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Aflatoxins in Pistachio, Detection and Prevention

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ABSTRACT: Almonds, hazelnuts and pistachios belong to the most popular tree nuts. Pistachio seeds are very appreciable owing to their high nutritional value. Pistachios are the lowest in calories, and fat, they are among the highest in fiber content for a nut (3 grams/ serving). Thirty pistachios make a satisfying healthy snack for just approximately 100 calories per serving a wise choice when watching your weight. Nevertheless, they are most of the time are not fresh, which this leads to the growth of toxic compounds such as mycotoxins. Aflatoxins (AFs) are the most toxic mycotoxins and Pistachios can easily be contaminated with aflatoxin. Contamination of pistachios by fungi may occur at three different stages; when the nuts are on the tree, when nuts are dehulled, and washed and sorted, and in storage, especially when nuts are stored under adverse conditions of temperature and relative humidity. For detection AF, there are several techniques, TLC, HPLC and ELISA are quite accurate methods for this purpose, but But these methods are expensive and time consuming, while optical methods are faster. Laser induced fluorescence spectroscopy (LIFS) which is used in HPLC systems is improved to enhance the sensitivity of aflatoxin measurements. LIFHPCE, MECC and mass spectroscopy are other used techniques to separate AFB1**.**

***Keywords*:** Aflatoxin, Detection, Mycotoxin, Pistachio, Prevention.

INTRODUCTION

Pistachio (Pistacia vera L.) is one of the most famous and popular nut trees in the world. It is mainly cultivated in Iran, USA, Turkey, Syria, Italy, Tunisia and Greece (Kashaninejad et al., 2006). Global data shows that Iran and USA are the main source of pistachio production in the world, while it is recorded for Iran with the production of about 230,000 MTs and export of about 180,000 MTs in 2014, for the USA with the production of around 230,000 MTs and export of about 120,000 MTs in the same year; Therefore, pistachio nut has a great economic value for Iran. Three species of Pistachio, namely Pistacia mutica, P. khinjuk, and P. vera, have been reported in Iran, but Pistachio vera is cultivated in this country. More than 60 pistachio varieties (from Pistachio vera species) are cultivated in different regions of Iran, but the O'hadi (or Fandoghi), Momtaz, Badami (or Sefid), Akbari, Ahmad-Aghayi and Kalle-Ghuchi are the major commercial varieties due to the good quality and adaptation to Iran's climate (panahi et al., 1381, Gheibi and javadi khosraghi, 1384) .

Pistachios are hard-shelled fruits and their seeds are an important source of nutrients for humans and animals. They contain unsaturated fatty acids and sterols (Arena et al., 2007; Venkatachalam and Sathe, 2006). They also contain about 23% proteins and high amounts of carbohydrates and minerals (Kuçukoner and Yurt, 2003). The pistachio is a well-used nut as an ingredient in icecreams, cakes and in the confectionery industries, owing to its flavour and deep green colour. Among nuts, pistachios have a high nutrient density; and provide an excellent source of copper, manganese and vitamin B6; pistachios offer a high amount of total polyphenols antioxidants; and are the only nut to contain significant amounts of lutein and zeaxanthin .

Unfortunately this delicious popular nut is most of the time consumed after being stored and not fresh, this leads to the growth of post-harvest moulds such as the genus Aspergillus and consequently mycotoxin production, mainly aflatoxins (AFs). AFs are fungal metabolites produced by strains of Aspergillus, namely A. flavus, A. parasiticus and, rarely, A. nomius (Payne, 1998; Pitt et al., 1993). A. flavus, the most common AF-producing species, is very common

on pistachios (Molyneux et al. 2007). They may be regarded as a quadruple threat: as a potent toxin, a carcinogen, a mutagen and a teratogen (Ueno and Ueno, 1978; Sweeney and Dobson, 1998).

