

MYCOTOXINS AND MYCOTOXIGENIC FUNGI IN IMPORTED PISTACHIO NUTS IN EGYPT

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ABSTRACT

A survey was carried out to obtain data on the occurrence of mycotoxins and the mycotoxin-producing potential of fungi isolated from 48 samples of imported pistachio nuts (24 sterilized salted and another 24 unsalted pistachio nuts) imported in Egypt (collected from Cairo retail markets). Four genera, i.e., *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* spp. were isolated. The most frequently isolated fungi were *Aspergillus* species. The predominant fungi present in samples was *Aspergillus niger*, followed by *Aspergillus flavus*, *Aspergillus ochraceus*, *Penicillium* Spp., *Fusarium* Spp. and *Alternaria* spp. Aflatoxins were determined in 24 salted and 24 unsalted kernels. The highest level of aflatoxins found was B₁ at range of 14.25 - 30.45 µg/Kg in relation of (a_w) 0.75 - 0.80 and 12.05 - 28.82 µg/Kg with (a_w) 0.61 - 0.65, respectively. Ochratoxin A and Zearalenone were found only in one sample of salted kernel at concentration of 34.21 µg/Kg and 51.02 µg/Kg in this respective order. Whereas, Zearalenone was found in 3 samples of unsalted kernel at range of 42.18 - 74.82 µg/Kg. The effect of processing on the aflatoxins present naturally in pistachio kernels was studied by preparation phostocia " an orient favorable sweet ". It was found that direct heating for 30 min destroyed most if not all of aflatoxins.

INTRODUCTION

Mycotoxins are metabolites of filamentous fungi that cause deleterious effects in animals and, in some circumstances humans. They are at the present, considered to be some of the most dangerous contaminants in foods and feeds (Cole, 1981; and Smith and Moss, 1980). The most toxic of these mycotoxins are aflatoxin B₁, ochratoxin A, T-2 toxin, fumonisins, zearalenone and possibly citrinin (CAST, 1989). However, aflatoxins, the most studied and widely known mycotoxins, are among the most potent mutagenic substances known; there is extensive experimental and epidemiological evidence that they induce liver cancer (WHO, 1998).

The major aflatoxin-producing fungi are *Aspergillus flavus* and *Aspergillus parasiticus*. Under favorable temperature and humidity conditions these fungi grow on certain foodstuffs, most commonly groundnuts, dried fruits, tree nuts (almonds, pecans, walnuts and pistachio). The rate and degree of aflatoxin contamination are dependent on temperature, soil humidity and storage conditions (Boutrif, 1999). The legal tolerance limit of pistachio nut is 5µg/kg for aflatoxin B₁ and 10µg/kg for total aflatoxins set by the European Commission Regulation EC 98/53, Blanc (2001). The joint FAO/WHO (1994a) Expert Committee on Food Additives (JECFA) concluded that there is no significant difference in risk to human health between the maximum levels of 10 µg/Kg and 20 µg/Kg for aflatoxin B₁ in food and from 0 to 50 µg/Kg for total aflatoxins. Up till now the Food and Drug Administration

(FDA) action levels are only given for total aflatoxins for foods and feeds, and has not established action levels for others mycotoxins. The FDA have issued guidance levels for foods and feeds producers in the USA which are not enforceable like action levels (FDA, 2003).

However, real risk of consuming food products can be evaluated by analyzing the content of mycotoxins and contrasting them with legally accepted levels. This study attempted to assess the market situation with respect to mycotoxins in pistachio nuts through detecting residues of aflatoxins, ochratoxin A and zearalenone. The incidence of toxigenic and other fungi and as well the water activity of pistachio nut kernels was investigated. The fate of processing on the stability of aflatoxin B in pistachio kernels was also studied. It is worthy to report that this study is the first and only investigation carried out in Egypt that dealt with mycotoxins in imported pistachio nuts.

MATERIALS AND METHODS

I - Chemicals

Aflatoxins, Ochratoxin A and Zearalenone standards were purchased from Sigma Chemical Company, St. Lewis, Mo, USA. Other solvent were of the highest purity that commercially available.

