

Research Article

Effect of Harvesting Time and Delay in the Hulling Process on the Aflatoxin Content of Pistachio Nuts

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Harvesting time is one of the important factors that affect the quantitative and qualitative characteristics of pistachio nuts. Delaying in harvest time decreased intact nut percentage and increased cracked nut percentage. Furthermore, harvest time had significant effects on overmaturity, and the highest and lowest values were observed at the last time (Oct. 22) and at the first harvest time (Sep. 6). Results of harvesting time on aflatoxin contamination (without delay in processing) showed that the highest aflatoxin content was 0.33 ppb on October 22 and the lowest was 0.1 ppb on Sep. 6. Effects of processing delays on aflatoxin content showed that 0 and two-day delays had no significant effects, but 12, 24, and 48-hour delays caused a significant increase in aflatoxin content. Delaying the harvest time increased the splitting percentage. Delay in processing time, increased nut staining, and aflatoxin content. The effects of harvest time and delays in processing showed that the highest stained nuts (11.3%) were found at the last harvest time and after a 48-hour delay in processing time. The lowest measured aflatoxin content at harvest was 0.1 ppb and the highest was 66.10 ppb on Sep. 6 (without delay in processing) and Oct. 22 (with 48 hours' delay in processing), respectively. The hulling process of the harvested pistachio nuts must be carried out immediately after harvest. Processing delays must not be more than 24 hours after harvest. Considering the lowest early splitting, hull cracking, over maturity, shell staining, and aflatoxin content, September 6 is recommended as the best time to harvest pistachio nuts.

1. Introduction

Today, the export of pistachios has been greatly affected by the amount of aflatoxin [1]. Therefore, there is a need to monitor the product before and after harvest for decreasing the aflatoxin contamination in the pistachio yield [2, 3]. Research shows that contamination with toxin-producing agents and related metabolites begins in the field and can spread during processing (especially in the floating tank and drying of the crop) [4, 5].

One of the factors that facilitate contamination in the product is the regular and irregular cracks created in the upper skin (hull) of pistachio fruit, through which the fungal spores penetrate around the fruit and grow in suitable conditions and produce the toxin and even fungal growth. The production of the toxin can increase during processing

time. As long as the soft surface of the fruit is intact and without cracks, it acts as a barrier against the penetration of fungal agents and even some insects. After ripening of the crop, if the crop of each cultivar is not harvested at the right time according to the environmental conditions, overgrowth of the crop will cause cracking of the soft hull of the fruit, and with increasing delay in harvesting, the rate of fruit cracking (regular and irregular) will increase [6].

The presence of the crack in the soft hull of the fruit reduces the quality of the fruit due to the appropriate conditions for the entry of fungal spores and some insect damage. If a product contaminated with fungal agents is stored for some time after harvest in suitable environmental conditions in terms of temperature and humidity, fungal agents will grow and increase the possibility of aflatoxin production [7, 8].

The results of the research for determining the best harvest time for the Ohadi pistachio variety showed that the highest dry weight of the crop is created in the first week of October, the highest weight of fruit kernels in the second week of October, and the maximum percentage of fruit splitting in the third week of October. The highest amount of over matured fruit was in the fourth week of October, and the lowest one was observed in the first and second weeks of September [9].

The best time to harvest the product is based on the appropriate quality indicators: the percentage of peeling, the percentage of splitting fruit, shell skin color, dry kernel weight, and total fat and sugar content, which is usually about two to three weeks after the skin peels off easily. Due to the high moisture content of pistachio fruit at harvest time (40–50%) and the splitting shell (fruit splitting), pistachio fruit is very sensitive to physical and mechanical damage, and its quality is affected by various methods of the peeling process. On the other hand, delays in the process can have a significant impact on increasing microbial load and product quality [10].

The dry weight of the kernel and the amount of raw fat, such as splitting and shell discoloration, indicate that pistachio fruit is physiologically mature when the soft hull is easily separated. Harvesting before or after the appropriate time of ripening causes uncompleted kernel development or staining and improper appearance of the shell. Staining of the shell starts from the lower part of the fruit and around the connection point of the fruit tail, and over time it spreads to the abdominal part [2]. Because these spots do not penetrate deep into the shell, they are likely to be the result of a reaction of various substances (such as tannins) caused by the rupture and amputation of cells near the soft surface of the skin.

Information on the hull softness and shell color indicates that among the different fruits of a tree, there is a difference of 4–5 weeks at the rate of physiological development. The highest quality of the product is obtained within one week after facilitating the separation of the soft hull from the shell [6]. Early splitting is the main cause of fungal contamination and the production of aflatoxin toxins, so it seems that very small fruits do not cause fungal contamination but provide a way for fungi to enter [11].