The economic loss resulting from fungal and mycotoxin contamination of nuts is difficult to estimate. However, judging from the widespread occurrence of fungal and mycotoxin contamination and the large number of nuts affected, one can assume that such losses must be large. These losses constitute direct nut losses, human illness and reduced productivity, and livestock losses from deaths and lower growth rates. Additional economic losses include indirect costs of various systems for control of mycotoxins in nuts reduced value of rejected nuts costs of detoxification to recover acceptable products, and, occasionally, from loss of export markets.

In this review, we briefly consider the main groups of mycotoxins in pistachio and conditions of their production, afterward we describe the instrumental detection techniques and ways to prevention of aflatoxigenic.

Main Groups of Mycotoxins in Pistachio

Mycotoxins are secondary fungal metabolites which are found in a wide range of food and crops, such as cereals, spices, coffee, nuts or dried fruits (Zöllner and Mayer-Helm, 2006).

Aflatoxins

Aflatoxins are a family of closely related secondary metabolites produced by some strains of moulds (i.e., Aspergillus flavus and Aspergillus parasiticus). These are among the most toxic mycotoxins (Molina and Giannuzzi, 2002; Passone et al., 2010; Sardin et al., 2011) and are such highly toxic and carcinogenic compounds that even low levels of contamination are important. Among 20 types of aflatoxins, only aflatoxins B1, B2, G1 and G2 play a vital role in foods and feeds (Aycicek et al., 2005; Juan et al., 2008). AFB1 known as a powerful hepatocarcinogenic and genotoxigenic substance and has been classified as group 1 carcinogen (Lyon and IARC, 1993). Different food commodities including cereals, nuts, dried fruit, cocoa, spices, oil seeds, pulses and beans are contaminated with AF (Tabibi and Salehian., 1974). The Pistachio nut is a commodity with the highest risk for aflatoxin contamination (Dini et al., 2013). Since aflatoxin contamination is unavoidable and unpredictable (Lopez-Garcia et al., 1999), permitted aflatoxin levels are regulated in many countries worldwide.

Institute of Standards and Industrial Research of Iran (ISIRI) has set a MTL of 5 and 15 ng/g for AfB1 and total aflatoxins, respectively (ISIRI. 2010). European Commission regulating set limits for AFB1 and total AFs 2 and 4 to 10 ng/g respectively in pistachio (Moss MO., 2002 and T H E European Commission., 2010)

Due to the significant health risks associated with the presence of aflatoxins in foods, it is important to establish a data collection on the occurrence of these toxins in nuts as valuable foods.

Other Mycotoxins. Information about mycotoxin contamination of nuts other than aflatoxins (recently reviewed in (Rodrigues et al., 2012)) and OTA is limited.

Conditions of Mycotoxins Contamination in Pistachios

Contamination of pistachios by fungi may occur at three different stages. First stage may occur while the nuts are on the tree. In that case, ripe pistachios are more susceptible to contamination by wind borne and insect borne spores of fungal species (Cheraghali and Yazdanpanah, 2010). After harvesting, when nuts are dehulled, washed and sorted, there is also the condition for contamination. The washing water can be a source of contamination, and if the nuts are allowed to remain wet, they will be contaminated. The third stage of contamination can be in storage, especially when nuts are stored under adverse conditions of temperature and relative humidity (Khosravi et al, 2007). Briefly, concentration of aflatoxins is generally low, while the nuts are on the tree, although existence of high humidity and high temperature within bulk bins provide ideal conditions for contamination of cracked pistachios fruits (Doster and Michailides, 1994). In general, nuts with thick shells (e.g. macadamia nuts) are better protected against the intrusion of moulds. Other nuts, like pistachios, are more prone to mould infestation due to shell splitting at the end of maturation (Pitt and Hocking, 1997). Sorting and elimination of split nuts can decrease the contamination of mycotoxins in the lot significantly (Shahidi Bonjar, 2004).

Whereas high humidity and high temperature are more suitable for toxin production (Studies performed on hazelnuts and pistachios suggested that optimum temperature and RH for AF production is 25–30□ C and 97–99%, respectively (Diener and Davis, 1967; Simsek et al., 2002)), thus Mediterranean countries' climate is favourable for mould infestation and so mycotoxin synthesis (Hadidane et al., 1985; Bacha et al., 1988) .

