The Effects of Segregating Stained Nuts on Reducing the Aflatoxin Content of Ahmad-Aghaei, Fandoghi and Kale-Ghoochi Pistachio Cultivars

Ahmad Shakerardekani (PhD)[1,2*], Fatemeh Mirdamadiha (MSc)[1]

[1] *Pistachio Research Center, Horticultural Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Rafsanjan, Iran*

[2] *Pistachio Safety Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

Information

Article Type:

Original Article

Article History:

Received: 17 Aug. 2018
Accepted: 1 Dec. 2018
DOI:10.22123/phj.2019.144715.1012

Keywords:
Aflatoxin
Contamination
Pistachios
Variety

Corresponding Author:

Ahmad Shakerardekani

Email: shaker@pri.ir
Tel: +98- 3434225203

Abstract

Introduction: The physical specifications of pistachios after processing are associated with the presence of aflatoxin. The classification of pistachio nuts based on the appearance of raw dried pistachios could have a very important effect on the amount of aflatoxin.

Materials and Methods: Samples of three commercial pistachio cultivars (Ahmad-Aghaei, Fandoghi, and Kale-Ghoochi) were categorized to different groups, which were large, medium, tiny, damaged, non-split yellow-brown stain and dark-greyish stain. The thin layer chromatography method was applied for the measurement of aflatoxins at the wavelength of 366 nm.

Results: The yellow-brown stained nuts in Ahmad-Aghaei, Fandoghi and Kale-Ghoochi cultivars were 2.3, 4.6 and 1.1%, and the grey-black stained nuts were 4.6, 4.7 and 1.2%, respectively. By completely separating stained nuts, the amount of aflatoxin in Ahmad-Aghaei, Fandoghi and Kale-Ghoochi lots decreased more than 89.0%. There were significant differences ($p<0.05$) in the aflatoxin amounts of stained pistachio cultivars.

Conclusion: This study pointed out that physical separation of the stained nuts from the pistachio lots could reduce the amount of aflatoxin in the final lots.

►Please cite this article as follows:

Shakerardekani A, Mirdamadiha F. The effects of segregating stained nuts on reducing the aflatoxin content of ahmad-aghaei, fandoghi and kale-ghoochi pistachio cultivars. Pistachio and Health Journal. 2018; 1 (4): 32-37

1. Introduction

Nuts are subject to contamination by different mycotoxins [1] .Aflatoxins are metabolites of different strains of *Aspergillus* species such as *A. flavus* and *A. parasiticus*. The amount of contaminated pistachio could be decreased with the application of suitable sorting methods after harvesting [2-6]. There are several reports about the relationship between the frequency of early split, cracked, damaged and stained pistachio nuts and the presence of aflatoxin. [7- 11]. A higher degree of staining occurs with a delay in harvesting in the damaged hull pistachio. Some researchers reported that the physical specifications of pistachios after processing are associated with the aflatoxin content [12- 15]. Separation of damaged and stained nuts from the pistachio lots are usually done manually or using a color sorter. This process is important as it reduces aflatoxin contamination in the product.

Shakerardekani *et al.* (2012) reported that the aflatoxin level of the yellowish-brown nuts of the Fandoghi, Ahmad Aghaei, and Kale-Ghoochi cultivars were 136.2, 632.4 and 120.3 ppb, respectively, while dark-greyish pistachios contained 28.7, 292.8, 7.3 ppb, respectively. Therefore, the aim of this study was to prove the importance of segregating stained nuts in reducing the aflatoxin content of three commercial pistachio cultivars.

2. Materials and Methods

2.1. Sampling

Fresh pistachio nuts harvested from the orchard of the Pistachio Research Center (Station No.2) in 2016. Dehulling (hull separation) was carried out in the pilot processing unit. Nuts were then sun-dried until 5% moisture content. The pistachio nut samples of Ahmad-Aghaei, Fandoghi and Kale-Ghoochi cultivars were collected based on the amount that entered the hand sorting section (including 10 batches of each variety with each batches weighing 50- 100 kg). Three kg of Fandoghi, Ahmad-Aghaei, and of Kale-Ghoochi samples were selected from all the batches for laboratory analysis. Before analysis, all stained nuts were separated from the unstained nuts. The stained pistachios were grouped based on the color of the stain into either yellow-brown or dark-grey nuts. The unstained pistachio nuts were divided into different groups including tiny, medium, large, damaged and non-split.