II – Samples:

Forty eight commercially available market basket samples (one kg each) of salted and/or unsalted (24 each) were randomly collected from local markets in Egypt. By stipulating food, the legislature intends to refer solely to edible constituents, for pistachio, which have an edible nut kernel and an inedible (oyster) shell, this is clearly the case. Accordingly, the determination of mycotoxin residues were then directed to the edible nut kernels. Open shell pistachios were used for this study and was completely shelled by hand. Pistachio nuts are imported to Egypt from different countries like Turkey, Syria and United States of America.

III – Mycotoxin production (in vitro):

The term "in vitro" refers to mycotoxin production in the laboratory by species isolated from pistachio nuts, using synthetic culture media and controlled culture condition. The isolated identified strains of *Aspergillus* (*flavus*, *niger* and *ochraceus*), *Penicillium* spp. and *Alternaria* spp. were inoculated into 100 ml of each Yeast Extract (YES) (Davis *et al.*, 1966) and Wickerham (Wick) media (Raper and Thom, 1968) and incubated for 14 days at 25 °C (Jiménez *et al.*, 1991). Strains of *Fusarium* spp. were incubated in Rice medium in 500 ml Erlenmyer flasks for 22 days at 25 °C (Jiménez *et al.*, 1988).

Media used:

- 1- YES (Yeast extract, 20 g/L; sucrose, 50 g/L; pH 5.5) (Davis *et al.*, 1966).
- 2- Wick (yeast extract, 2.0 g/L; peptone, 3.0 g/L; dextrose 2.0 g/L; sucrose, 30.0 g/L; corn steep solid, 5.0 g/L; NaNO₃, 2.0 g/L; K₂HPO₄·3H₂O, 1.0 g/L; MgSO₄·7H₂O, 0.5 g/L; KCl, 0.2 g/L; FeSO₄·7H₂O, 0.01 g/L; pH 5.5) (Raper and Thom, 1968).
- 3- Rice (rice, 50 g and water, 50 ml) (Jiménez *et al.*, 1991).

IV – Mycotoxins analysis in pistachio kernels:

All samples (kernels) were fine grounded with molenex mil. Aflatoxins, ochratoxin A and zearalenone residues were determined. Aflatoxins were extracted, derivatized with trifluoroacetic acid (TFA), and quantified by high-pressure liquid chromatography (HPLC) with a C₁₈ reversed-phase column, fluorescence detector (Ex. 365 nm and Em. 450 nm) and mobile phase, water-methanol-acetonitrile (62:20:18). By using method of AOAC (1995) (49.2.19A).

Ochratoxin A content was achieved according to AOAC (1995) (49.5.03) using solid phase extraction C₁₈ bonded Silica gel, fluorescence detector (Ex. 333 nm and Em. 460 nm) and mobile phase, water- cetonitrile - acitic acid (99:99:2).

Zearalenone was determined in all samples by TLC (Thin Layer Chromatography) method using chromatographic plates and mobile phase, toluene- ethyl acetate- chloroform 85%- Formic acid (45:25:25:5) as described by L'vova et al., (1998). The resulting zones on TLC plates were examined and marked under long ultraviolet wavelength (366 nm). The marked areas were determined by the densitometer using (CS-9000 Daul wavelength flying-spot scanning Densitometer, photomode reflection and beam zigzag, SHIMADZU), (Microanalyses Center, Faculty of Science, Cairo University), using wavelength range 200-400 nm. Standard curve of zearalenone was used to calculate the detected concentration.

V – Isolation and Identification of fungi:

All samples of pistachio nuts (whole pistachio, shell and Kernel) were investigated for the occurrence of fungi. One hundred of each were taken and their surfaces were sterilized with a solution of 2% sodium hypochlorite, and subsequently washed twice with sterile distilled water. Five of these kernels were placed in aseptic conditions on potato dextrose agar plates. After 7 days of incubation at 28°C, samples were examined for fungal count and the molds present isolated and identified according to Jimenez et al. (1990).

VI – Determination of water activity (^aw) in pistachio kernels:

Water activity (^aw) was determined by using the methods described in AOAC, (1995) by equilibrium moisture absorption of microcrystalline cellulose at 35 °C for 24 hours.

