

## STABILITY OF AFLATOXINS IN NATURALLY CONTAMINATED PISTACHIO NUTS INCORPORATED IN SOME LOCAL SWEET DISHES OF PAKISTAN

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The possible effects of conventional Pakistani cooking practices on the integrity and stability of naturally occurring aflatoxins in a complex food matrix of sweet dishes were observed. Three conventional local sweet dishes of pistachio nuts, naturally contaminated with different levels of aflatoxins were studied. Prior to cooking there was a decrease in recovery of aflatoxins from the various types of food matrices in all the three sweet dishes. Some variations were observed from the initial contents and the recovery from the precooked contents of naturally contaminated aflatoxins of two sweet dishes. Some variation in the recovery of aflatoxins in the milk based sweet dish was also observed.

**Key words:** Aflatoxins, Pistachio, Food, Pakistani cooking.

### Introduction

Aflatoxins are well known for their potent carcinogenic, hepatotoxic, mutagenic and teratogenic activities in experimental animals with similar effects in human, based on circumstantial evidence (IARC 1987). Aflatoxin B<sub>1</sub> is one of the most potent naturally occurring carcinogen known (IARC 1987). The important factors to the disintegration of aflatoxin is the matrix, physico-chemical characteristics and additives of the food (Scott 1984). Food processing destroys the aflatoxin to some extent (Scott 1984). Various methods of cooking e.g. frying, roasting, conventional and microwave baking, etc., have shown varying degree of destruction of aflatoxins (Scott 1984; Tabata *et al* 1992; Farag *et al* 1996). Generally, roasting destroys 50% of contaminated aflatoxins (Jemmali and Lafont 1992). Normal cooking of rice destroyed about 49% of aflatoxin B<sub>1</sub> and no differences were observed between naturally contaminated rice or rice that contained added aflatoxin B<sub>1</sub> (Rehana *et al* 1979). Pressure cooking and cooking with excess water destroyed aflatoxin B<sub>1</sub> in the rice, 73% and 82%, respectively, providing further evidence of the influence of water on the instability of aflatoxins during heat processing (Rehana *et al* 1979).

Edible nuts are consumed in almost every part of the world either directly as snacks or in food. Pistachio nuts are also consumed as such for snacks as well as a nutritive food ingredient in Pakistan. It is consumed throughout the year, particularly in the winter and by women during parturition and lactation periods. In Pakistan, many food product especially sweet dishes contain pistachio nuts not only as a

decorative, but as a nutritive and active component of the recipe as well. Pistachio is one of the most vulnerable agricultural commodity to fungal infection, particularly to *Aspergillus flavus* and *A. parasiticus* and hence to aflatoxins contamination (Mojtahedi *et al* 1979; Sommer *et al* 1986; Doster and Michailides 1994). Previous studies have shown high levels of aflatoxins in locally available pistachio nuts (Ahmad *et al* 1994). If contaminated with aflatoxin, a safe food is converted to a health-threatening commodity.

### Materials and Methods

Preliminary studies have shown low levels of aflatoxins in whole pistachio nut. Broken pistachio nuts (grits) are economically suitable for local manufacturers of commercial sweetmeats, confectioneries, bakery products and ice cream. Consumers of these products are generally low income and middle class groups. The low-income group also consumes this type of pistachio nuts in homemade products.

Broken pistachio nuts were obtained from five different shops of city wholesale market. The samples were collected from the stores during the sorting process of various grades of pistachio nuts and were mixed together. Due to difficulty associated with determination of a representative sample, the following procedure was adopted. Five kilograms of the sorted and mixed pistachio nuts were ground to pass the mesh size of ASTM 40. These were then reduced to representative size through riffle sample divider and cascade sample divider to be finally used in the preparation of sweet dishes. In pre-parations of all sweet dishes, the pistachio added, was from the same lot representing the randomly divided samples.

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Chemicals and solvent used in the analysis were of analytical grades. Aflatoxins standards were purchased from Sigma Chemical Company, MO, USA. Pre-coated Thin Layer Chromatograph plates of Silica gel 60 (layer thickness 0.25mm, 20cm) on glass or aluminum, without fluorescent indicator were purchased from E Merck, Darmstadt, Germany. The 700 watts microwave oven (Frigidaire model RCM 5130 with a temperature probe) was used.