Mycotoxins contamination of foodstuffs and feedstuffs has been studied in Iran (Shephard et al., 2002; Cheraghali et al., 2005; Kamkar, 2005; Yazdan- panah et al., 2006).

Detection of Aflatoxins Contamination

Thin-layer chromatography (TLC) is the most important method for aflatoxin detection (Stroka et al., 2002; Cheraghali et al., 2007; Ghali et al., 2009). High Performance Liquid Chromatography (HPLC) is another most important method for aflatoxin detection that reported by authors (Pearson, 1999). Also, some researchers have used immunochemical based assays methods such as Enzyme- Linked Immune Sorbent Assay (ELISA) for AF detection in pistachios (Albani, 2007; Siahi Shadbad et al., 2012; Karimi et al., 2015).

Although all methods based on TLC, HPLC and ELISA are quite accurate, but they are expensive, time consuming and unsuitable for in-line application. However, optical methods seem to have the potential for rapid detection of AFs contamination in pistachio contamination. AFB1 and AFB2 emit fluorescence in bright-blue region of the spectrum (425–480 nm), and AFG1 and AFG2 emit fluorescence in blue–green range (480–500 nm) (Abramczyk, 2005). The bright greenish yellow fluorescence (BGYF) under UV-excitation has been used to detect AFs in pistachio nuts in 1980 (Pasikatan AND Dowell, 2001), the obtained results show a strong relationship between BGYF and AFs concentration (Demtröder, 2003). UV lamps are used for excitation of sample and usually charged coupled device (CCD) camera is used in vision system (Abramczyk, 2005). There are many reports on attempts made to device rapid, quantitative and inexpensive fluoremetric method for the purpose of measuring AFs based on the extraction of aflatoxin (Farsaie et al., 1978; Pearson et al., 1999; Roze et al., 2007; Lunadei et al., 2013). In agar medium in the presence of Aspergillus flavus and Aspergillus parasiticus, aflatoxin concentration variations are measured (Lunadei et al., 2013). Since laser invention, many researchers have used different laser systems for spectroscopy applications (Alcaide-Molina et al., 2009; Pedarnig, 2014). Fluorescence spectroscopy was improved by the employment of ultraviolet lasers; this method is named laser induced fluorescence spectroscopy (LIFS) which is used in HPLC systems to enhance the sensitivity of aflatoxin measurements. This method is capable of detecting a few hundred of femtogram of each of four commonly seen aflatoxins (Liang, et al., 2009). For sensing AFB1, a system of laser induced fluorescence high performance capillary electrophoresis (LIFHPCE) was developed. Micellar electro kinetic capillary chromatography (MECC) is employed to separate AFB1, then the separated AFB1 is excited by a UV laser (375 nm) and lastly fluorescence photons (440 nm) are detected by a photo multiplier tube (PMT) (Simeon, et al., 2001). From other used method to determination of aflatoxins in nuts, we can reffer to mass spectroscopy. The importance of mass spectrometry for the confirmation of mycotoxin identity is emphasised, e.g. in (Zöllner and Mayer-Helm., 2006).

Moreover during the last years, single analyte methods for the detection and quantification of mycotoxins are more and more replaced by multi-target methods for the simultaneous determination of different yet co-occurring classes of mycotoxins. The majority of these methods are based on the combination of high- or ultra-high-performance liquid chromatography with tandem (e.g. (Sulyok et al., 2006; Spanjer et al., 2008; Ren et al., 2007; Sulyok et al., 007)) or high-resolution (Zachariasova, 2010) mass spectrometry.

Control or Reduce Aflatoxin Risk in Pistachios

Identification of Aflatoxin Risks and Suitable Control Measures In various stages of production to consumption, Can be summarized as follows:

Step 1: On Farm

Risk: Most aflatoxin contamination occurs in the orchard and is associated with damage caused to the fruit's hull, mainly early splitting, prior to harvesting. The exposed nut becomes susceptible to infestation by A. flavus spores, leading to aflatoxin accumulation. Subsequent invasion of early-splitters by insects, particularly the navel orangeworm, compounds the problem.

