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The Effects of Storage Period and Relative Humidity on Tombul Type Hazelnut Produced in Turkey

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Abstract: Aflatoxin development was studied in Tombul type hazelnut inoculated with *Aspergillus parasiticus* NRRL 2999 and control, stored at 20 ± 2°C at 85 ± 2 and 95 ± 2% relative humidity (RH) on '10th 20th and 30th days. The mould-inoculated samples had different amounts of aflatoxins (B+G) ranging from 0.0 to 13092 μg/kg. At 85 ± 2% *and* 95 ± 2% RH with 20 ± 2°C storage temperature, The detected total amount of aflatoxins were 325 μg/kg and 13092 μg/kg respectively, while no aflatoxin formation was found in control group.

Key words: Hazelnut, aflatoxin, storage, relative humidity

Introduction

Aflatoxins, the secondary metabolites produced by toxigenic strains of *Aspergillus flavus* and *A. parasiticus*, are known to be involved in some toxic, carcinogenic teratogenic and mutagenic diseases in human and animals (Whyllie and Morehouse, 1977; Richard *et al.*, 1993; Gourama and Bullerman, 1995).

Hazelnut on tree is protected by a strong peel (Mehlenbacher, 1994). Although strong peeled fruits are sensitive against mould contamination less than the others, time after time hazelnut contaminated with mould may be confronted. Naturally dehulled hazelnut can he contaminated more frequently (Eke and Goktan, 1987). Aspergillus generally grows on dead cells, following harvest and produce aflatoxin when temperature and moisture are adequate. Although toxic mould present in the food sometimes aflatoxin may not be seen, in the same way aflatoxin may not be present in a food which seems healthy. A number of factors Physical and chemical, affect the aflatoxin formation among which the most important ones are environmental conditions, especially temperature and moisture (Goldblatt, 1971; Hill *et al.*, 1983; Chiou *et al.*, 1984, Sert, 1985; Lacey, l989).

In this study regarding the contamination of hazelnut with mould and aflatoxin formation, when it is stored tinder inadequate conditions and consequent public health risk, the importance of hazelnut in Turkey's export, the economic loss, when it is rejected due to aflatoxin, the effect of relative humidity on aflatoxin formation were investigated

Materials and Methods

Hazelnuts were obtained from Giresun Hazelnut Research Institution Directorate (Turkey) and were dehulled.

Aflatoxin analysis: The analysis method outlined by Majerus and Zakaria (1992) for strong shelled fruits, with sensitive limit of 0.5 μg/kg, was used.

The hazelnut samples were milled with Waring Bander for 20 sec and then filtered through a 2mm mesh. Twenty five grams sample was transferred to a 250-ml flask, which 100-ml methanol water (85:15, v/v) was added and then closed tightly and shaken with Electromeg M 22 for 30 min. The sample was then filtered through Whatman No 1. Approximately 5O ml of filtrate was transferred to a separating flask (250 ml) into which 50 ml NaCl solution of 10% and 25 ml hexane were added and then the mixture was stirred for 1 min. Aflatoxin was extracted in two portions with 25 ml methylendichlorur and aqueous phase and then shaken for one minute. Methylene dichloride phase was sieved through Whatman No: 1 on which was placed 5 g dehydrated sodium sulfate. Filtrate was collected in 100 ml joje and evaporated using rotate evaporator (Heidolph-511). The extract was purified using 65xl0 mm glass colon. The sample extract that dissolved in 3ml methylendichlorur was transferred into colon, the remainder part in joje was redissolved twice using I ml methylendichlorur each and repassed through colon. 10 ml of toxin was extracted twice. It was then dissolved in 1 ml methylendichlorur to apply thin layer chromatography. Glass layers of 20x20 cm^2 were used. Silica-60 (Merck No: 7731) was applied on the layers (Anon., 1985).

The aflatoxin standards were prepared according to Anonymous (1975) using spectrometer (Shimadzu UV-160). Aflatoxin standards were diluted with benzene acetonitrile to obtain definite ration of μg/ml aflatoxin B_1, B_2, G_1 and G_2. Both the standard toxin solutions and the extracts were applied on TLC layers with microshrynge. The amount of aflatoxin was determined as outlined in Anon (1975).

