

## The effect of gamma radiation on the growth of *Aspergillus flavus* and the production process of aflatoxin B1 in pistachio Sample

Zahra Latif (MSc)<sup>1</sup>, Abolfazl Salehizadeh (PhD)<sup>2</sup>, Gholamreza Shahhosseini (PhD)<sup>3</sup>

<sup>1</sup> Laboratory expert in Radiation Application Development Company, Atomic Energy Organization

<sup>2</sup> Radiation Application Development Company, Atomic Energy Organization, Tehran, Iran

<sup>3</sup> Nuclear Science and Technology Base - Nuclear Agriculture Research Institute, Karaj, Iran - Director of Veterinary Medicine and Animal Science and Fisheries Research Group

Information	Abstract
<p><b>Article Type:</b> Review Article</p>	<p><b>Aim and objectives:</b> Pistachios rank as Iran's second most significant non-oil export and are among the agricultural products most susceptible to <i>Aspergillus flavus</i> and aflatoxin contamination. Aflatoxin B1, a highly potent naturally occurring carcinogen, is the most toxic variant of mycotoxins.</p> <p><b>Methodology:</b> <i>Aspergillus flavus</i> and aflatoxin B1 in pistachios pose a significant challenge for international exports. Therefore, the early detection of these toxic compounds is crucial for managing pistachio contamination. Various methods exist for preventing and treating contaminated pistachios.</p> <p><b>Results:</b> Gamma-ray irradiation is an approved technique for pistachio preservation in many countries. It has proven effective in safeguarding against pathogenic bacteria without compromising the physical and chemical properties of the nuts. The required dosage of gamma radiation is primarily determined by the level of contamination and the specific harmful microorganisms present. However, excessive gamma radiation can adversely affect the pistachio's attributes, including its color and flavor.</p> <p><b>Conclusion:</b> This review article aims to determine the effective dose of gamma-ray radiation necessary to inhibit the growth of <i>Aspergillus flavus</i> and the production of aflatoxin B1 in pistachios, thereby reducing microbial load while maintaining the quality characteristics of the product.</p>
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<p><b>Corresponding Author:</b> <i>gholamreza shahhosseini</i></p> <p><b>Email:</b> gshahhosseini@yahoo.com</p> <p><b>Tel:</b> +98- 9122626227</p>	

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## 1. Introduction

Pistachios are a significant agricultural product in regions including Iran, America, Syria, Turkey, Greece, and Italy, and they hold a prominent position in global markets [1]. Iran, in particular, is the leading exporter of pistachios worldwide [2]. However, the presence of high levels of aflatoxin in these nuts poses a serious challenge to their global marketing and export potential. As a result, pistachios are frequently tested for microbial quality, including assessments of fungal contamination and aflatoxin levels, due to their susceptibility to contamination [3,4]. Mycotoxins, which are secondary metabolites produced by fungi, pose significant risks to both human and animal health, resulting in various diseases and substantial economic losses. The contamination of food and nutrition with mycotoxins represents a critical global issue [5]. Research indicates that the early ripening of pistachios may increase their exposure to spores from aflatoxin-producing fungi, resulting in a significant risk of aflatoxin contamination [3,4]. Aflatoxins represent a significant group of mycotoxins primarily generated by two fungal species: *Aspergillus flavus* and *Aspergillus parasiticus* [1,6,7]. The four primary types of aflatoxins are AFB1, AFB2, AFG1, and AFG2 (see Figure 1). Numerous studies indicate that aflatoxins can be generated in the field and during storage, and they are known to be carcinogenic, mutagenic, and capable of suppressing the immune system [8,9]. The potential presence of *Aspergillus* species in pistachios warrants a thorough investigation due to the risk of aflatoxin production. Critical factors that contribute to the germination of *Aspergillus flavus* and subsequent aflatoxin production include the early maturity of pistachios and ambient

