Aflatoxin Contamination of Pistachio and the Problems and Strategies of Decontamination; A Review Study

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Abstract

It goes without saying that the most important problem of the country' pistachio exports is the contamination of pistachios with *Aspergillus flavus* and aflatoxin. The liver and kidney are the tissues targeted by aflatoxin that leads to carcinogenic effects. The contamination of pistachios with this toxin can threaten the main source of foreign exchange earnings, and prevent the producers from competing in the global market. In Iran and other pistachio-producing countries, contamination by *Aspergillus* species occurs during the growth phase as well as during or after the processing phases. As much as 2.5% of the world's agricultural products are contaminated with mycotoxins. At present, the maximum allowed levels for aflatoxin B1 and total aflatoxin are 1-20 ng g^{-1} and 0-35 ng g^{-1}, respectively. The Iranian Institute of Standard and Industrial Research has set the highest acceptable levels of aflatoxin B1 and total aflatoxin in pistachios at 5 and 15 ng g^{-1}, respectively. Mechanical separation techniques, thermal inactivation, and irradiation are among the methods of decontamination and fungus control after pistachio harvesting. Ionizing radiation decontamination is a highly efficient, safe and environmental-friendly process that can be used for decontamination.

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1. Introduction

Given its high nutritional value and significant economic role, pistachio is of special importance among nuts. According to the reports released by Food and Agriculture Organization (FAO), the world's pistachio production was 548759 million tons in 2002. Pistachio is Iran's second most important non-oil export product and has an important role in the development of the national economy and food industry. In general, Iran is in the first rank of pistachio production with 300,000 million tons per year, followed by the United States with 127010 million tons, Turkey with 40,000 million tons, Syria with 39208 million tons, and China with 26,000 million tons. The next ones are. Between 2009 and 2011, about 20,000 tons of pistachios were exported from Iran to the European Union, most of which was from Kerman and Rafsanjan (1- 3).

Pistachio is rich in nutrients (Table 1) that are essential for human's diet (4, 5). Iran, with a cultivated area of around 440,000 hectares of pistachio orchards, accounts for about 57% of world production and over 60% of global exports of this product; Iran is known as the largest and the most important pistachio-producing producing. The most important properties and benefits of pistachio include antioxidant and anti-inflammatory properties, as well as benefits for heart, nervous system, and vision, etc. (6, 7).

Iran is the world's largest pistachio producer. However, sometimes due to aflatoxin contamination of pistachios, human health and pistachio marketing status face numerous problems, and this has caused challenges for pistachio exports of Iran and some other countries. Major markets for pistachio include Iran, Asian countries, Central Asia, and the Persian Gulf countries (1).

Fungi are one of the main causes of post-harvest health problems. Food contamination with mycotoxins has become a major problem across the world. Mycotoxins are mostly produced by fungi in areas with hot and humid climates. They can also be found in temperate climates. Exposure to mycotoxins is mainly oral. However, it is likely to occurs through the skin and inhalation. The disease caused by being exposed to mycotoxins is known as Mycotoxicosis (8, 9). Food storage conditions or animal feed are factors affecting the presence of mycotoxins that can be controlled. Other factors include climatic conditions as well as the characteristics of the fungal species, etc.

Table 1- Nutritional value of dried and roasted pistachios, slat-free (in 100 grams)

Energy	567 Kcal	**Vitamin E**	1.42 mg	**Phosphorus**	469.0 mg
Carbohydrate	29.38 g	**Vitamin C**	3.0 mg	**Potassium**	1007 mg
Protein	20.95 g	**Thiamine (B_1)**	0.70 mg	**Sodium**	6.0 mg
Fat	44.82 g	**Riboflavin (B_2)**	0.23 mg	**Selenium**	10.0 µg
Water	1.85 g	**Niacin (B_3)**	1.37 mg	**Copper**	1.29 mg
Fiber	9.90 g	**Pantothenic acid (B_5)**	0.51 mg	**Zinc**	2.34 mg
Sugar	7.74 g	**Choline (B_4)**	71.40 mg	**Manganese**	1.24 mg
Cholesterol	0.0 mg	**Vitamin B_6**	1.12 mg	**Calcium**	107.0 mg
Iron	4.03 mg	**Folate (B_9)**	51.0 µg	**Magnesium**	109.0 mg
Vitamin A	13.0 µg	**Vitamin K**	13.20 µg	**-**	-