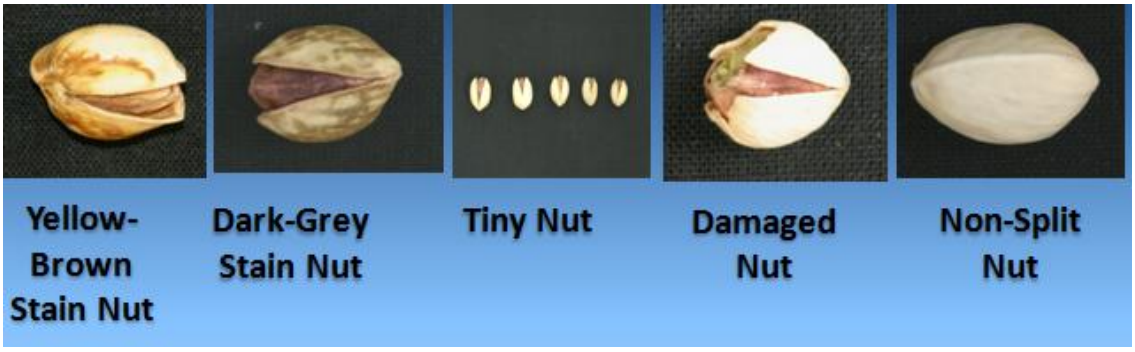

Fig 1. The yellow-brown stain nut, dark-grey stain nut, tiny nut, damaged nuts and non-split nuts used in this research

2.2. The Average Weight of Each Pistachio Nut

The weight of pistachio nuts of each group was divided to their number and the average weight of each pistachio nut in every group was computed.

2.3. Aflatoxin Measurement

The Association of Analytical Communities (AOAC) method of aflatoxin analysis was used for pistachio aflatoxin extraction and the clean-up procedures [16]. The thin layer chromatography (TLC Scanner 3; Camag Scientific Inc., Wilmington, NC) was applied for the measurement of the aflatoxins from all the three cultivars at the wavelength of 366 nm. The detection limit (LOD) was 0.4 ng/g. For aflatoxin extraction, 125 ml methanol, water (55:45, v/v) and 3 g Nacl were poured into each flask, incubated on a shaker (30 min) and filtered through Whatman No. 4 filter paper. Fifty ml filtrates were poured in a funnel, an equal amount of chloroform was poured and the mixture was rotated for 1 min. The extraction was repeated with 50 ml portions of chloroform. Chloroform extracts were mixed and evaporated in a rotary evaporator to a final volume of 2 ml. The extracts were transferred to a borosilicate vial and dried under a gentle stream of nitrogen and dissolved in 200 µl benzene: acetonitrile (98:2, v/v), spotted on TLC plate adjacent to known aflatoxin standards (Sigma-Aldrich, Italy). The plates were developed in chloroform: acetone (9:1, v/v). Aflatoxins were measured directly on TLC plates with a scanning densitometer [17, 18]. The aflatoxin (B1 and total) amount of each cultivar was measured after the separation of either yellow-brown (first stage) or dark-greyish nuts (second stage). The aflatoxin reduction was calculated in each case.

2.4. Statistical Analysis

All measurements were done in triplicate. The Tukey's multiple comparison test was used to compare the means in different groups of Ahmad-Aghaei, Fandoghi and Kale-Ghoochi cultivars. The Minitab software version 16.2.2.0 (Minitab Inc. USA) was used to analyze the data.

3. Results

3.1. The Average Weight of Each Pistachio Nut

Regarding weight, tiny pistachios are similar to damaged ones. Medium pistachios are similar to non-split pistachios, and large pistachios are very similar to stained pistachios (Table 1). In other words, they will be separated simultaneously by the segregation of physical approaches.

3.2. Comparison of the Total Aflatoxin (ppb) in Different Cultivars

In all cultivars, the greatest amount of aflatoxin is associated with yellow-brown stain pistachios, and dark-grey stain pistachios have by far fewer aflatoxins (Table 2).

3.3. The Percentage of Aflatoxin Decrease after Sorting

The amount of aflatoxin B2 in all samples was less than aflatoxin B1 and the importance and toxicity of the latter aflatoxin is higher. Therefore, only results related to aflatoxin B1are shown in Table 3. As can be seen, at least 89.5% of aflatoxin of each parcel after complete segregation of stain pistachio nuts is reducible (Table 3).