As harvest time approaches, the population density of aflatoxin-producing fungi in pistachio orchards increases, and there seems to be a correlation between the increase in spore density of these fungi and the period of pistachio fruit growth and development [12].

Harvesting time is one of the most important factors in the quality and aflatoxin contamination of pistachio. Usually, an intact hull is the best barrier that prevents pistachio infection by *Aspergillus flavus* and *Aspergillus parasiticus* in the orchard. Therefore, after early splitting or cracking of matured nuts, infection would increase rapidly [13].

Decreasing hull moisture content after early splitting or cracking denotes time passing and increasing of mold contamination probability. Usually, the pistachio harvest duration is from Sep. 6 to Oct. 22 in Kerman province. Harvested pistachio are delivered to processing plants for

hulling and drying immediately. However, delays in the hulling process under high relative humidity and high pistachio aflatoxin content can increase rapidly in processing plants. The purpose of this research was to determine the effects of different harvesting times and delays in processing on pistachio appearance and aflatoxin content of pistachio yield.

2. Materials and Methods

In order to investigate the effects of harvest time and the delay time of the peeling process on the degree of product contamination with aflatoxin, the present study was conducted for two years. This research was carried out on a one-hectare plot of pistachio trees of the Ohadi variety at the Kerman Pistachio Research Station (Kerman, Iran).

The treatments included harvest time (four levels of 6 September, 21 September, 10 October, and 22 October) and delayed peeling time (five levels of 0, 2, 12, 24, and 48 hours). At each time of harvest, 30 trees were selected and, with three repetitions (a total of 90 trees at any time), sampling related to the percentage of early splitting pistachios, the percentage over matured pistachios, the percentages of split and non-split pistachios, and the percentage of shell discoloration was carried out.

At each harvest time, the entire crop of each of the replications was harvested separately and stored in bulk in the open air. After delayed peeling time, three 10 kg samples were randomly selected and peeled using a pistachio peeler and washed by immersion in water. After washing, the samples were sun-dried for 72 hours. Then, the product of each of the treatments was collected separately and transferred to the laboratory to determine the amount of aflatoxin. The total aflatoxin levels of each sample were determined by HPLC according to the Shakerardekani and Mirdamadiha [2] method.

To calculate the early splitting, cracked, over-matured, and stained nut percentage, 100 nuts (three replications) were selected and the number of related parameters were counted. The observations were statistically plotted with a basic plot of randomized complete blocks (with three replications) using Minitab software (ver. 20.3), and the averages were compared in the form of a Tukey test at a probability level of 5%.

3. Results

3.1. Early Splitting, Hull Cracking, and Overmaturing Percentage of the Pistachio Nut. Early splitting, cracking, over-maturing, and staining percentages of pistachio nuts increase with delays in harvesting time. These parameters had significant differences between different times of harvest (Table 1). The results show that when the harvest time shifts from September 6 to October 22, the rate of early splitting increases from 0.33 to 1.03%. There is no significant difference between the harvest times of September 21 and October 10, and the harvest times of October 10 and October 22, but the difference between the rest of the harvest times is significant. As we approach the end of harvest time, hull

TABLE 1: Early splitting, cracking, and overmaturing parentage of the pistachio nut in different harvesting times.

Harvest time	Early split (%)	Hull crack (%)	Overmatured (%)
Sep. 6	0.33 ± 0.01c	21.1 ± 1.0c	0.0 ± 0.0d
Sep. 21	0.79 ± 0.1b	38.3 ± 2.0b	4.2 ± 0.2c
Oct. 10	0.98 ± 0.1ab	40.9 ± 2.0b	9.5 ± 0.5b
Oct. 22	1.03 ± 0.03a	43.4 ± 1.0a	17.2 ± 1.0a

*Noncommon letters in each column and row are significantly different.

TABLE 2: Effect of processing delays on stained nut percentage of the Ohadi pistachio cultivar.

Harvest time	Processing delay (hrs)				
	0	2	12	24	48
Sep. 6	1.30 ± 0.10j	2.00 ± 0.1ij	2.70 ± 0.20hi	3.30 ± 0.30fgh	4.70 ± 0.2cd
Sep. 21	3.00 ± 0.10gh	3.30 ± 0.3fgh	3.70 ± 0.20efg	3.70 ± 0.20efg	4.30 ± 0.30cde
Oct. 10	3.10 ± 0.10gh	3.30 ± 0.3fgh	3.70 ± 0.20efg	4.00 ± 0.20def	6.70 ± 0.20b
Oct. 22	3.70 ± 0.20efg	4.30 ± 0.30cde	4.70 ± 0.20cd	5.00 ± 0.30c	11.30 ± 0.30a

*Noncommon letters in each column and row are significantly different.