humidity levels ranging from 78% to 88%. It is imperative to monitor and control these conditions to mitigate the risk of contamination and ensure the safety and quality of pistachios. [9]. *Aspergillus flavus* is indeed frequently cited in scientific literature as one of the most prevalent fungi responsible for food contamination. It exhibits a particular affinity for contaminating nuts, oilseeds, and grains. These products, rich in fats and carbohydrates, provide an ideal natural substrate for the growth of aflatoxin-producing fungi. The high nutrient content of these foods makes them especially susceptible to contamination. Consequently, it is crucial to implement stringent monitoring and control measures to prevent the proliferation of *Aspergillus flavus* and ensure food safety. [8]. Consumers increasingly prioritize food safety and health. One effective method to prevent aflatoxin production is by inhibiting fungal growth [10,11]. Food irradiation, often referred to as cold pasteurization, involves exposing food to ionizing radiation energy [12]. This technique, utilizing medium doses (1-10 kg), effectively eliminates microbial contamination and extends the shelf life of various foods, including fruits, vegetables, seafood, poultry, and spices [13].

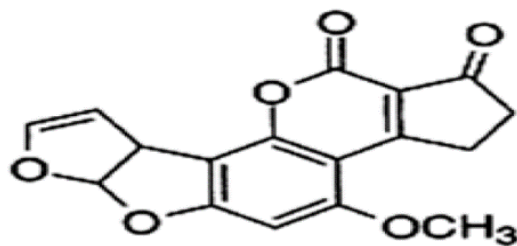
This research addresses a critical challenge in the export of Iranian pistachios, specifically the risk of aflatoxin contamination, primarily caused by the fungus *Aspergillus flavus*. Given the significance of maintaining food safety and quality, gamma radiation has emerged as a promising method for controlling microbial growth and extending shelf life without leaving harmful residues. This study aims to identify the optimal gamma radiation dose that effectively reduces or eliminates aflatoxin B1 production

while preserving the quality attributes of pistachios. Key aspects of the study will include: The dose-response relationship: Investigating various doses of gamma radiation to determine the threshold that sufficiently inhibits aflatoxin B1 production by *Aspergillus flavus* without compromising the sensory and nutritional qualities of the pistachios. Assessing the physical (appearance, color, texture), chemical (nutritional composition, oil content), and sensory properties (taste, aroma) of the pistachios post-irradiation. Microbial analysis: Conducting microbiological assessments to evaluate the reduction in fungal load and overall microbial contamination in the irradiated pistachios. Establishing the shelf life of gamma-irradiated pistachios compared to non-irradiated samples to demonstrate the preservation benefits of the treatment. Ensuring that the proposed doses comply with international food safety regulations and standards for radiation treatment of food products. Through this comprehensive investigation, the study aims to provide valuable insights and recommendations that could enhance the safety and marketability of Iranian pistachios, thereby addressing both public health concerns and economic challenges in the export sector.

## 2. Materials and Methods

### *Aspergillus flavus* and aflatoxin contamination

*Aspergillus flavus* contamination frequently occurs in various foods and poses a significant threat to both human and animal health [14]. This mold produces aflatoxins B1 and B2, while *Aspergillus parasiticus* produces aflatoxins G1 and G2 [15]. A 2023 study by Chasti et al. highlighted that during the pre-harvest stage, products are vulnerable to pest and insect attacks, leading to quality degradation [16]. The resultant damage from these insects allows molds to penetrate pistachios, facilitating aflatoxin production [17,18]. Table 1 displays the aflatoxin levels in various Iranian pistachio cultivars, determined using Duncan's multi-range test ( $\mu\text{g}/\text{kg}$ ) [19]. In Iran, the permissible limit for aflatoxin B1 is 8 ppb, and the total aflatoxin limit is 10 ppb, or nanograms per gram [20]. In 2023, Amirafazl et al. identified insect and pest damage as a major factor contributing to aflatoxin production in numerous agricultural products [21].



Aflatoxin B1

**Fig. 1.** Chemical structure of aflatoxin B1

**Table 1.** Comparison of aflatoxin levels in some Iranian pistachio cultivars based on Duncan's multi-range test ( $\mu\text{g}/\text{kg}$ )

Pistachio cultivars	Aflatoxin production rate	Duncan Group
Shahpasand	0.8	a
Abbas Ali	0.6	ab
ohadi	0.6	ab
Ahmad Aghaei	0.2	ab
Kalle Ghoochi	0.4	ab
khanjari	0.2	ab
Akbari	0	b

Similar letters after the mean percentage in each column indicate no significant difference at the 5% level (Duncan's multiple range test method).