VII – The effect of processing on aflatoxins levels in pistachio nuts products (Phostocia):

Preparation of phostocia:

Phostocia "it is an oriental favorable sweet" was produced in the laboratory and prepared from natural contaminated salted pistachio nuts (kernels, the edible parts) - containing residues of aflatoxin B₁, B₂, G₁ and G₂ by concentration of 30.45, 10.08, 29.34 and 9.49 µg/Kg, respectively - as follows:

Naturally contaminated pistachio kernels (500 g) was roasted (140°C) on stove with constant stirring for 10 min. The sugar (250 g) was added to the water (250ml) in a boiling pan and heated on a stove with thoroughly stirring until melting of the sugar. Five grams of citric acid was added to the sugar solution and the stirring continued until it become brown (130°C) (caramel).Some cold water was added to stop the browning. The

roasted pistachio was then added to the brown sugar solution (caramal) and mixed thoroughly at 110°C for 5min. The pistachio caramal-mixture was scraped down on a greased surface and pressed until a thin layer was formed (5cm). The layer was left until it become warm then cut to cubs then stored in dry place at room temperature.

Determination of aflatoxins:

Phostocia cubs were grounded with molinex mill and mixed well. A representable 50 g of fine grounded and well mixed sample was analysed for mycotoxins using the TLC technique (AOAC, 49.2.19A, 1995). The resulting zones on the TLC plate were examined and marked undere long ultraviolet wavelength (360 nm). The marked areas were determined by using wavelength range 200-400 nm, photomode reflection and beam zigzag. Standard curve of aflatoxins was used to calculate the detectable concentration by the Densitometer (as mentioned before).

RESULTS AND DISCUSSION

Mycotoxin in pistachio nuts :

During the current investigation, kernels of salted and unsalted pistachio nuts were tested for mycotoxin residues (Table, 1). The present data revealed that aflatoxins were detected in 5 samples (20.8%) and 3 samples (12.5%) at levels ranged between 29.45 – 79.36 and 12.05 – 28.82 µg total aflatoxins/kg of salted and unsalted kernels, in this respective order. All the four types of aflatoxins were found in five salted ones at levels varing between 14.25 – 30.45 µg B₁, 8.81 – 10.08 µg B₂, 5.08 – 29.34 µg G₁ and 1.99 – 9.49 µg G₂ /kg. The range of total aflatoxins detected was 29.45 – 79.36 µg/kg for the positive salted pistachio nut kernels. Aflatoxin B₁ was only detected in the three unsalted kernels showing ranges between 12.05 to 28.82 µg /Kg.

Table (1): Water activity and detection of mycotoxins In Pistachio nut (kernels) (µg/Kg)

Samples	Water activity	Number of samples		Mycotoxins detected	Range Ppb
		analysed	Positive (%)		
Salated Pistachio Nuts	0.75-0.80	24	5 (20.8)	Aflatoxins B1	14.25-30.45
				Aflatoxins B2	8.81-10.08
				Aflatoxins G1	5.08- 29.34
				Aflatoxins G2	1.99- 9.49
				Ochratoxin	34.21
Unsalted Pistachio nuts	0.61-0.65	24	3 (12.05)	Zearalinone	51.02
				Aflatoxins B1	12.05 - 28.82
				Zearalinone	42.18 - 74.82

Similar concentration and incedence as well were given by Dickens and Welty (1975) who determined the total aflatoxins of 46 samples of pistachio kernels and found that 8 samples (17.4%) contained ranges of 34 to 97 µg/kg. In the same concern, Boutrif *et al.* (1977) found that one sample

(5.3%) of 19 Tunisian pistachio nut samples contained 22 µg aflatoxin B₁/Kg. However, lower levels of aflatoxins and incidence as well were reported by Tabata *et al.* (1993) who examined 165 samples of pistachio nuts in Japan and found that 2 (1.2%) of which contained > 10 µg of total aflatoxins/Kg. Apergi *et al.* (1995) in Greece analysed 25 samples of pistachio nut imported from Argentina and found that the average detected level of aflatoxin B₁ was 1.3 µg/Kg and the maximum level of total aflatoxins was 7.6 µg/Kg. Recently, AbdulKadar *et al.* (2002) reported that 20% of pistachio nut imported to Qatar showed levels varying from 0.1 – 20 µg aflatoxins/Kg.