Mycotoxins are universally distributed and are known as the natural microflora of air and soil. Pure aflatoxin is a yellowish white crystal that is colorless solid and is a major group of mycotoxins. It contaminates a wide range of foods such as nuts, rice, wheat, barley, cereals and some other agricultural products (10, 11).

Aflatoxins are a group of furanocoumarins derived from polyketides. They are secondary metabolites produced by some species of Aspergillus, especially *Aspergillus flavus* and *Aspergillus parasiticus*. There are four main types of *Aspergillus flavus* including B_1, B_2, G_1, and G_2. The reason for this naming is due to the blue and green colors produced by fluorescent light under ultraviolet light (12, 13).

Among the types of aflatoxins, most concerns are about aflatoxin B_1. *Aspergillus flavus* becomes more compatible with the aerial parts of plants (leaves, flowers and fruits) and produces only aflatoxin B. However, *Aspergillus parasiticus* becomes compatible with the soil environment and produces aflatoxins G and B. *Aspergillus flavus* has always been mentioned as a food contaminant in scientific sources. Almost all types of *Aspergillus parasiticus* produce aflatoxins. The toxicity percentage of *Aspergillus flavus* varies depending on the branch, background and geographical origin. Containing high fat and carbohydrates, pistachio is one of the most susceptible products and the most suitable natural environment for the growth of aflatoxin-producing fungi in the world. This toxin is produced by productive fungi in the stages of production, storage and food processing (14, 15).

A number of studies and monitoring programs have been conducted in several countries to obtain a general pattern of aflatoxin contamination in foods. In at least 100 countries, there are rules for major mycotoxins in goods and foods; many of these rules have been adopted for aflatoxins. However, the maximum level of tolerance varies greatly in different countries. Poor storage conditions are likely to contribute to fungal growth and increase toxin contamination. Aflatoxins are heat resistant, and it is very unlikely to convert them from toxic forms to non-toxic ones. According to the FDA, although food contamination is inevitable, applying various monitoring devices can minimize it (16, 17).

Given their relatively long history of being studied and the problems of their presence in the environment, aflatoxins are known to be the most popular mycotoxins. It is difficult to prevent pre-harvest aflatoxin production, so aflatoxins are considered a constant hazard in animal feed. All pistachio-producing countries face aflatoxin contamination. As a result, these condition not only create uncertainty

for pistachio consumers, but also lead to excessive costs in production and loss of income of producers, distributors and other beneficiaries (18, 19). Studies have indicated that all *Aspergillus parasiticus* and 40% of *Aspergillus flavus* isolated from agricultural products have the potential to produce aflatoxins. Nowadays, contamination of agricultural products with mycotoxins is one of the most important health problems of the world community, and given the serious dangers of mycotoxins, different countries have adopted special rules for the production and consumption of food imports. Most of the countries in the world are highly sensitive to food contamination by *Aspergillus* fungi and the aflatoxin resulting from them (8, 20, 21).

2. Materials and Methods

In order to find all the published studies on aflatoxin contamination ways and decontamination methods, various databases have been reviewed including PubMed, Google scholar, Scopus, ISI Web of Science by implementing the time constraint from 2005 to 2020. For the searching process, the keywords of the articles conducted in this field have been used. Moreover, the database of thematic titles was used. Some of these keywords include pistachio contamination, aflatoxins, *Aspergillus flavus*, decontamination of aflatoxins, etc. In addition, the list of sources used in all articles published in this field has been reviewed to include other possible sources in the study and to ensure the completeness of the sources.