Aflatoxins were determined as described in the Methods of the AOAC (1990), Section no.26.025, except that the defatting by hexane of the acetone extract of the sample was performed. It was observed under long wavelength UV light in an enclosed viewing cabinet and quantified by visual comparison with standards of TLC plates (AOAC 1990, Section 26.031). All positive findings of aflatoxin B<sub>1</sub>, naturally present were confirmed by spraying the TLC plates with 50% sulphuric acid or making derivative with trifluoroacetic acid (AOAC 1990, Section no. 26.025).

*Preparation of sweet dishes. Pista-Pak (sweet dish).* Pista-pak was made with 75% wheat flour (25% w/w), sugar (32% w/w), vegetable cooking oil (33% w/w) and pistachio nuts (10% w/w). Sugar syrup was prepared in hot simmering water (around 95°C) to which ground pistachio (from representative samples) was added and mixed for five minutes at the same temperature. Vegetable cooking oil was separately heated to 180°C and 75% wheat flour was fried for 8 to 10 min, to a light brown colour. To this, hot sugar pistachio syrup was added with a vigorous stirring and mixing to a uniform consistency. Within a time span of 4 min, it was poured into a square pan (15cms x 15cms) to set with a layer thickness of approximately 2cm. When cooled, it was divided into 9 equally divided pieces of 25cm each. Such three preparations were made and five analyses of each were performed.

*Kheer (similar to rice pudding but of thicker consistency).* Cleaned, washed and aflatoxins-free rice (20% w/w) was boiled to cook for 10 min. Raw milk (with no aflatoxins contaminatin, 50% w/w) was separately boiled for 5 min. Cooked rice was added to the boiling milk with stirring and the ground pistachio nuts (10% w/w) and sugar (20% w/w) were added and heated for 15 min with a gentle stirring for further thickening of the content of a uniform consistency. The resulting product called Kheer was poured into a dish to settle. Arbitrarily, the content was divided into 50g portions and each was analyzed for aflatoxins. Four such preparations were made.

*Nan-Khatai (cookie-like sweet biscuit).* Seventy-five percent wheat flour (28% w/w), semolina (5% w/w) and baking powder (5% w/w) were mixed to which ground sugar (26%

w/w) and ground pistachio (10% w/w) were added through mixing. Hydrogenated oil (26% w/w) was added to the mixture and kneaded to dough of a uniform consistency. Portion of 20g each of the dough was converted to shape of a flying saucer (like a small pancake) called Nan-Khatai and was placed in the baking pan, previously greased and lined with brown paper. The pan was placed in the preheated oven (180°C) for 5 min for Nan Khatai to bake. It was cooled and analyzed for aflatoxins in a group of three each. Five such preparations were made.

Similar procedures were used for the microwave cooking at the power setting similar to that of the normal gas stove cooking, as to give the final products of Pista-Pak, Nan-Khatai and Kheer. Number of samples and preparations were same as mentioned earlier.

## Results and Discussion

*Pista-Pak.* The initial quantity of aflatoxin B<sub>1</sub> in the ground pistachio nut used was 40±1.3µg kg<sup>-1</sup> and 50±0.3µg kg<sup>-1</sup> for the gas stove process and 50±0.6µg kg<sup>-1</sup> for the microwave oven process. The recovery of quantities of aflatoxin B<sub>1</sub> were reduced to 35±2.1µg kg<sup>-1</sup> and 45±3.4µg kg<sup>-1</sup>, when mixed with the ingredients of the Pista-Pak prior to the cooking by the gas stove and to 45±1.6µg kg<sup>-1</sup> for the microwave oven, respectively. Recoveries in the aflatoxin B<sub>2</sub> were similarly reduced in the ingredients of Pista-Pak prior to the cooking in both processes. In the gas stove cooking process the exposure of aflatoxin for 5 min to a temperature of 95°C followed by further heating of 4 min at 120°C destroyed only 39.4% and 22.2% of the aflatoxin B<sub>1</sub> and 20.4% of aflatoxin B<sub>2</sub>. In the microwave cooking process 45.8% of aflatoxin B<sub>1</sub> and 52.2% of aflatoxin B<sub>2</sub> was destroyed in the Pista-Pak.

