

Recognition and Determination of Contaminated Pistachios to *Aflatoxin* in Processing Stage

^{1*} Ali tajabadipour, ² Hossein Afshari and ¹ Hossein Hokmabadi

¹ Iran pistachio Research Institute, Rafsanjan, Iran

² Islamic Azan University, Damghan Branch, Damghan, Iran

Abstract: Pistachio is one of the most important horticultural products of Iran with special economic importance. In average 70% of Iran pistachio are exported. Aflatoxin is secondary metabolites of fungus such as *Aspergillus flavus* and *A. parasiticus*. If favorite condition is prepared for stabilization of fungi spores producing Aflatoxin, the first pistachio contamination to Aflatoxin occurs in orchard. The most important way for penetration of mentioned fungus is cracking in pistachio hull in orchard. The most dangerous kind of cracking is early splitting. The shells of most pistachio nuts split naturally in the orchard prior to harvest. Sometimes the hull is attached to the shell so that it splits with the shell, exposing the kernel to moulds and insects. This is called an "early split". Although the importance of early splitting for mould, aflatoxin and insect contamination is well established, very little is known concerning the physical property of early split pistachio. In order to determination appearance and physical properties of contaminated pistachios this researched was carried out on three commercial Iranian pistachio cultivars of Owhadi, Kalleghochi and Ahmadaghahi in processing stage in two mechanized pistachio processing and two sampling times. Sampling were done from non-stain and stained split pistachio on the surface of water, stained and non-stained of pistachio under the water (sank pistachio), small pistachio, deformed pistachios, shelled pistachio and intact pistachio from the end of processing. Stained pistachios were divided in 3 groups including group 1 (amount of stain 1-30%), group 2 (amount of stain 31-60%) and group 3 (amount of stain more 60%). The amount of aflatoxin was measured by scanner-TLC in each sample. The results indicated that stained pistachio on the surface of water had the highest amount of aflatoxin. With increasing of stain level on shell, the amount of aflatoxin increased remarkably. The amount of aflatoxin in stained sinked pistachios of groups 1, 2, 3, was 490, 1364.7 and 1782 ppb respectively. Small, deformed and shelled pistachios had negligible amount of aflatoxin and final pistachios (intact pistachio from the end processing) showed any contamination to aflatoxin.

Key words: Aflatoxin- processing stage, Pistachio, shell staining, floated and sinked pistachio

INTRODUCTION

Pistachio is one of the most important Horticultural products of Iran with special economic importance. In average 70% of Iran pistachio are exported. Iranian pistachio is well-known in the world horticultural market, and for this reason producing high quality pistachio nuts with low production cost is an important task for the Iranian pistachio industry. However Pistachio nut contamination to *Aspergillus* species and their toxins are the most serious problems in pistachio production, consumption and export processing (Emami et al, 1977; Mirabolfathy, 1981; Tajabadipour, 1997; 1999; Mojthahedi et al, 1978; 1979; Moradi and Javanshah 2005; Thomson and Mehdy, 1978). *Aspergillus flavus* is the primary cause of aflatoxin contamination of pistachio (Mojtehedy et al, 1979). Infection by *Aspergillus flavus* and aflatoxin production in

pistachio nuts have been documented before harvesting and in pistachio orchards (Doster and Michailides 1995; Emami et al, 1977; Thomson and Mehdy, 1978).

The shells of most pistachio nuts split naturally in the orchard prior to harvest. Fortunately, the hull covering the shell usually remains intact, protecting the kernel from invasion by moulds and insects. Nuts that are poorly protected by hulls are most prone to contamination in the orchard. Sometimes the hull is attached to the shell so that it splits with the shell, exposing the kernel to moulds and insects. This is called an "early split". In some countries the proportion of early splits can be as high as 30 percent. In a study done in the United States, approximately 1 to 5 percent of the nuts were early splits. When early splits were examined in one study about 20 percent of the samples (50 nuts each) were found to be contaminated with aflatoxin, while there

was no contamination in nuts with hulls that remained intact in the orchard (Doster and Michailides, 1995; Sommer et al., 1986). Although the importance of early splitting for mould, aflatoxin and insect contamination are well established, very little is known concerning the physical property of early split pistachio. By using of characteristic of existence stain on shell and pistachios size can separate %90 early split pistachios (Sommer et al., 1986). Mechanical separation method separate %90 early split pistachios successfully and only %5 of normal pistachios remain in this sample. Early split pistachios have differences in weight, length, width and diameter significantly (Sommer et al., 1986). Early split pistachios were informed earlier in orchard have shriveled hull, lower specific gravity, smaller kernel and shell and stain on shell in comparison with early split pistachios that were inform in the near of harvest time and as intact pistachios. These special characteristics were used for separation of contaminated pistachio in processing stages (Doster and Michailides, 1999). Although the importance of early splitting for mould, aflatoxin and insect contamination are well established, very little is known concerning the physical property of early split pistachio. this researched was carried out in order to determination appearance and physical properties of contaminated pistachios.