Pathways of aflatoxin contamination

Aflatoxins are commonly produced in pistachios before harvesting process and in the orchard. Any delays in harvest, lack of proper processing, and improper storage conditions are likely to result in the increased fungal growth and aflatoxin production. Factors such as splitting and 88-78% of humidity are essential for the germination of *Aspergillus flavus* and the production of aflatoxins. *Aspergillus flavus* is a plant pathogen that is rarely found in healthy pistachio kernel. Studies have indicated that pistachios with early splitting are likely to expose pistachio kernels to fungal spores with high levels of aflatoxin contamination. The most important factor for the entry of aflatoxin-producing fungi into pistachio fruit and their growth and development and ultimately the production of aflatoxin is the cracking of the outer skin of pistachio in the orchard. Early splitting is the most dangerous type of splitting where both green and bony skin crack at the same time, and the kernel is exposed to the influx of aflatoxin-producing fungi (22, 23).

Inadequate storage conditions are likely to lead to the occurrence or increase of *Aspergillus* contamination. The growth of *Aspergillus flavus* and the production of aflatoxin in storage conditions are influenced by different parameters such as

gaseous composition of the environment, aquatic activity, acidity, microbial interaction and storage duration. In general, water activity of less than 0.7 at 25 °C (relative humidity of pistachio kernel is less than 7%) and relative humidity of less than 70% have been recommended for long-term storage of pistachios.

Health effects of aflatoxins

Aflatoxins were first identified in agricultural products in the early 1980s and were identified as the most potent natural carcinogens, mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus*. Chemically, aflatoxins are known as one of 18 polycyclic sulfur-free compounds that emits light strongly against fluorescence rays. The liver and kidney are the tissues commonly targeted by aflatoxin and lead to carcinogenic effects. The International Agency for Research on Cancer has listed aflatoxin B_1 on its list of potential human and animal carcinogens; it has the highest zootoxic and carcinogenic potential among aflatoxins in agricultural products and plays an important role in the etiology of liver cancer, especially in individuals carrying hepatitis B surface antigen.

Aflatoxin B_1 is converted to -B_1 epoxide and hydroxylated metabolites such as aflatoxin M_1 in the first phase of metabolism by cytochrome P450. The epoxide form of aflatoxin B1, which is highly active, can be connected to RNA, DNA and proteins; this binding is highly associated with the carcinogenic effect of aflatoxin (20, 24). Foods are contaminated with aflatoxins B_1 and B_2, and they are then consumed by humans or animals. These compounds are hydroxylated in one's liver and converted to aflatoxins M_1 and M_2.

Aflatoxin M_1 is less toxic than aflatoxin B_1. Studies have indicated that the toxicity of aflatoxin M_1 is about one tenth of the toxicity caused by aflatoxin B_1. Aflatoxin is toxic, carcinogenic, mutagenic, and it weakens the immune system as well. Aflatoxin is soluble in methanol, chloroform, acetone, and acetonitrile (8, 9). Aflatoxin B_1 epoxide is an active agent in the carcinogenicity of aflatoxin B_1. Moreover, in general, hot climatic conditions, such as high temperatures and humidity, lead to fungal growth and aflatoxin production. Absorption of aflatoxins has been associated with liver cancer, and it has also been indicated that it is likely to increase the risk of mutations (10, 25).

Limits and laws of aflatoxins in Iran and the European Union

Given the significant health risks associated with the presence of aflatoxins in food, maximum restrictions have been made in the European Union and other countries of the world. As a result, a large number of formal controls have been established by ACCP during the production and distribution (import and export), so that the legal limits of aflatoxin are guaranteed. Mycotoxins have been recognized a dangerous group with the highest reports in Europe in 2010 and

recent years. Implementing a series of interventions to reduce aflatoxin contamination is essential ensuring the consumer's health and maintaining producer profits (26, 27).