On the other hand, for more higher levels and lower incidence of total aflatoxins and/or aflatoxin B₁ were given by other investigators. Steiner *et al.* (1992) quantitated the aflatoxin content of the shells of the three lots of the highest contaminated pistachio nuts imported from Iran to Switzerland. The shells of 2 lots contained an average of 0.81 µg aflatoxin B₁; the others were considered to be free from aflatoxin. They concluded that shells do not seem to be convenient substrate for fungi to produce aflatoxins and do not as well contribute to a considerable concentration of aflatoxins in the sample. The corresponding kernels contained an average of 107 µg aflatoxin B₁/Kg. Tabata *et al.* (1993) found levels of > 1000 and > 100 respectively in 1 (0.6%) and 2 (1.2%) samples of 165 pistachio nut sample imported from Iran. Also, Candlish *et al.* (2001) examined a range of ethnic foods including pistachio nuts for the presence of total aflatoxins. Samples were obtained from local retail outlets and distributors in Glasgow, England. Three samples of pistachio nuts contained significant levels of total aflatoxins in concentrations varying from 15 to 259 µg/Kg. Moreover, the highest incidence and levels of total aflatoxins were given by AbdulKadar *et al.* (2002) in pistachio nut kernels as being 289 µg/Kg, in 33% of the examined samples imported to Qatar.

As regards other mycotoxin detected in the present investigation, it is evident from the same table that ochratoxin A was found only in one sample (4.2%) of salted kernel at concentration of 34.21 µg/Kg. Also, zearalenone was found in another one sample (4.2%) kernels of salted pistachio nuts at concentration of 51.02 µg/Kg and in three samples (12.5%) of unsalted ones at a range between 42.18 to 74.82 µg/Kg. Concerning the detection of both ochratoxin A and/or zearalenone in pistachio kernel, no reports are available update. However, ochratoxins was produced by isolates of *Aspergillus ochraceus* relatively common in early splits in California (Cigler, 1972) and from those isolated from pistachio nuts in Turkey (Denizef *et al.*, 1976).

The legal tolerance limit of pistachio nut set by the European Commission Regulation EC 98/53 (Blanc, 2001) is 5 µg/Kg for aflatoxin B₁ and 10 µg/Kg for total aflatoxins. Applying these limits to the current detected levels, it can be concluded that 79.2% of salted pistachio nut samples and 87.5% of unsalted ones are within the tolerable level. On the other hand 5 samples of salted pistachio kernels (20.8%) and 3 (12.5%) of unsalted pistachio kernels exceeds such legal tolerance limits. It is worthy to report that implementation of EC Regulation since 1 January 1999 has revealed major practical short comings with the limits set for pistachio resulting in a very high percentage of pistachio imports to Europe being rejected. So, they are

asked to take a close look at the regulation and its directive and proposes amendments to make them more precise and fairer in terms of balancing consumer and producer risk. In this respect, the FDA, (2003) currently employs an aflatoxin tolerance level in pistachio nut of 20 µg/Kg (ppb). This is strongly supported by the JECFA committee in 1997, who concluded from a quantitative risk assessment on aflatoxins that there is no significant difference in risk to human health between the maximum levels of 10 µg/Kg and 20 µg/Kg for aflatoxin B₁ (WHO, 1998).

From this presentation and in view of action level used by the FDA (20 µg aflatoxins/Kg), 79.2% and 87.5% of salted and unsalted pistachio nut samples under the current investigation are still within such action level.

Determination of the water activity (^aw):

Regarding water activity content (^aw) in salted pistachio nuts (kernel) and unsalted ones, the present data indicate that water activity (^aw) level ranging between 0.75 – 0.80 and 0.61 – 0.65, respectively as shown in table (1). Our results meet the legal tolerance limit of 0.7 as set up by the Codex Alimentarius Committee on Food Hygiene, which has proposed an ^aw standard of 0.7 for peanuts to prevent contamination with aflatoxins. The ^aw standard of 0.7 is also useful for safe storage of other agricultural products (FAO, 1990).