Table 1. The average weight (g) of each pistachio nut of three different cultivars of Ahmad-Aghaei, Fandoghi and Kale-Ghoochi*

Cultivar	Yellow– brown	Dark- grey	Large	Medium	Tiny	damaged	Non-split
Ahmad-Aghaei	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.1	0.8 ± 0.0	0.6 ± 0.0	0.8 ± 0.0	1.0 ± 0.0
Fandoghi	1.0 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	0.9 ± 0.0	0.7 ± 0.0	0.7 ± 0.1	0.8 ± 0.0
Kale-Ghoochi	1.0 ± 0.0	1.0 ± 0.0	1.2 ± 0.1	1.1 ± 0.0	0.9 ± 0.1	0.9 ± 0.0	1.1 ± 0.1

*Standard deviation values are given after±

Table 2. Comparison of the total aflatoxin (ng/g) in different groups of Ahmad-Aghaei, Fandoghi and Kale-Ghoochi cultivars*

Cultivar	Yellow-brown	Dark-grey	Large	Medium	Tiny	Damaged	Non-split
Ahmad-Aghaei	666.6± 4.5^{a**}	297.7± 1.1^{b}	1.3± 0.3^{d}	2.6± 0.4^{d}	6.0± 0.4^{d}	9.4± 0.7^{d}	7.6 ± 0.5^{d}
Fandoghi	141.4± 3.1^{c}	8.8± 1.0^{d}	0.3± 0.1^{d}	1.1± 0.2^{d}	1.2± 0.3^{d}	5.9± 0.6^{d}	0.3± 0.1^{d}
Kale-Ghoochi	122.8± 2.1^{c}	7. 3± 0.7^{d}	0.1± 0.0^{d}	0.1± 0.0^{d}	0.1± 0.0^{d}	0.8± 0.1^{d}	0.1± 0.1^{d}

*Standard deviation values are given after±

**Means with different letters in each column are significantly different (P≤0.05)

Table 3. The effect of sorting on aflatoxin B_1 reduction of different pistachio cultivars of Ahmad-Aghaei, Fandoghi and Kale-Ghoochi cultivars*

Cultivar	Initial aflatoxin (ppb)	after complete segregation of yellow stain (ppb) (first stage)	Reduction (%)	after complete segregation of grey-black stain (ppb) (second stage)	Reduction (%)
Ahmad-Aghaei	26.6± 0.8	12.7± 0.5	59.4± 1.9	1.6± 0.3	96.4± 3.4
Fandoghi	6.9± 0.5	1.0± 0.3	82.5± 2.5	0.6± 0.2	89.5±3.1
Kale-Ghoochi	1.7± 0.3	0.2± 0.1	89.4± 2.1	0.1± 0.1	95.1± 2.9

*Standard deviation values are given after±

4. Discussion

Based on the average weight of each pistachio nut in the different categories, it is clear that the tiny pistachios and large pistachios are similar to damaged and stained pistachios, respectively. Therefore, they will be separated altogether by the physical method separation.

The comparison of the amount of measured aflatoxin in the samples taken from each lot indicates the non-homogenous distribution of aflatoxin in contaminated pistachio nuts [19, 20].In fact, the amount of aflatoxins will depend on the specific distribution and the numbers of contaminated nuts. The results show that the sensitivity of Ahmad-Aghaei cultivar to fungi growth is more than other cultivars. It is found that the segregation of stained nuts is very important for the final decrease.

According to the results, the amount of aflatoxin in stained pistachio nuts are significantly more than other groups, and the highest amount of aflatoxin was detected in the yellow-brown stained Ahmad-Aghaei cultivar. By completely separating stained nuts, the amount of aflatoxin in Ahmad-Aghaei, Fandoghi and Kale-Ghoochi lots were decreased 96.4, 89.5and 95.1%, respectively. Therefore, physical sorting in pistachio lots for the separation of stained pistachio nuts can help us provide a better product for industries and consumers. These results were in agreement with those reported by Doster and Michailides (1995) and Bonjar (2004) [9, 21] .

5. Conclusion

The result showed that the highest and lowest aflatoxin content was found in yellow-brown stained nuts of Ahmad-Aghaei and Kale-Ghoochi cultivar, respectively. The findings of this study pointed out that separation and removal of the yellow stained nuts (first stage) from the pistachio lots decreases more than 50% of the aflatoxin content, while after segregation of grey-black nuts (second stage) the aflatoxin reduction increased to more than 90%.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank the Pistachio Research Center for its financial support.