### The effect of gamma rays on the microbial quality of pistachios

Gamma irradiation is an effective method for eliminating insects and rodents [22,23]. This process is valuable not only for reducing microorganisms and increasing shelf life but also for preserving the physical, chemical, nutritional, and biological properties of foods [24]. Appropriate doses of gamma radiation are crucial for achieving these goals. However, a downside is the potential formation of oxidized lipids, resulting from reactions between cell membrane fats and oxygen radicals, which can cause an unpleasant taste [24]. According to the FAO, around 25% of global agricultural products are contaminated with mycotoxins, leading to economic losses. Preventing aflatoxin contamination during harvest and storage isn't always possible, necessitating food and feed disinfection. Ensuring food safety remains a critical 21st-century challenge for the WHO and humanity [25]. Studies show that gamma irradiation at doses of 5 and 9 kGy on pistachios can completely eradicate aflatoxin throughout storage [20]. Doses up to 10 kGy pose no

toxicity, nutritional, or microbiological risks, making gamma irradiation highly effective for controlling mold growth and preventing mycotoxin production. [24,26-30].

## 3. Result

### Irradiation of food

Food irradiation is a contemporary preservation method utilizing ionizing radiation to control or decrease harmful organisms in food. Endorsed as safe by the WHO, FAO, IAEA, and Codex Alimentarius, it effectively extends shelf life while maintaining sensory and safety qualities. Unlike some chemical disinfectants, which can harm agricultural products, irradiation offers a safer alternative. Methyl bromide, for example, was phased out due to ozone layer damage. Over four decades of research have revealed numerous food industry applications for radiation, including sterilizing grains, preventing vegetable sprouting, preserving perishable quality, and disinfecting meats, seafood, and eggs. As more countries adopt this technology, interest in its potential applications grows. Food irradiation involves

exposing packaged or bulk items to controlled ionizing radiation doses, reducing harmful microorganisms by disrupting microbial cells through generated free radicals. Free radicals, formed when radiation interacts with food, are highly reactive yet transient. However, they can persist longer in hard, frozen, or dehydrated foods, such as bones, necessitating higher energy levels to effectively eliminate microbes. The effectiveness of this radiation process hinges on several factors, including the structure of the microorganism, its sensitivity to radiation, and its DNA composition. The effects of irradiation on food can be classified as direct or indirect. Direct effects occur when radiation damages the DNA molecules of microorganisms, leading to cell death, particularly in low-moisture foods. In contrast, indirect effects arise from the radiolysis of water, which generates free radicals such as hydroxyl and hydrogenated atoms that attack cells, with an especially pronounced impact on high-moisture

foods like fresh produce and meat. When microbes in food are exposed to irradiation, the energy disrupts the bonds in DNA and RNA, resulting in genetic damage that halts reproduction and leads to cell death. DNA's double-helix structure, made up of sugar-phosphate backbones and nucleotide base pairs (cytosine, thymine, adenine, and guanine), is particularly susceptible to this damage. The extent of the damage is influenced by the state of the food; for instance, frozen foods generally require higher radiation doses to kill microbes compared to fresh foods. Additionally, the process's efficiency varies depending on the organism's ability to repair DNA, its sensitivity to radiation, and its DNA content. Larger organisms, such as parasites and insects, can be eliminated with lower doses of radiation due to their larger DNA amounts, while bacteria and viruses, which possess smaller DNA, typically require higher doses for effective eradication.