Counts and types of isolated fungi detected on pistachio nuts:

Samples of unsalted and/or salted pistachio nuts were examined for the probability of their infection by fungi directly after collection from the local market. Results in table (2) show the averages of the total fungal count per one hundred sterilized pistachio nuts (whole pistachio, shell and kernel). These results clearly indicated that the total fungal count for sterilized unsalted and salted whole pistachio nut, shell and/or kernel were 220 & 150, 160 & 120 and 200 & 180 per 100 nuts in this respective order. The percentage of fungal species detected were calculated in different parts of pistachio nuts. It is evident from the same table that the isolated fungi associated with sterilized unsalted and salted whole pistachio nuts belonged to two genera; *Aspergillus spp.*, (78 and 79%) and *Alternaria spp.*, (22 and 20%) in this order. *Aspergillus niger* predominated in both sterilized unsalted and salted whole pistachio being 34% and 49%, respectively followed by *Aspergillus flavus* recording 29% and 30% in this respective order. *Aspergillus ochraceus* was only isolated from the whole sterilized unsalted ones recording 15%. On the other hand, fungi isolated from sterilized unsalted and salted shell belong to three genera namely *Aspergillus spp.* 69% & 76%, *Penecillium spp.*, 16% & 13% and *Alternaria spp.* 14% & 13% respectively. It is obvious from the same table that *A. niger* predominated in both sterilized unsalted and salted shell recording 43%, for each *A. flavus* 27% & 33%, *Penecillium spp.*, 16% & 13% and *Alternaria spp.* 14% & 13% in this order.

Concerning the distribution of isolated fungi in sterilized unsalted kernels, it is obvious from the same table that *A. niger* is still the predominate isolate recording 44% followed by *A. flavus* 31% then *Penecillium spp.* 16% and *Fusarium spp.* 9%. In case of salted kernels, the presented data show the same trend of distribution *A. niger* 39% followed by *A. flavus* 31% then

Penecillium spp. 16%. *Aspergillus ochraceus* was only detected in salted kernels recording 14% of the total isolated fungi. The presence of the different species of fungi detected in pistachio nuts may be initially from the orchard and nuts that are not infected in the orchard may become infected during transport and handling.

Table (2) : Average counts and types of fungi isolated from sterilized unsalted and/or salted pistachio nuts

Sample	Parts of Pistachio	TFC	Identified fungi (%)					
			<i>Aspergillus Spp</i>			<i>Penicillium</i>	<i>Fusarium</i>	<i>Alternaria</i>
			<i>flavus</i>	<i>niger</i>	<i>ochraceus</i>	<i>Spp.</i>	<i>Spp.</i>	<i>Spp.</i>
Unsalted	Whole	220	29	34	15	---	---	22
	Shell	160	27	43	---	16	---	14
	Kernel	200	31	44	---	16	9	---
Salted	Whole	150	30	49	---	---	---	20
	Shell	120	33	43	---	13	---	13
	Kernel	180	31	39	14	16	---	---

TFC : Average total fungi count/100 nuts

Similar results were shown by Jimenez *et al.* (1991) who found that 9.8%, 21.4%, 18.6%, 0.1% and 2.6% of kernels were infected with *A. flavus*, *A. niger*, *Penecillium spp.*, *Fusarium spp.* and *Alternaria spp.*, respectively. In the same respect, the current results of pistachio kernels coincide with those given by Doster and Michailides (1994), they reported that a total of 14 *Aspergillus* species were isolated from the kernels of pistachio nut, from 11 commercial orchards in the United State (California) mainly early splits. The authors explained that the early splits are a typical nuts that have split hulls, exposing the kernel to invasion by molds and insects. They added that *A. niger* was the only *Aspergillus* species that occurred frequently. However, *A. flavus* or *A. parasiticus* (Potential producer of the mycotoxin, aflatoxins) were found in early splits from most orchards, and *A. ochraceus* (Potential producer of the mycotoxin, ochratoxins) were found in all orchards. They found that early splits with rough, shriveled hulls had more than twice the *A. niger* infection and more than three times as much *A. flavus* or *A. parasiticus* infection as early splits with smooth hulls. On the other hand, the predominance of *A. niger* was also reported by other investigations in nut orchards. For pecans in Georgia (Huang and Handin, 1976) in almond kernels from California (Purcell *et al.*, 1980) and pistachio nuts from Iran (Doster *et al.*, 1993). *A. niger* was the most common *Aspergillus* species isolated.