The preparation of medium with different relative humidity: The mediums with different relative moisture were prepared in jars of 5 L. With saturated salt solutions the relative moisture in jars were maintained (Coksoyler, 1984).

Inoculation *of A. parasiticus* spores: *Aspergillus parasiticus* NRRL 2999 strain was obtained from TUBITAK-MAM Food Research Dept. and was inoculated into hazelnuts.

The spores were brought out by the method of Çoksöyler (1984). Hazelnut and spores were mixed as calculated 10^7 spores per hazelnut in jar following activation in 200 ml 0.005 % Tween 80 solution and its amount was determined with Thoma slide (Kosker, 1976).

Statistical analysis: The data obtained were subjected to variance analysis. The significant means were compared according to Duncan's new multiple range test.

Results and Discussion

Aflatoxin was not found in control group, which was not inoculated, during storage period, in either relative humidity conditions, but compounds (stain) unlike aflatoxin were detected. It was reported that these stains can be characterized as metabolic compounds synthesized by indigenous micro flora of hazelnut (Sert, 1984).

Hazelnut that was inoculated with *A. parasiticus* NRRL 2999 strain was divided into two groups and stored at 85% and 95% relative humidity (RH) for 30 days. Analyses were carried out on 10th, 20th and 30th days and the results are summarized in Table 1.

As can be seen in Table 1, while there was no toxin

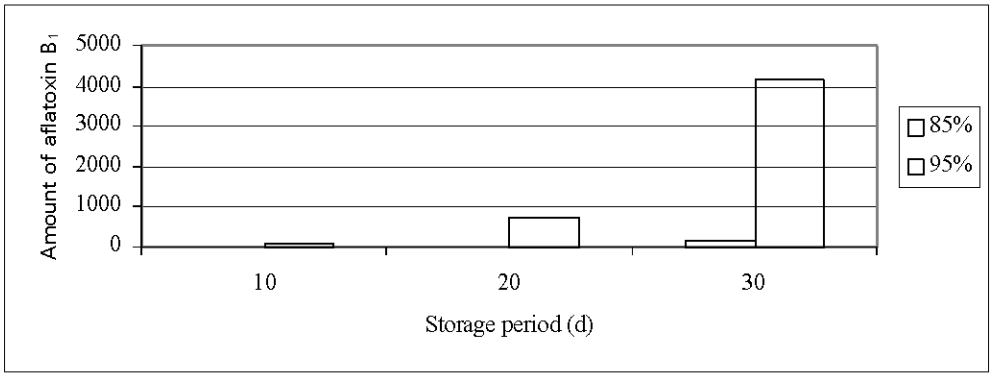

Fig. 1: The effect of storage period x relative humidity interaction on Aflatoxin B_1 hazelnut samples

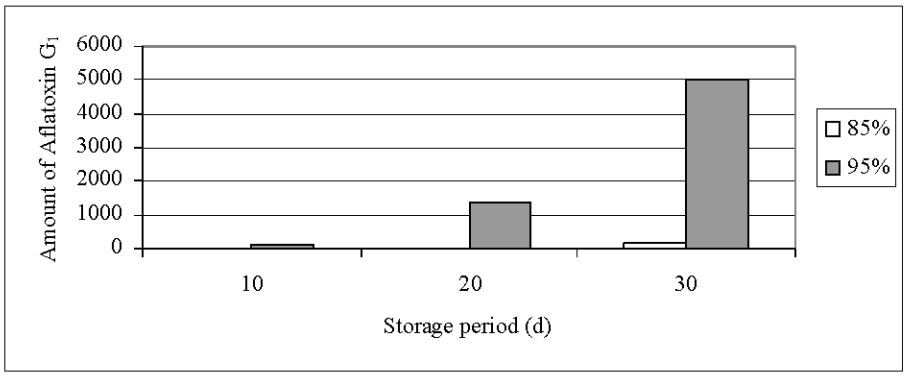

Fig. 2: The effect of storage period x relative humidity interaction on Aflatoxin G_1 in hazelnut samples