TABLE 3: Effect of processing delay hours on total aflatoxin content (ppb) of the Ohadi pistachio cultivar.

Harvest time	Processing delay (hrs)				
	0	2	12	24	48
Sep. 6	0.10 ± 0.01h	0.10 ± 0.02h	0.11 ± 0.01h	0.014 ± 0.02h	0.16 ± 0.01h
Sep. 21	0.16 ± 0.02h	0.17 ± 0.02h	3.30 ± 0.30e	3.50 ± 0.30de	7.90 ± 0.20c
Oct. 10	0.18 ± 0.02h	0.20 ± 0.02h	0.25 ± 0.02h	2.42 ± 0.20f	10.10 ± 0.10b
Oct. 22	0.33 ± 0.03h	0.47 ± 0.02gh	1.20 ± 0.20g	4.30 ± 0.30d	66.10 ± 1.0a

*Noncommon letters in each column and row are significantly different.

cracking and overmaturation of nuts increase in the range of 21.1–43.4% and 0.0–17.2%, respectively, and the difference between harvest times is significant.

3.2. Delay in Processing of Fresh Pistachios

3.2.1. Shell Staining. One of the main signs of delay in pistachio processing is the staining of its shell, which is presented in Table 2. According to the results, the amount of staining on the shell at the first harvest time (September 6) is the lowest, which is significantly different from the rest of the harvest time. The longer the delay (until 48 hrs) in the peeling process, the greater the amount of stained shell. The highest amount of staining is observed after 48 hours' delay in peeling and the last harvest time (October 22), which is equivalent to 11.3%.

3.2.2. The Amount of Aflatoxin. There is no significant difference in terms of aflatoxin content at all harvest times without or with 2 hrs delay in processing start ($p \leq 0.05$) (Table 3). Further delays (2–48 hrs) increased the amount of aflatoxin significantly. The highest amount of aflatoxin was observed at the last harvest time (October 22) and 48 hours' delay in processing, which was 66.1 ppb.

4. Discussions

Regarding the pistachio nut market, developed countries have more restrictive standards for aflatoxin limits [14]. The total allowable aflatoxin in the USA, Iran, and EU is 15, 15,

and 10 ng/g, respectively [15]. Pistachio nuts could be contaminated with aflatoxin from maturity until storage [16]. Aflatoxin was detected in orchards at maturity and increased until harvest [4]. Both early and late harvesting times decreased the quality of pistachio nuts. Nuts harvested too late are clearly vulnerable to hull cracking, shell staining, mechanical injuries, insects, and bird attacks [9]. There is a direct relationship between harvest time and the amount of aflatoxin. Shakerardekani and Mirdamadiha [2] reported that processing delays increased shell staining and aflatoxins.

Early splitting is one of the parameters that cause fungi to attack pistachio kernels and produce aflatoxins. Mohammadi Moghadam et al. [17] reported that potential producers of aflatoxin (*A. flavus* and *A. parasiticus*) were found in early split nuts. Several studies correlate high levels of aflatoxin with early split pistachio nuts [3–5, 9]. Hull cracking, like early splitting, provides the conditions for fungal growth and aflatoxin production. Of course, the risk of hull cracking is less than that of early splitting. The hull cracking may not be in the direction of splitting. Therefore, fungi are less likely to reach the kernel [5]. Early-split nuts have more moldy nut kernels than nuts with cracked hulls because early-splitting has a tendency to occur earlier than nuts with cracked hulls [9].

The pistachio nuts suspected of aflatoxin contamination have several physical characteristics such as shell discoloration that could help to remove them during processing. Based on our results of research, there is a relationship between the amount of stained shell and the amount of aflatoxin. Tajabadipour, Afshari, and Hokmabadi [18]

reported that stained nuts had the highest amount of aflatoxin. With the increasing stain levels on the shell, the amount of aflatoxin increased significantly. Panahi and Khezri [9] found that nuts from the delayed harvest time were more likely to have more shell staining than those from the earlier harvest time.

Maturity can also be considered the most critical stage for aflatoxin contamination in the preharvest stages [4]. Overmaturation in addition reduces the firmness of the superficial soft skin as a kernel protector and increases the chances of fungal growth and aflatoxin production. Matured nuts soften and can be physically damaged due to the cracking of the hull, increasing the attack risk of aflatoxin-producing fungi [4].

5. Conclusion

Since the amount of aflatoxin affects human health, a delay in pistachio processing is not acceptable. Pistachio farmers should harvest their yield as soon as the product is ripped. The hulling and drying of the harvested pistachio nuts must be done immediately after harvest. If a delay is happening, it must not be over 24 hours after harvest. Based on the results (lowest early splitting, hull cracking, over maturity, shell staining, and aflatoxin content), the best time to harvest pistachio nuts is September 6.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

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