Controls: Cultivation of pistachio varieties, which are not susceptible to early splitting. Carry out early harvesting to reduce the levels of early-split fruit. Pre-harvest aflatoxin contamination can be significantly reduced by applying Integrated Phytosanitary Management (IPSM), which aims to minimize the fungal spore counts and navel orangeworm levels in the orchard. Removal or burial of tree litter is highly recommended. Then planned early harvesting of pistachios is the most important practice to reduce the levels of early-split nuts exposed to A. flavus contamination. Nut contact with the soil during harvesting should be avoided. Harvested nuts should be transported to the processing plant as soon after harvest as possible.

Step 2: De-huller

Risk: The process of de-hulling can predispose healthy nuts to subsequent fungal contamination due to the release of large volumes of A. flavus spores from the plant and fruit debris.

Control: Direct airflow away from the fresh pistachios and through a chlorinated water tank to eliminate the fungal spores.

Step 3: Floatation Tank

Risk: Incorrect management of water flotation can lead to further A. flavus contamination. The addition and continuous circulation of contaminated water within the flotation tank system can further contaminate the nuts. Leaving the sorted nuts in the flotation tank for too long can also lead to excessive fungal contamination.

Controls: Use chlorinated water in the flotation system and replace dirty water on a regular basis.

Step 4: Washing under Sprayers

Risk: The circulation of contaminated, dirty water from the flotation tank to this washing step will increase aflatoxin levels by infecting healthy nuts.

Controls: Same as Step 3.

Step 5: Drying (Mechanical/Solar)

Risk: Delayed drying of the wet nuts can lead to the development of A. flavus on the nuts and subsequent aflatoxin contamination.

Controls: Rapid drying of the pistachios to remove excess water is essential to prevent growth of A. flavus on the wet nuts.

Step 6: Storage

Risk: Improper storage conditions can lead to aflatoxin contamination.

Controls: Storage of pistachios in clean jute bags at a suitable temperature and relative humidity will prevent any aflatoxin accumulation.

Step 7: Screening by Size (Gravity Separator)

Levels of aflatoxin may be reduced at this step due to the mechanical removal of small, shriveled nuts, which are more likely to be contaminated with aflatoxin.

Step 8: Sorting (by Hand or Electronic Eye)

Levels of aflatoxin will be significantly reduced at this step due to the removal of shell-stained, discolored and defective pistachios, which contain high levels of aflatoxin.

Step 9: Packaging

No risk of aflatoxin contamination, but inappropriate packing may make the nuts susceptible to future contamination if re-wetting occurs.

Step 10: Transportation and Export

No aflatoxin contamination is likely at this stage, provided that transportation and export conditions protect the nuts from excessive heat and fluctuations in moisture. It is also important to select lots that meet the customer's aflatoxin specification.

CONCLUSION

Pistachio is a famous and popular nut tree in the world, so it is important to be fresh and healthy. Therefore detection of aflatoxins contamination of pistachios (aflatoxins are among the most toxic mycotoxins) is a concern. For this purpose there are several methods, for example TLC, HPLC and ELISA, optical methods, Laser induced fluorescence spectroscopy (LIFS) which is used in HPLC systems, and LIFHPCE, MECC and mass spectroscopy are other used techniques to separate AFB1. Moreover during the last years, single analyte methods for the detection and quantification of mycotoxins are more and more replaced by multi-target methods for the simultaneous determination of different yet co-occurring classes of mycotoxins. The majority of these methods are based on the combination of high- or ultra-high-performance liquid chromatography with tandem .Moreover, there are ways to reduce the risk of aflatoxin contamination from farm to store such as: Cultivation of pistachio varieties, which are not susceptible to early splitting, planned early harvesting of pistachios, use chlorinated water in the de-hulling, flotating and washing system, rapid drying of the wet pistachios and finally providing appropriate conditions during storage and packaging and transporting stage.

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