*Kheer.* In the gas stove cooking process for the preparation of Kheer, naturally contaminated aflatoxin B<sub>1</sub> in ground pistachio nut used was 40±0.5µg kg<sup>-1</sup> and 50±0.9µg kg<sup>-1</sup>, respectively and aflatoxin B<sub>2</sub> was 4±0.7µg kg<sup>-1</sup> and 5±1.5µg kg<sup>-1</sup> respectively. Whereas the initial content of aflatoxin B<sub>1</sub> was 50±1.0µg kg<sup>-1</sup> and aflatoxin B<sub>2</sub> was 6±2.3µg kg<sup>-1</sup> in the pistachio used for the microwave cooking process. The recovery of aflatoxin B<sub>1</sub> prior to the cooking of the Kheer was reduced to 25±0.7µg kg<sup>-1</sup> and 29±1.5µg kg<sup>-1</sup> for the gas stove process and 43±2.3µg kg<sup>-1</sup> for the microwave process. Likewise, recovery of the aflatoxin B<sub>2</sub> in the pre-cooked Kheer was also reduced for all the cooking process. The quantity of aflatoxin B<sub>1</sub> in the Kheer after the cooking process on the gas stove was further reduced to 11.8±0.6µg kg<sup>-1</sup> and 15.7±0.3µg kg<sup>-1</sup>, respectively. Similarly the quantity of the aflatoxin B<sub>1</sub> was reduced to 24.5±0.9µg kg<sup>-1</sup> in the



microwave oven process. The aflatoxin B<sub>1</sub> was also reduced in both the cooking processes.

*Nan Khatai.* The initial quantity of aflatoxin B<sub>1</sub> were 40±0.4µg kg<sup>-1</sup> and 50±1.0µg kg<sup>-1</sup> for the ground pista powder used in the preparation of Nan Khatai by the gas oven method and 50±0.9µg kg<sup>-1</sup> for the microwave oven method. These initial quantities of aflatoxin B<sub>1</sub> were reduced to 35±6.3µg kg<sup>-1</sup> and 47±6.1µg kg<sup>-1</sup>, respectively in the final ingredients prior to baking by gas oven. No reduction was observed in the recovery of aflatoxin B<sub>1</sub> in the precooked Nan Khatai for the microwave oven. The percent destruction of aflatoxins for gas oven method was lower than that for the microwave oven cooking process.

In this study, the extent of aflatoxin destruction in the presence of food matrix before and after cooking was determined. It revealed interesting results regarding the effects of Pakistani conventional cooking on the stability and distribution pattern of naturally contaminated aflatoxins in food. It was expected that the oil-flour-based product (pista pak) may have produced similar results to that of heated oils like palm, coconut and corn in which aflatoxins B<sub>1</sub> is moderately destroyed (Scott 1984). Somehow, the sweet dish, pista pak with over 50% oil and high temperature of 150°C with the cooking time of 10 min had not significant decrease in the aflatoxins content. This may be due to the protection provided by the matrix of the food. In the preparation of Nan-Khatai, the process involved kneading, dough making and addition of sodium bicarbonate (baking soda) as a food additive. Several authors have studied

the effect of bread making on the integrity of aflatoxins in artificially contaminated wheat (El-Banna and Scott 1983; Scott 1984) or maize flour (Seenappa and Nyagahungu 1982). Variable destruction of aflatoxins during preparation of dough (both kneading and fermentation) have been reported by Jemmali and Lafont (1972) and they also observed the transformation of aflatoxins B<sub>1</sub> to G<sub>1</sub>, but this was not confirmed by El-Banna and Scott (1983). We neither found any conversion of aflatoxin B<sub>1</sub> to aflatoxin G<sub>1</sub> nor loss of aflatoxins in the precooked Nan-Khatai. It has been reported that actual baking process caused loss up to 25% only, but these averaged 36% for Egyptian bread, which is a flattened loaf and is baked at a higher temperature (El-Banna and Scott 1983). We also found that higher amount of aflatoxin was destroyed by the microwave process as compared to gas oven baking. It has been reported that food additives of various kinds affect the stability of aflatoxins (Scott 1984; Tabata *et al* 1992) particularly that of the artificially contaminated aflatoxins. The quantity of aflatoxins in Nan-Khatai in the presence of 5% baking soda after the kneading and dough making but prior to cooking was not significantly reduced, with the initial values of 35±6.3µg kg and 47±6.1µg kg, respectively. Hence, the effect of baking powder in a complex food matrix like Nan-Khatai was not destructive on the aflatoxins as it is with the pure aflatoxins in a test tube, as reported by other workers (Tabata *et al* 1992). It has been suggested by other workers (Scott 1984; Tabata *et al* 1992; Tabata *et al* 1994) that this may be due to the loss of H<sub>2</sub>O<sub>2</sub> after reaction with food and the protective effect of food