Given their increasing awareness of the dangers and effects of carcinogenesis, mutagenicity, and teratogenicity of mycotoxins in humans and animals, a large number of countries have adopted the highest permitted levels of mycotoxins in foods. Nuts and the products made from them are a main part of the consumables in one's daily diet. Thus, it is important to control their presence and percentage in food. As much as 2.5% of the world's agricultural products are contaminated with mycotoxins; this leads to huge economic losses. The aflatoxin limit has been set in 76 countries around the world, and currently the maximum permissible levels for *Aspergillus flavus* B_1 and total *Aspergillus flavus* have been set to be 1-20 ng g^{-1} and 0-35 ng g^{-1}, respectively, (8, 28). As early as 1998, EU member states have set *Aspergillus flavus* B_1 and total *Aspergillus flavus* limits at 2 and 4 ng g^{-1}, respectively. In 1988, the European Union declared the highest level of aflatoxin B_1 for a range of agricultural products for human consumption at 2 ng g^{-1}. The European Union has recently raised the limit.

As for almond and pistachio kernels for direct human consumption or use in food products, the limit has been increased aflatoxin B to 8, and for total aflatoxin has increased from 4 to 10 ng g^{-1}. It is not always possible to prevent aflatoxin contamination before or after the harvesting process or and during the storage phase. Due to the serious dangers of mycotoxins and given the contamination of agricultural products with aflatoxins, special rules and regulations have been set for the production, consumption, and import of foods. In the United States, for example, foods and drugs containing more than 20 ppb of total aflatoxin and 15 ppb of aflatoxin B_1 cannot be traded for either export or import. Aflatoxins have many laws in many countries. In the European Union, 4 ppb is the highest allowed level of total aflatoxin, while in the United States and the FDA, the allowed level of aflatoxin for nuts has been set to be 20 ppb. In Iran, the highest allowed levels of *Aspergillus flavus* B_1 and total *Aspergillus flavus* in pistachios have been set to be 5 and 15 ng g^{-1}, respectively (14, 26).

Determination of aflatoxin content

Determining the aflatoxin content of pistachios and other nutrients has been conducted by common analytical techniques such as thin-layer chromatography, high-performance liquid chromatography, spectrophotometry and spectrofluorimetry (29, 30). These methods require expensive tools and equipment, high costs, preparation of samples, separation time of attached samples in compounds. Immunochemical methods using antigen-antibody reactions are selective, sensitive, and rapid techniques that are relatively inexpensive, and they conducted in a short time. These

methods are also used to monitor the levels of aflatoxins; Moreover, ELISA test kits are economically viable and easy to apply, and their results can be comparable to the results made from thin-layer chromatography and high-performance liquid chromatography (8, 31).

Decontamination and fungal contamination control methods

Prevention, especially by reducing fungal growth and the production of toxins in sensitive foods, has been recognized as the most effective way to limit fungal contamination. In practice, this can reduce fungal contamination in growing crops through proper drying and storage of harvested crops. It is not always possible to prevent aflatoxin contamination before or after the harvesting process and even during the storage phase.

Today, the isolation of aflatoxin-contaminated is conducted according to their sensory properties, such as color. However, this method is not a completely specific one, and there is always the possibility of errors during the process. Moreover, incorrect harvesting time, transportation of peeled pistachios, and delay in the process result in some cases of contamination in pistachios that do not change the characteristics of pistachios, and therefore they are not separable (11, 32). Aflatoxin is also produced during the post-harvest period or after processing, so the presence of *Aspergillus* species in pistachios is required to be closely monitored for aflatoxin production. The incidence of aflatoxin contamination in nuts is low. Aflatoxin levels can be quite different; that is to say, a small percentage may have high levels of aflatoxin. However, it has been estimated that in every 28250 walnuts, 25600 almonds, and 25000 pistachios, one has a high level of contamination and can spread the contamination. However, a pistachio with the aflatoxin concentration of 60000 ppb can infect as much 4.5 kg of healthy pistachios (33, 34).

There are several methods for decontamination and fungal control after the harvesting harvest. They include extraction by using a solvent, mechanical separation, thermal inactivation, irradiation, and selective chemical adsorption. Although these chemical methods almost completely reduce the concentration of mycotoxins, they also reduce the nutritional value of nuts. Despite the significant efforts made to prevent contamination, there is still a growing trend of food-borne diseases (14, 35). Proper sanitation measures can be taken to reduce contamination, yet the most important pathogens cannot be removed from many farms at present; they cannot be removed by primary processes, especially in foods sold raw. There are several methods of decontamination. The most diverse treatment is the treatment with ionizing rays.