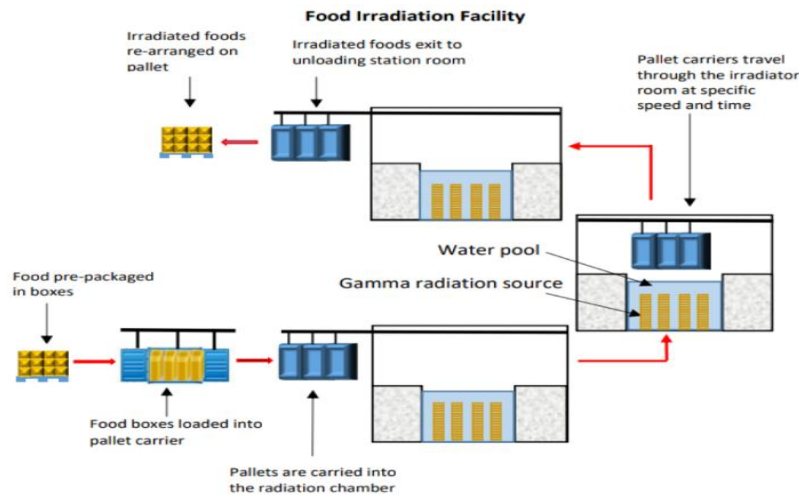
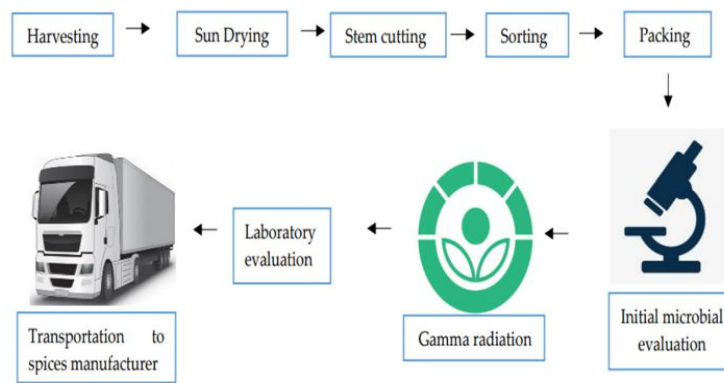


Fig. 2. A schematic diagram of a food irradiation center.



**Fig. 3.** Diagram of pistachio irradiation process.

## 4. Discussion

### Consumer understanding of food irradiation

One of the primary challenges facing the development of food irradiation is the pervasive misconception among consumers that irradiated food becomes radioactive and poses serious health risks. Additionally, some individuals harbor concerns about the quality of irradiated food, while others mistakenly believe that this preservation method results in significant nutrient loss. Consumers must understand that all food preservation techniques—whether they involve refrigeration, freezing, or heating—can have some impact on the natural properties of foods. For instance, heat-based preservation methods can lead to the destruction of certain vitamins and nutrients, with the extent of loss varying depending on the specific technique utilized. In contrast, irradiation does not involve heat, which allows for the preservation of nutrients more effectively than methods that do. Food irradiation offers significant advantages for both the food industry and consumers. When consumers are provided with clear and adequate information about food irradiation, their willingness to accept irradiated foods increases.

Numerous surveys conducted worldwide have consistently revealed a positive consumer response toward the acceptance of irradiated foods. Making this information readily available is essential for overcoming misconceptions and fostering greater acceptance of this valuable food safety technology [44,45,46].

## 5. Conclusion:

Higher doses of gamma radiation effectively reduce *Aspergillus flavus* and aflatoxin B1 in pistachio products, but excessive doses can adversely affect their chemical and quality attributes, such as taste, color, and aroma. Thus, it is crucial to identify an optimal irradiation dose. Studies indicate that gamma radiation can significantly lower the microbial load in pistachio products. Some research suggests a dosage of three kilograms for specific pistachio types stored for three months; however, after six months, aflatoxin levels may surpass the standard limit [20,36,37]. To preserve pistachio quality and ensure export to European countries, a dose of 5 kg is recommended [47,48]. This dose not only inhibits microorganism growth but also retains flavor, extending storage duration without increasing aflatoxin levels [47,48]. Exportable pistachios to the EU must contain a

maximum of 10 ppb of total aflatoxin and 8 ppb of aflatoxin B1 [20]. Research shows that doses of 5 and 7 kg of gamma rays effectively eliminate spores of aflatoxin-producing fungi, preventing both their growth and aflatoxin production. A 2021 study by Mina Makari and colleagues found that a 6 kg dose was most effective for eliminating *Aspergillus flavus* and preventing aflatoxin B1 production, while a 2 kg dose preserved pistachio quality [49]. Additionally, research by Masoume Alinjad and colleagues in 2021 suggested that light gamma

radiation up to 2 kg maintains the physicochemical, structural, and sensory properties of pistachios. Overall, the optimal irradiation dose is 5 kg, which ensures no growth of aflatoxin-producing fungi during nine months of storage, corroborated by findings from Sepideh Khorasani and colleagues in 2017 [20].

### conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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