Generally, it is quite well recognized that 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 molds and insects. Moreover, 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". High humidity and high temperature within bulk bins provide ideal conditions for the infection of early split nuts, which dramatically

increases the incidence and level of aflatoxin contamination, until nuts are mycologically stabilized by drying or refrigeration (Boutrif, 1999)

Screening trial for the detection of mycotoxigenic fungi:

It is recognized that, the best media for toxin production depend on the mold species and/or strains under investigation and as well to the components of the tested medium. More than one media might be necessary to ensure that biosynthetic capabilities of the tested organisms are detected. Accordingly the toxicity of 103 fungal strains isolated from the tested salted and unsalted pistachio nut was studied (Table, 3). Fungal isolates were assessed for mycotoxin production using three liquid media to favor the toxic metabolites production from the tested strains, and which will then be extracted and detected using TLC technique. Yeast-Extract Sucrose (YES) (Davis et al., 1966) and Wickerham (Wick) (Raper and Thom, 1968) media were used for cultivation *Aspergillus* strains and *Alternaria* spp., and Rice medium only for *Fusarium* spp.

Table (3): Detection of "in vitro" mycotoxins production by fungi isolated from pistachio nuts.

Moulds	Number of isolates				Medium*	Mycotoxins detected
	Salted nuts		Unsalted nuts			
	Examined	Positive	Examined	Positive		
<i>A. flavus</i>	16	7	8	5	Yes, Wick	Aflatoxin B ₁ , B ₂ , G ₁ , G ₂
<i>A. niger</i>	30	2	28	3	Yes, Wick	Aflatoxin B ₁ , B ₂
<i>A. ochraceus</i>	1	1	2	--	Yes, Wick	Ochratoxin A
<i>Penicillium</i> spp.	2	--	3	--	Yes, Wick	ND
<i>Alternaria</i> spp.	2	--	5	--	Yes, Wick	ND
<i>Fusarium</i> spp.	3	1	3	2	Rice	Zearalenone

*Medium = YES: Yeast Extract Sucrose medium, Wick: Wickerham Liquid medium, Rice: Autoclaved rice medium.
ND = Not Detected

It is clear from this table that the spectrum of identified toxins from the extracts of the tested fungi include aflatoxins, ochratoxin A and zearalenone. It is evident from the present data that the both Yeast-Extract Sucrose (YES) or Wickerham (Wick) liquid media allowed consistent growth and aflatoxins production as well, from 21 (20.4 %) mycotoxigenic strains of the total isolates (103). It is also clear from the given data that concerning *Aspergillus* spp. the majority (57%) of toxigenic strains belonged to *A. flavus* which were found to be aflatoxin producers yielding the four types (B₁, B₂, G₁ and G₂). *Aspergillus niger* follows, (23.8%) producing aflatoxin B₁ and B₂, then *A. ochraceus* (4.8%) yielding ochratoxin A all in both (YES) and (Wick) liquid media. *Fusarium* spp. representing 14.3% of the total toxigenic strains produced zearalenone in Rice medium. Isolated strains of *Penicillium* spp. and/or *Alternaria* spp. did not produce mycotoxins in the YES, Wick and/or Rice media, used.

From the presented data it is obvious that both the (YES) and/or (Wick) media were suitable liquid media for the production of mycotoxins from mycotoxigenic strain and in particular reference to aflatoxins. This might be

probably attributed to that these two tested media contained the required different ingredients in concentrations needed for the good growth of the tested fungi and toxin elaboration as well. We can also, indicate from the current results the predominance of the mycotoxigenic strains of *Aspergillus flavus* producing the four types of aflatoxins. This agrees with the data of the survey study (Table 1) where the predominance of aflatoxins in pistachio nuts (kenel) was recorded.

It is obvious from the present investigation that the incidence of mycotoxigenic fungi particularly aflatoxin-producing *Aspergillus spp.* seems to be alike in both salted and unsalted pistachio nuts (kernels) showing percentages of 20.4% for each. In this concern, Jiménez *et al.* (1991) reported that the highest percentage of contamination with *A. flavus* was found in unpacked pistachio nuts (88%), although only 3 (4.5%) of 66 isolates from these samples produced aflatoxins. The same author added that, all *A. flavus* isolates were found to be aflatoxin-producing (6 strains yielded aflatoxins B₁, B₂, G₁ and G₂, 8 strains produced aflatoxin B₁, 9 strains yielded aflatoxins B₁ and B₂ and the remaining yielded aflatoxins B₁ and G₁). This is the only report available in the literature. However, fungal contamination and subsequent production of aflatoxins can occur in pistachio nuts in the field at harvest and during post harvest operations. Contamination can also be associated with storage in the country of origin and as well as the importing country ought to be considered (Boutriff, 1999).