Food irradiation is a physical method that involves exposing packaged or bulk food to ionizing energy; this process is sometimes called cold pasteurization. Since inactivation of microorganisms is performed at the lowest temperature,

unlike thermal pasteurization, food safety can be improved by irradiation, and in most cases, the shelf life of the product is maintained without significant changes in its nutritional, chemical and physical properties. Food irradiation, up to a dose of 10 kg, has been conducted since 1981, and later, doses above 10 kg were considered safe for certain products. The joint experts from the Radiation Safety Committee, FAO, WHO, and the International Atomic Energy Agency (IAEA) have suggested that the use of radiation is suitable for food disinfection. Irradiated food at a dose lower than 10 kg has no toxic hazards; it has a minor effect on foods.

Decontamination with ionizing rays is a highly efficient, safe and environmental-friendly process. In comparison to microorganisms that have not seen rays, low- and medium-dose radiation-surviving microorganisms are more sensitive to environmental stresses or subsequent food processing. Treatment with radiation is an emerging technology in many countries around the world (35, 36).

3. Conclusion

Pistachio is Iran's second non-oil export product, and it plays an important role in the development of the national economy and food industry. In general, hot climatic conditions such as high temperatures and humidity lead to fungal growth and aflatoxin production. The presence of fungi in food products may not only affect the quality and quantity of the product but also lead to negative effects on consumer's health. Food contamination with mycotoxins has become a major problem worldwide. Aflatoxins are a group of furanocoumarins derived from polyketides. They are secondary metabolites produced by some species of Aspergillus, especially *Aspergillus flavus* and *Aspergillus parasiticus*. There are four main types of *Aspergillus flavus* including B_1, B_2, G_1, and G_2. Aflatoxin B_1 is on the list of possible carcinogens for humans. Generally, aflatoxins are produced in pistachios before the harvesting process and in the garden. Any delays in harvest, lack of proper processing and improper storage conditions can lead to increased fungal growth and aflatoxin production. Several methods have been proposed for decontamination and control of fungi after the harvesting process. Irradiation has been recognized as one of the most suitable methods.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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References

1- Saremi H, Okhovat M. Effect of Aflatoxin produced by *Aspergillus* flavus in reduction of our pistachio marketing all over the world. **2008**.

2- Dini A, Khazaeli P, Roohbakhsh A, Madadlou A, Pourenamdari M, Setoodeh L, Askarian A, Doraki N, Farrokhi H, Moradi H. Aflatoxin contamination level in Iran's pistachio nut during years 2009- 2011. Food Control. **2013**;30(2):*540-4*.

3- Ghorbanian A, Mahroudi A, Eslami H, Dolatabadi M. Evaluation of Activated Carbon Prepared from Pistachio Shell as an Adsorbent for the Removal of Tetracycline Antibiotics from Aqueous Solution: Kinetic and Isotherm Study. Pistachio and Health Journal. **2020**;3(1):*58-75*.

4- Dreher ML. Pistachio nuts: composition and potential health benefits. Nutrition Reviews. **2012**;70(4):*234-40*.

5- Bulló M ,Juanola-Falgarona M, Hernández-Alonso P, Salas-Salvadó J. Nutrition attributes and health effects of pistachio nuts. British Journal of Nutrition. **2015**;113(S2):*S79-S93*.

6-Gulati S, Misra A, Pandey RM, Bhatt SP, Saluja S. Effects of pistachio nuts on body composition, metabolic, inflammatory and oxidative stress parameters in Asian Indians with metabolic syndrome: a 24-wk, randomized control trial. Nutrition. **2014**;30(2):*192-7*.

7- Fantino M, Bichard C, Mistretta F, Bellisle F. Daily consumption of pistachios over 12 weeks improves dietary profile without increasing body weight in healthy women: A randomized controlled intervention. Appetite. **2020**;144:*104483*.