References

1. Bayman P, Baker JL, Mahoney NE. Aspergillus on tree nuts: incidence and associations. MYCPAH. **2002**;155(3):*161-9*.
2. Cheraghali AM, Yazdanpanah H, Doraki N, Abouhossain G, Hassibi M, Ali-abadi S, Aliakbarpoor M, Amirahmadi M, Askarian A, Fallah N, Hashemi T, Jalali M, Kalantari N, Khodadadi E, Maddah B, Mohit R, Mohseny M, Phaghihy Z, Rahmani A, Setoodeh L, Soleimany E, Zamanian F. Incidence of aflatoxins in Iran pistachio nuts. Food Chem Toxicol. **2007**;45(5): *812-6*.
3. Set E, Erkmen O. The aflatoxin contamination of ground red pepper and pistachio nuts sold in Turkey. Food Chem Toxicol. **2010**;48(8-9):*2532-7*.
4. Pour RS, Rasti M, Zighamian H, Garmakhani AD. Occurrence of aflatoxins in pistachio nuts in Esfahan province of Iran. J Food Saf. **2010**;30(2):*330-40*.
5. Arino A, Herrera M, Estopanan G, Rota MC, Carraminana JJ, Juan T, Herrera A. Aflatoxins in bulk and pre-packed pistachios sold in Spain and effect of roasting. Food Control. **2009**;20(9):*811-4*.

6. Udomkun P, Wiredu AN, Nagle M, Müller J, Vanlauwe B ,Bandyopadhyay R. Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application–A review. Food Control. **2017**;76:*127-38*.
7. Sommer NF, Buchanan JR, Fortlage RJ. Relation of early splitting and tattering of pistachio nuts to aflatoxin in the orchard. Phytopathology. **1986**;76(7):*692-4*.
8. Pearson TC, Slaughter DC. Machine vision detection of early split pistachio nuts. Transactions of the ASAE. **1996**;39(3):*1203-7*.
9. oster MA, Michailides TJ. The relationship between dateof hull splitting and decay of pistachio nuts by Aspergillus species. Plant Disease. **1995**;79(8):*766-9*.
10. Adibian M. Aflatoxins in Pistachio, Detection and Prevention. JNASCI. **2016**;5(1):*27-33*.
11. Shakerardekani A, Karim R, Mirdamadiha F. The effect ofsorting on aflatoxin reduction of pistachio nuts. J Food Agric Env. **2012**;10(1):*459-61*.
12. Ahmadi F, Tajabadipour A. Investigation of aflatoxin contamination in indehiscence and mechanical splitting pistachios. IJNRS. **2011**;1(2):*31-6*.
13. Doster MA, Michailides TJ. Aspergillus molds and aflatoxins in pistachio nuts in California. Phytopathology. **1994**;84(6):*583-90.*
14. Hadavi E. Several physical properties of aflatoxin-contaminated pistachio nuts: Application of BGY fluorescence for separation of aflatoxin-contaminated nuts. Food Additives & Contaminants: Part A. **2005**;22(11):*1144-53*.
15. De Mello FR, Scussel VM. Development of physical and optical methods for in-shell Brazil nuts sorting and aflatoxin reduction. Journal of Agricultural Science. **2009**;1(2):*3*.
16. de Pena DG, Arredondo JJM. Modification of the method 1 AOAC (CB-method) for the detection of aflatoxins. Bulletin of environmental contamination and toxicology. **1992**;49(4):*485-9*.
17. Sheibani A, Ghaziaskar HS. Pressurized fluid extraction for quantitative recovery of aflatoxins B1 and B2 from pistachio. Food Control. **2009**;20(2): *124-8*.
18. AOAC. Official Method, 968.22**1988**.
19. Luttfullah G, Hussain A. Studies on contamination level of aflatoxins in some dried fruits and nuts of Pakistan. Food Control. **2011**;22(3):*426-9*.
20. Wesolek N, Roudot A. Monte carlo modeling for aflatoxin b1 distribution in pistachio samples: A prerequisite for sampling plan validation. J Food Technol Res. **2014**;1(1):*1-20*.
21. Bonjar GHS. Incidence of aflatoxin producing fungiin early split pistachio nuts of Kerman, Iran. J Biol Sci. **2004**;4(2):*199-202*.