect of new thermal treatment technologies in drying and roasting operations on the quality and marketability of pistachios has been discussed and compared with the conventional method. As a result of the thermal processes, it can be expected that there is a close relationship between increasing microstructure size and lipid oxidation rate. According to studies, laminated and metallized films are the proper type of packaging for pistachios. Also, different packaging systems such as vacuum and modified by reducing the oxygen level inside the packaging can improve the shelf life of pistachios with maintaining quality. It is expected that advanced technologies will be handled in the pistachio processing industry further in the future.

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