MATERIALS AND METHODS

In this study, in order to investigate role of physical and appearance properties of pistachios in processing stag, an orchard, 70 hectare Owahdi, Kalleghochi and Ahmadaghahi cultivars 30-year-old was selected in Rafsanjan area. Mentioned orchard had suitable management of horticulture, nutrition, irrigation and control of pests and weeds. Pistachio processing was done in a processing system of mechanization consisting stage of separation hull, washing, absorption moisture, windy hollowed, pistachio with hull separator, optic separation of stained pistachios and pistachios with hull and to dry sunny in a loge. Time to dry of pistachios (to reach to 4-6 percentage moisture) was about 2 days. Dried pistachios were divided into broken

and shelled pistachios and small and large pistachios by visual. Sampling were done in 8 replications from harvest time starting (September) in one day interval in 2 successive years. Types of sampling were including:

- 1- Small pistachios by visual separation
- 2- Deformed and shelled pistachios, by visual separation
- 3- Non-stain and stained split pistachio on the surface of water
- 4- Stained and non-stained of pistachio under the water (sank pistachio). Stained pistachios were divided into 3 groups including group 1 (amount of stain 1-30%), group 2 (amount of stain 31-60%) and group 3 (amount of stain more 60%).
- 5- Intact pistachio from the end of processing.

The aflatoxin content in the samples was extracted as detailed below. The pistachio kernels were dried on oven at 50°C; finally powdered samples (20gr) were vigorously shaken by hand with 100ml methanol: water (55/45 V/V) for 30 minutes.

In order to deletion of lipids and other pigments 40ml hexane was added and shaken by hand for 15 minutes. The contents were centrifuged at 2000 rpm. Then the methanolic layer was extracted with chloroform (50ml) thrice. The chloroformic phase was then filtered through layer of cheesecloth and anhydrous Na₂SO₄ to remove fungal tissue and to dehydrate the extracts respectively.

The extracts were evaporated in benmary. The residue were dissolved in chloroform and analyzed for the presence of aflatoxin on silicagel 60 thin layer chromatography (TLC) plates. The developing solvent was chloroform: methanol (97:3). The separated aflatoxin was quantified by fluorodensitometric measurement of extracts spots with R_f value and fluorescence similar to aflatoxin standard. The detection limits of the technique were 2-3 µg/kg of aflatoxins reference standard. Stock solution of aflatoxins were prepared in chloroform and stored in darkness at 4°C. Data were analyzed as RCBD (Randomized Complete Block Design) by the SAS software. The means were separated using Duncan's Multiple Range Test.

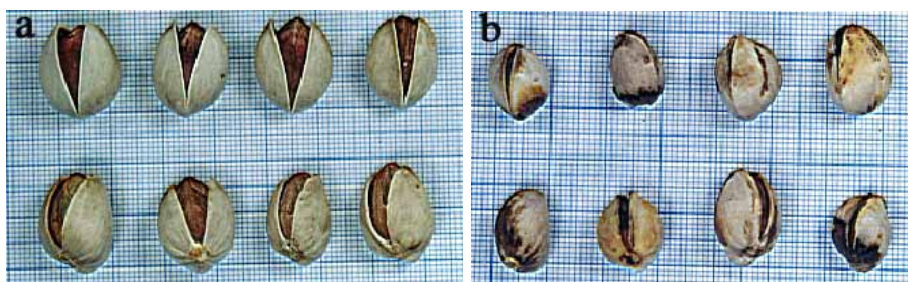




Fig 1- Stained pistachios were divided into 4 groups including: a) 0% Of shell surface discolored b) More than 60% Of shell surface discolored c) 31 to 60% Of shell surface discolored d) 1 to 30% Of shell surface discolored

RESULTS AND DISCUSSION

Results indicated that stained and floated pistachios had the highest amount of aflatoxin. (2015.4 ppb) (table1). Floated pistachios had physiological disorders that their hulls are cracked before harvest time (mainly early split pistachios). Because of cracking in hull, the moisture of hull and kernel were decreased and consequently specific gravity became lower than intact pistachios. Therefore, these pistachios float on water the same as blank pistachios. Doster and Michailides (1995) expressed, hull and kernel moisture of early split pistachios with shriveled and dried hull were 17 and 9 percentage respectively. Doster and Michailides (1995) asserted early split pistachio with shriveled and dried hull had contamination more twice to *A. niger* and more threefold to *A. parasiticus* and *A. flavus*. Early split pistachios with soft and smooth hull amount of aflatoxin in floated pistachio with unstained shell in without stain on water pistachios is than its mount in stained on water pistachios. However (In any case) this amount is very more from permissible limit of aflatoxin in pistachio. Therefore, it must were certainly separated from intact pistachios. By reason of lack special appearance characteristics (absence stain on shell), only method their separation, usage from physical characteristics (special gravity) be. In all of groups of stained sinked pistachios were observed aflatoxin. Therefore property of existence stain on shell is very important in separation of contaminated pistachios to aflatoxin. Sommer et al. (1986) stated by using of property of existence stain on shell and

pistachios size can separate 90 percentage early split pistachios in processing stage. The amounts of aflatoxin in stained sinked pistachio in groups 1, 2, 3 were 490, 1364 and 1781 ppb respectively (table 1). Of course these differences by reason of much different of aflatoxin amount in samplings weren't significant.