8- Abdolshahi A, Marvdashti LM, Salehi B, Sharifi-Rad M, Ghobakhloo S, Iriti M, Sharifi-Rad J. Antifungal activities of coating incorporated with Saccharomyces cerevisiae cell wall mannoprotein on *Aspergillus flavus* growth and aflatoxin production in pistachio (Pistacia vera L.). Journal of Food Safety. **2019**;39(2):*e12608*.

9- Siahmoshteh F, Siciliano I, Banani H, Hamidi-Esfahani Z, Razzaghi-Abyaneh M, Gullino ML, Spadaro D. Efficacy of Bacillus subtilis and Bacillus amyloliquefaciens in the control of *Aspergillus parasiticus* growth and aflatoxins production on pistachio. International Journal of Food Microbiology. **2017**;254:*47-53*.

10- Mohammadi Moghadam M, Rezaee S, Mohammadi AH, Zamanizadeh HR, Moradi M. A Survey on Contamination of Iranian Pistachio Cultivars to *Aspergillus* Section Flavi and Aflatoxin. Journal of Nuts. **2020**;11(1):*13-22*.

11- Ortega-Beltran A, Moral J, Picot A, Puckett RD, Cotty PJ, Michailides TJ. Atoxigenic *Aspergillus* flavus isolates endemic to almond, fig, and pistachio orchards in California with potential to reduce aflatoxin contamination in these crops. Plant Disease. **2019;**103:*905-12*.

12- Aldars-García L, Sanchis V, Ramos AJ, Marín S. Single vs multiple-spore inoculum effect on growth kinetic parameters and modeled probabilities of growth and aflatoxin B1 production of *Aspergillus flavus* on

pistachio extract agar. International Journal of Food Microbiology. **2017**;243:*28-35*.

13- Shaterian Mohammadi A, Aminian H, Jamshidnia A. Effects of smoke produced from smoldering plants on the *Aspergillus flavus* growth and production of aflatoxin in pistachio. Journal of Food Safety. **2020;**40:*12847-**52.***

14- Abdolshahi A, Shabani AA, Mortazavi SA, Marvdashti LM. Aflatoxin binding efficiency of Saccharomyces cerevisiae mannoprotein in contaminated pistachio nuts. Food Control. **2018**;87:*17-21*.

15- Kaminiaris MD, Camardo Leggieri M, Tsitsigiannis DI, Battilani P. AFLA-pistachio: Development of a mechanistic model to predict the aflatoxin contamination of pistachio nuts. Toxins. **2020**;12(7):*445*.

16- Mohammadi Moghadam M, Tavakkoli AR, Mohammadi AH, Moradi M, Hokmabadi H. Effect of Irrigation Systems on the Contamination of *Aspergillus flavus* and Aflatoxin Production in Shahpasand Pistachio Cultivar. Journal of Nuts. **2020**;11(3):*237-44*.

17- Moghadam MM, Rezaee S, Mohammadi AH, Zamanizadeh HR, Moradi M, Fani SR. The Potential of Aflatoxin Production in the *Aspergillus* Section Flavi Isolates of Pistachio in Iran. Journal of Fasting & Health. **2020;**4:*84-9*.

18- Hassanzadeh Davarani F, Ashrafizadeh M, Saberi Riseh R, Ghasemipour Afshar E, Mohammadi H, Razavi SH, Mandegary A, Mohammadinejad R. Antifungal nanoparticles reduce aflatoxin contamination in pistachio. Pistachio and Health Journal. **2018**;1(2):*26-33*.

19- 19. MT GL, Luo Y, Ortega-Beltran A, Jaime R, Michailides T. Quantification of the aflatoxin biocontrol strain Aspergillus flavus AF36 in soil, and nuts and leaves of pistachio by real-time PCR. Plant Disease. **2020**;4: *52-59*.