**Table 1**  
Aflatoxins (µg kg<sup>-1</sup>) in sweet dishes prepared from naturally aflatoxin contaminated pistachio nuts by gas stove cooking process

Sweet dishes	Recovery before cooking		Recovery after cooking		% Destruction	
	AFB <sub>1</sub>	AFB <sub>2</sub>	AFB <sub>1</sub>	AFB <sub>2</sub>	AFB <sub>1</sub>	AFB <sub>2</sub>
Pista Pak (Initial Content = 40µg kg <sup>-1</sup> )	35±2.1	ND*	21.2±8.8	ND	39.4%	ND
Pista Pak (Initial Content = 50µg kg <sup>-1</sup> )	45±3.4	4.9±1.2	35.1±3.8	3.9±2.8	22.2%	20.4%
Nan Khatai (Initial Content = 40µg kg <sup>-1</sup> )	35±6.3	2.7±1.8	24.0±9.5	1.6±1.0	31.4%	40.7%
Nan Khatai (Initial Content = 50µg kg <sup>-1</sup> )	47±8.1	3.7±1.6	34.1±8.5	3.4±1.8	27.5%	8.1%
Kheer (Initial Content = 40µg kg <sup>-1</sup> )	25±0.7	2.9±1.0	11.8±0.6	1.2±0.4	52.8%	58.6%
Kheer (Initial Content = 50µg kg <sup>-1</sup> )	29±1.5	2.3±1.0	15.7±0.3	1.6±0.1	45.9%	30.4%

\*Not within detectable limits.



**Table 2**

Aflatoxins ( $\mu\text{g kg}^{-1}$ ) in sweet dishes prepared from naturally aflatoxin contaminated pistachio nuts by microwave cooking process

Sweet dishes	Recovery before cooking		Recovery after cooking		% Destruction	
	AFB <sub>1</sub>	AFB <sub>2</sub>	AFB <sub>1</sub>	AFB <sub>2</sub>	AFB <sub>1</sub>	AFB <sub>1</sub>
Pista Pak = 50 $\mu\text{g kg}^{-1}$	50 $\pm$ 5.6	6.7 $\pm$ 1.8	27.1 $\pm$ 4.3	3.2 $\pm$ 1.5	45.8%	52.2%
Nan Khatai = 50 $\mu\text{g kg}^{-1}$	50 $\pm$ 6.7	5.6 $\pm$ 1.3	16.3 $\pm$ 1.3	2.7 $\pm$ -.8	67.4%	51.8%
Kheer = 50 $\mu\text{g kg}^{-1}$	43 $\pm$ 1.3	4.9 $\pm$ 1.7	24.5 $\pm$ 0.9	4.2 $\pm$ 1.3	43.0%	14.3%

components (like starch, reducing saccharides, protein, amino acids, but not inorganic salts or non-reducing saccharides). It is interesting to note that during the cooking process the added 5% baking powder in Nan-Khatai had some destructive effects on the content of the aflatoxins. The difference was higher for Nan-Khatai cooked by the microwave oven.

The Kheer (rice pudding) has a more complex food matrix due to presence of milk in rice and also a different cooking process than that of the above two preparations. It is very interesting to note that the initial content of the aflatoxin B<sub>1</sub> prior to cooking was less than that had been expected for the added representative sample of the pistachio nuts. It showed a decrease in the aflatoxins content from 35 $\pm$  $\mu\text{g kg}^{-1}$  to 25 $\pm$  $\mu\text{g kg}^{-1}$ . This is probably due to the binding of the aflatoxins to milk-added food matrix and is made unavailable to the present detection method of analysis that is also reported by other workers (Scott 1984). Further loss was observed in the aflatoxins content of the kheer. Similar observation has been reported in which 83% of aflatoxins present in contaminated Aleppo pine nuts remained after cooking a pudding, (similar to porridge) which is widely used in Tunisia (Boutrif *et al* 1997). Nevertheless, the study shows that a considerable amount of aflatoxins is stable in these types of food processing.