Table 1: The amount of aflatoxin determined in hazelnut samples stored in 85 and 95% relative humidity at 20±2 °C

Storage period (Day)	Relative humidity (%)	Aflatoxin amount (μg/kg)			
		B_1	B_2	G_1	G_2
10	85	0.0	0.0	0.0	0
10	95	95.8	0.0	114.7	0.0
20	85	0.0	0.0	0	0.0
20	95	728.4	237.9	1357.9	423.1
30	85	167.6	0.0	157.8	0.0
30	95	4191.1	237.9	5021.3	634.6

Table 2: The result of Duncan's multiple test applied on aflatoxin amounts determined during storage*

Storage Period (Day) -	Aflatoxin amount (μg/kg)			
	B_1	B_2	G_1	G_2
10	517.908 c	0.000	243.883 c	0.000
20	1564.488 b	110.300 a	958. 183 b	198.312b
30	2311.160 a	79.317 b	1807.654 a	370.183b

* Averages (n=8) followed by different letters are statistically different ($p<0.05$)

Table 3: Duncan's multiple test applied on aflatoxin amounts determined during storage*

Relative humidity (%)	Aflatoxin amount (μg/kg)			
	B1	B2	G1	G2
85	667.589 b	15.698 b	353.928 b	61.697 b
95	2261.418 a	110.714 a	1.656.182 a	317.300 a

* Averages (n=8) followed by different letters are statistically different ($p<0.05$)

development in samples stored at 85%RH on 10th and 20th days, a dramatic increase in toxins B_1 and G_1 on 30th day. The aflatoxin types B_2 and G_2 was not determined at 85% RH until 30th day, but they increased dramatically starting from 20th day at 95% RH. On the other hand, toxin formation in samples stored at 95% relative humidity increased during storage, starting from 20th day.

The variance analysis revealed that the interaction between relative humidity and storage period had a significant ($p<0.01$) effect on all the aflatoxin types that were investigated.

The average amounts of toxin types B_1, B_2, G_1 and G_2, and the results of Duncan's multiple range test are given in Table 2 and 3.

It is evident from Table 2 that during the storage period the amounts of toxin types B_1, G_1 and G_2 increased significantly. The amount of toxin type B_2 was determined to decrease significantly following 20th day. A fluctuation during storage period in aflatoxin amount due to storage conditions was reported by several authors (Ashwort *et al.*, 1987; Mogan *et al.*, 1984; Özkaya and Coksoyler, 1988). It was reported that the change in amount of aflatoxin during storage was caused by decomposition of chemical structure of toxin, increase in free fatty acids, the lack of nutritional compounds for mould growth, the dominance of other indigenous mould strains (Eke and Okten, 1987).

It is evident from Table 3 that the amount of aflatoxin formed in those stored at 85% RH were significantly ($p<0.05$) higher than those stored at 85% RH. This result was in well accord with many researchers, who reported that relative humidity played important role in both mould growth and toxin

formation (Sanchis, 1988; Gourama and Bullerman, 1995). Özkaya and Coksoyler (1988) stored hazelnut at 30°C and found that rapid mould growth and more aflatoxin formation occurred in hazelnut samples stored at 100% RH than stored at 91% RH. This is in consistence with the results obtained in present study.

The effects of storage period and relative humidity interaction on aflatoxin formation were displayed in Fig. 1 and 2.

It is evident that an apparent decrease occurred in Aflatoxin B_1 starting from 20th day, stored at 85% RH conditions, whereas the toxin amount in that of 95% RH increased.

As well as aflatoxin B_1, a higher amount of Aflatoxin G_1 was found in hazelnut samples stored at 95% RH than in that stored at 85% RH.

In conclusion, it has been determined that in addition to mould concentration, RH plays important role in aflatoxin formation due to storage period. The higher amount of aflatoxin was formed at 95% RH than at 85% RH.

Following suggestions can be concluded from obtained findings to protect hazelnut from aflatoxin contamination:

1. Hazelnut should be stored in separated compartments rather than a whole.
2. Controlled conditions should be applied and precautions should be taken to prevent contamination of *Aspergillus* strains.
3. Farmers should be informed about precautions to be applied during both harvest and storage.

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