20- Fani SR, Moradi M, Probst C, Zamanizadeh HR, Mirabolfathy M, Haidukowski M, Logrieco AF. A critical evaluation of cultural methods for the identification of atoxigenic *Aspergillus flavus* isolates for aflatoxin mitigation in pistachio orchards of Iran. European Journal of Plant Pathology. **2014**;140(4):*631-42*.

21- Fallahzadeh RA, Eslami H, Eshaghpanah MS. A Review of Applied Disinfection Methods in Foods Industry and Pistachio Processing. Pistachio and Health Journal. **2020**;3(1):*32-44*.

22- Aldars-García L, Sanchis V, Ramos AJ, Marín S. Time-course of germination, initiation of mycelium proliferation and probability of visible growth and detectable AFB1 production of an isolate of *Aspergillus flavus* on pistachio extract agar. Food Microbiology. **2017**;64:*104-11*.

23- Holakouie Naieni K, Ghods B, Ghorbani R, Bagheri B, Abdolshahi A. Aflatoxin Contamination of Pistachio and Aflatoxicose: Knowledge, Attitude, and Practices of People in Damghan City, Iran. Journal of Nuts. **2020**;11(1):*91-9*.

24- Sayanjali S, Ghanbarzadeh B, Ghiassifar S. Evaluation of antimicrobial and physical properties of edible film based on carboxymethyl cellulose containing potassium sorbate on some mycotoxigenic

Aspergillus species in fresh pistachios. LWT-Food Science and Technology. **2011**;44(4):*1133-8*.

25- Rahimi P, Sharifnabi B, Bahar M. Detection of aflatoxin in Aspergillus species isolated from pistachio in Iran. Journal of phytopathology. **2008**;156(1):*15-20*.

26- Marin S, Ramos AJ, Sanchis V. Modelling *Aspergillus flavus* growth and aflatoxins production in pistachio nuts. Food microbiology. **2012**;32(2):*378-88*.

27- Bayman P, Baker JL, Mahoney NE. *Aspergillus* on tree nuts: Incidence and associations. Mycopathologia. **2002**;155(3): *161-9*.

28- Khorasani S, Azizi MH, Barzegar M, Hamidi-Esfahani Z, Kalbasi-Ashtari A. Inhibitory effects of cinnamon, clove and celak extracts on growth of *Aspergillus flavus* and its aflatoxins after spraying on pistachio nuts before cold storage. Journal of Food Safety. **2017**;37(4):*e12383*.

29- Wu Q, Xu H. Design and development of an on-line fluorescence spectroscopy system for detection of aflatoxin in pistachio nuts. Postharvest Biology and Technology. **2020**;159:*111016*.

30- Ortega-Beltran A, Bandyopadhyay R. Comments on" Trial summary on the comparison of various non-aflatoxigenic strains of Aspergillus flavus on Mycotoxin levels and yield in maize" by MS Molo, et al. Agron. J. **2019**; 111: *942-46*.

31- Moradi M, Fani SR. Review of aflatoxin in pistachio and its control strategies. Plant Pathology. **2018**;7(2):*22-33*.

32- Catalan L, Alvarez-Orti M, Pardo-Gimenez A, Gomez R, Rabadan A, Pardo JE. Pistachio oil: A review on its chemical composition, extraction systems, and uses. European Journal of Lipid Science and Technology. **2017**;119(5):*1600126*.

33- Marin S, Hodzic I, Ramos AJ, Sanchis V. Predicting the growth/no-growth boundary and ochratoxin A production by Aspergillus carbonarius in pistachio nuts. Food microbiology. **2008**;25(5):*683-9*.

34- Spanjer MC, Rensen PM, Scholten JM. LC–MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs. Food Additives and Contaminants. **2008**;25(4):*472-89*.

35- Khezri M, Heerema R, Brar G, Ferguson L. Alternate bearing in pistachio (Pistacia vera L.): A review. Trees. **2020**:*1-14*.

36- Tai B, Chang J, Liu Y, Xing F. Recent progress of the effect of environmental factors on *Aspergillus flavus* growth and aflatoxins production on foods. Food Quality and Safety. **2020**;4(1):*21-8*.