According to Mohoney and Rodriguez (1996) variability was found in the production of aflatoxins in pistachio nut when

**Table 3**

Cooking and exposure time of aflatoxins in naturally contaminated pistachio nuts incorporated in various sweet dishes

Product	Type of cooking	Temperature (°C)	Exposure time (minute)
Pista Pak	Gas stove	95	5
Nan Khatai	Gas stove oven	178	5
Kheer	Gas stove	96	15
Pista Pak	Microwave oven	105	8
Nan Khatai	Microwave oven	105	4
Kheer	Microwave oven	110	7

contaminated by *A flavus*. Wounded kernels are more prone to *A flavus* contamination than the whole or hulled pistachio. Stream process of sampling to observe the distribution of aflatoxin in pistachio products indicates that 90% of aflatoxin is contained in 4.6% of low quality fraction, and consequent removal of this fraction would reduce average aflatoxin from 1.2 to 0.12 $\mu\text{g kg}^{-1}$  for all product sold for human consumption (Schatzki and Pan 1996). Similarly, higher amount of aflatoxins was found in broken pistachio as compared to the better quality of whole kernel pistachio in the present studies.

Significant outcome of this work is the finding that unnecessary hazardous exposure of traditional Pakistani foods to aflatoxins is made through addition of contaminated pistachios nuts the foods are otherwise nutritious and healthful. Preliminary studies have shown circumstantial evidence of the correlation of aflatoxin B<sub>1</sub> and Hepatitis B virus in the etiology of liver cancer in Pakistan (Zuberi 1994). Therefore, it is emphasized that there is a need for both producer and consumer to control aflatoxin contamination of food intended for human consumption.

## References

- Ahmad M A, Khan B A, Shamsuddin Z A, Khan M A 1994 Problem of aflatoxins in edible nuts in Pakistan. *The First International Conference on Impact of Food Research on New Product Development*. Proceedings: 179-183, Karachi, Pakistan.
- AOAC 1990 Official Methods of Analysis, *Association of Official Analytical Chemists*. Washington, DC, USA, Vol 2 15th ed.
- Boutrif E, Jemmali M, Pohland A E, Campbell A D 1977 Aflatoxin in Tunisian Aleppo pine nuts. *J Assoc Off Anal Chem* 60 747-748.
- Doster M A, Michailides T J 1994 Development of *Aspergillus* molds in litter from pistachio trees. *Plant Disease* 78(4) 393-397.
- El-Banna A A, Scott P M 1983 Fate of mycotoxins during processing of foodstuffs. Aflatoxin B<sub>1</sub> during making of

- Egyptian bread. *J Food Prot* **46** 301-304.
- Farag R S, Rasheed M M, Hgger A A A A 1996 Aflatoxin destruction by microwave heating. *Int J Food Sci Nutr* **47** 197-208.
- IARC 1987 Aflatoxins. In: *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals in Humans Suppl 7*, International Agency for Research on Cancer, Lyon, France, pp 83-87.
- Jemmali M, Lafont P 1972 Evolution del aflatoxin B<sub>1</sub> au cours de la panification. *Cah Nut Diet* **7** 319-322.
- Mohoney N E, Rodriguez S B 1996 Aflatoxin variability in pistachios. *Appl Environ Microbiol* **62** 1197-1202.
- Mojtahedi H, Rabie C J, Lubben A, Steyn M, Danesh D 1979 Toxic *Aspergilli* from pistachio nuts. *Mycopathologia* **67**(2) 123-127.
- Rehana F, Basappa S C, Murthy V S 1979 Destruction of aflatoxin in rice by different cooking methods. *J Food Sci Technol* **16** 111-112.
- Schatzki T F, Pan J L 1996 Distribution of aflatoxin in pistachios. 3 Process streams distribution in pistachio. *J Agri Food Chem* **44** 1076-1084.
- Scott P M 1984 Effects of food processing on mycotoxins. *J Food Prot* **47**(6) 489-499.
- Seenappa M, Nyagahungu I K 1982 Retention of aflatoxin in ugali and bread made from contaminated maize flour. *J Food Sci Technol* **19** 64-65.
- Sommer N F, Buchanan J R, Fortlage R J 1986 Relation of early splitting and tattering of pistachio nuts to aflatoxin in the orchard. *Phytopathology* **76** 692-694.
- Tabata S, Kamimura H, Ibe A, Hashimoto H, Tamura Y 1994 Degradation of aflatoxins by food additives. *J Food Prot* **7**(1) 42-47.
- Tabata Sh H, Kamimura H Ibe A, Hashimoto H, Tamura Y, Nishima T 1992 Fate of aflatoxins during cooking process and effect of food components on their stability. *J Food Hyg Sco Jpn* **33** 150-156.
- Zuberi S J 1994 Primary liver cancer in Pakistan and aflatoxin. In: *Hazards of Mycotoxins in Human and Animal Health*. Ahmad M A, Shamsuddin Z A, Khan B A (eds), Proceeding 58-61.