From these results were inferred that by increasing amount of stain on shell, the amounts of aflatoxin were augmented and there are considerable differences between stained sinked water pistachios group 1 with pistachios of groups 1, 3. Therefore if stain level on shell was more %30, amount of aflatoxin in this pistachios increase considerably. Kinds of mentioned stains in this study were brown black (Doster et al., 1999). After classifying of stained pistachio on basis amount and extension level of stain on shell and gain similar result to this study, They stated pistachios with black brown stain after oily stain pistachios had the highest amount of aflatoxin in comparison with pistachio with medium brown and light brown stain. The results showed pistachios had little amount of aflatoxin (1 ppb) and this amount was lower permissible limit of aflatoxin (2 ppb). However by reason of quality reduction of commercial pistachios and probability contamination to aflatoxin were eliminated.

Of course, be notice that tiny pistachios in this study were without stain. On has done studies, stained pistachios and amount of high aflatoxin were (fraction) small pistachios.

Table 1: Aflatoxin amount (total) in different pistachio types (ppb)

Type of pistachio	Total of Aflatoxin amount (ppb)
flouted and stained Split pistachios	2015a
Sinked and stained (group 3) pistachios	1781a
Sinked and stained (group 2) pistachios	1364a
Sinked and stained (group 1) pistachios	490a
Flouted Split pistachios without staining shell	233.9b
Small pistachios	1c
Broken and shelled pistachios	0.4c
Sinked pistachios without staining shell	0c
Final pistachio	0c

The means with different letters have significantly different (P=0.05 by Duncan's multiple range test).

Done studies about role of pistachios size in amount of aflatoxin showed contradictory results. So that schateki and Pan (1997) reported with decreasing of pistachio size and weight, amount of aflatoxin increased. This increase of aflatoxin amount about tiny pistachios was not true and very tiny pistachios had little amount of aflatoxin (0.4 ppb). This topic is showing in processing stage, there was sufficient time for development of fungi and aflatoxin producing. Unstained shell of sinked pistachios and final pistachios (without shell staining) hadn't any aflatoxin (table 1). Pistachios with intact hull were without shell staining and set in group of sinked pistachios. Doster and Michailides (1995) resulted that 4-5 percentage of pistachios with irregular cracked hull had stained shell and pistachios with intact hull were without shell staining. Therefore total of pistachios with intact hull and the most of irregular cracked pistachios with soft hull hadn't any detected aflatoxin.

REFERENCES

Doster, M. A. and T.J. Michailides., 1995. The development of early split pistachio nuts and the contamination by molds. Aflatoxins, and insects. First international symposium on pistachio nut. 20-24 September 1994. Adana-Turkey-Acta Horticulture 419, 359-364.

Doster, M.A. and J. Michailides., 1999. Relationship between shell discoloration of pistachio nuts and incidence of fungal decay and insect infestation. Plant Disease. 259-262.

Emami, A., Suzangar, M., and Barnett, R., 1977. Contamination of pistachio nuts with aflatoxin while on the trees and in storage. Zeszyty problemowe postepow Nauk Rolniczych 189: 135-140.

Mirabolfathy M., 1981. Study of pistachio molds. Annual Report of Laboratory of Plant Pests and Diseases Research Institute, Rafsanjan, Iran, 21.

Mojtahedi H., Danesh D., Haghghi B. and Barnet R., 1978. Postharvest pathology and mycotoxin contamination of Iranian pistachio nuts. Phytopathology, 68:1800-1804.

Mojthahedi H., C. J. Rabie, A. Lubben, M. Steyn and D. Danesh, 1979. Toxic *Aspergillus* from pistachio nuts. Mycopathologia, 67: 123-127.

Moradi M. and A. Javanshah, 2005. Distribution of aflatoxin in processed pistachio nut terminals. IV International Symposium on Pistachios and Almonds.

Schatzki, T.F. and J. pan., 1997. Distribution of aflatoxin in pistachios. Distribution in small pistachios. J.Agre. Food. Chem. 45. 205-207.

Sommer, N.F., T.R. Buchanan and R.j. Fortge., 1988. Relation of early splitting and tattering of pistachio nuts to aflatoxin in the orchard. Phytopathology 76(7): 692-694.

Tajabadipour, A., 1997. Morphological identification of some of pistachios cultivars. The thesis of master science, Agriculture College of Tehran University. 177 p

Tajabadipour, A., 1999. Early splitting disorder in pistachios. Iran's Pistachio Research Institute Press.16 p

Thomson, S.V. and M.C. Mehdy., 1978. Occurrence of *Aspergillus flavus* in pistachio nuts prior to harvest. Phytopathology 68: 1112-1114.