Effect of processing on the stability of naturally contaminated pistachio nuts "kernels" with aflatoxins "phostocia":

The fate of aflatoxins during processing of contaminated cooked pistachio kernels (phostocia) is given in table (4).

As shown from this table that the initial levels of aflatoxins in phostocia were 30.45 µg B₁, 10.08 µg B₂, 29.34 µg G₁ and 9.49 µg G₂/kg. It is clear from the analysis of phostocia that the direct heating for 30 min destroyed most if not all of aflatoxins (aflatoxin B₁, B₂, G₁ and G₂). Trace amounts of aflatoxins were visually noticed on the TLC plate but can not be detected by the Densitometer. The shown destruction of aflatoxins might be attributed to the effect of direct heat and the prolonged treatment (30 min) such elevated temperature used in phostocia processing. The ingredients added (sugar, water, oil and/or citric acid) might probably effect the destruction levels of aflatoxina acheved in processed naturally contaminated pistachio kernels.

Table (4): The effect of processing on aflatoxins levels in pistachio kernels "phostocia"

Mycotoxin	Levels (µg/kg)	
	Initial	final
aflatoxin B ₁	30.45	ND*
aflatoxin B ₂	10.08	ND
aflatoxin G ₁	29.34	ND
aflatoxin G ₂	9.49	ND

*ND : Non detectable.

As phostocia is an oriental sweet, no data are available in the literature on such product. The authors compared their data with those given for other processed nuts products as peanuts and/or pecan. The current results coincide with those reported by Samarajeewa et al. (1990). They found that varying degrees of temperature is effective for aflatoxin B₁ degradation were noted in peanut and pecans products. An average of 58.79% destruction of aflatoxin B₁ in dry roasting peanut at 250-400° F for 5-30 min and 60-90% destruction of aflatoxin B₁ in pecans treated with dry roasting at 191° C for 15 min. In general, several factors – the nature of the process, the food matrix, moisture content of food stuff, additives and level of contamination – can affect experimental results on the decomposition or loss of mycotoxins during food processing (Scott, 1984).

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السموم الفطرية المنتجة من الفطريات المعزولة من الفستق المستورد في مصر
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تم عزل ٤ أجناس من الفطريات في ٤٨ عينة فستق مستوردة (٢٤ عينة فستق مملح و ٢٤ عينة فستق غير مملح) تم تجميعهم من أسواق محافظة القاهرة وهي *Aspergillus*, *Penicillium* و *Fusarium and Alternaria*. وقد أظهرت النتائج أن جنس *Aspergillus* هو الأكثر شيوعا وتواجد في العينات وكان *Aspergillus niger* هو النوع المساند يليه *Aspergillus flavus* ثم *Aspergillus ochraceus* و *Penicillium* ثم *Fusarium* ثم *Alternaria Spp.*

تم تقدير الميكوتوكسين في عينات الفستق وقد وجد أن أعلى مستوى للأفلاتوكسينات هو ب، بمستوى يتراوح ما بين ١٤,٢٥ - ٣٠,٤٥ ميكروجرام / كجم وكان النشاط المائي من ٠,٧٥ - ٠,٨٠ للفستق المملح و ١٢,٠٥ - ٢٨,٨٢ ميكروجرام أفلاتوكسين ب١ / كجم للفستق الغير مملح مع مستوى للنشاط المائي يقدر ب ٠,٦٥ - ٠,٦١ على التوالي .

أما عن الأوكراتوكسين أ ، الزيرالينون فقد وجدت في عينة واحدة من الفستق المملح عند تركيزات ٣٤,٢١ ، ٥١,٠٢ ميكروجرام / كجم ، على التوالي ، في حين أنه قد وجد الزيرالينون في ٣ عينات من الفستق الغير مملح وقد تراوح التركيز ما بين (٤٢,١٨ - ٧٤,٨٢) ميكروجرام / كجم .

تم تصنيع الفستق الملوّث طبيعيا بالأفلاتوكسينات لدراسة مدى تأثير التصنيع على تواجد هذه التوكسينات في المنتج النهائي . وقد وجد أن استخدام الحرارة المباشرة لمدة نصف ساعة تؤدي إلى التخلص من معظم إن لم يكن جميع الأفلاتوكسينات .