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INFLUENCE OF PROCESSING AND STORAGE OF PEANUT PRODUCT ON AFLATOXINS CONCENTRATION

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ABSTRACT: Peanut is considered one of the most important export crops in Egypt; however this export amount has regressed in the last few years because of pod rot infection and seed contamination with aflatoxins. Aflatoxin-containing kernels may be processed into peanut products and peanut meal. For this reason they can be a problem in processed peanut such as peanut butter. In this study aflatoxin levels in some peanut products in Egypt were estimated. Isolated fungi belong to 8 genera and 20 species, *Aspergillus niger and Aspergillus flavus* recorded higher occurrence in all three varieties, while *Aspergillus Terreus, Aspergillus tamarii, Chaetomium sp. Alternaria alternate* showed lower occurrence but *Aspergillus tamari* isolated from only Giza 6 variety and not appears in other two varieties. Pathogens map all over the main Egyptian peanut productive varieties was processed. And aflatoxin content during the manufacture steps of the positive samples was investigated. It was found that, if raw peanut contains low concentration of aflatoxin and under Codex safe level, butter may contains high concentrations of aflatoxin after storing for some while and not after processing. Aflatoxin test should be checked after production and before marketing.

INTRODUCTION

Aflatoxins are recognized as the most important mycotoxins produced by certain moulds of the genus *Aspergillus* growing on a number of raw food commodities, in particular, under high moisture conditions. Conditions of high humidity conducive to mould growth are inherent in some methods

of harvesting and drying raw food and are considered as major factors in mould contamination of these commodity **(Altenkirk *et al.*, 1974)**. Aflatoxins are highly toxic compounds and can cause both acute and chronic toxicity in humans and many other animals. Aflatoxins may be present in a wide range of food commodities, particularly cereals, oilseeds and tree nuts. It is important to recognize that, it is primary food commodities that usually become contaminated with aflatoxins by mould growth. These toxins are very stable in many foods and fairly resistant to degradation even through quite severe processes. Besides the possibility that aflatoxin-containing kernels may escape detection and be processed into peanut products and peanut meal. For this reason they can be a problem in processed foods, such as peanut butter **(Bennett & Klich, 2007)**.

Factors responsible for the high incidence of aflatoxin contamination of peanuts include poor agricultural practices during planting, harvesting, drying, transportation and storage of the product. These practices favour fungal contamination and growth, and aflatoxin production. Aflatoxins in peanut butter most often result from fungi growth on peanuts stored in warm, humid silos. The amount of aflatoxin present in peanut butter varies from brand to brand and batch to batch **(Moss, 1998)**.

Oliveira *et al.*, 2009 investigated the aflatoxin levels in peanut products traded in Brazil. Results showed that 44.2% of samples were positive for aflatoxin at levels of 0.5 to 103.8 µg/kg. The highest aflatoxin levels were observed in samples of ground peanut paste candy, unprocessed peanut, and salty dragee peanut. Peanut paste candies showed the highest number of samples positive for aflatoxin, and this fact warrants concern when considering that children are the main consumers of those products.

Nearly half of the United States peanut crop is used to make peanut butter. Americans spend almost $800 million a year on peanut butter. Peanut butter is not only found as a condiment, but is sandwiched in crackers, combined with chocolate, sold as "chips" for cookies **(Katz, 1997)**. Although nearly all peanut butter sold in the United States contains trace amounts of aflatoxin, these levels are far below the FDA's recommended safe level of 20 parts per billion. It is believed that there is

little danger from long-term exposure to low levels of aflatoxin. Low levels of aflatoxins are unavoidable, and there are precautionary measures in place to ensure that the peanut butter sold in the U.S. is safe **(Churchwell, 2011)**. Ingestion of low levels of aflatoxins over a long period has been implicated in primary liver cancer, chronic hepatitis, jaundice, cirrhosis and impaired nutrient conversion **(Bennett & Klich, 2007)**.

Peanut pod rot is a serious worldwide disease where it occurs on fruits that develop below ground. Since the flowers developed above ground to form the fruits underground, the pods are subjected to attack with numerous soil borne pathogens such as *Fusarium* spp., *Sclerotium* sp., *Rhizoctonia solani*, *Aspergillus* spp. *etc.* which causing different symptoms of pod rots. This study is aiming to process a peanut seeds and pathogens map all over the main Egyptian peanut produced varieties; estimation of aflatoxin levels in some peanut products in Egypt and investigate aflatoxin content during the manufacture steps of the positive samples.

Materials and Methods:

Eighteen samples of different products of peanut & peanut butter were collected from different markets for performing this study. Seeds of the three peanut cultivars (Giza 6, Gregorea, Valeancea) grown in Agricultural Research Center (ARC), Ismailia, Egypt (2011) have been chosen for peanut product processing investigation.

Determination of aflatoxins:

Total aflatoxins content in peanut and peanut products was determined according to **AOAC (2006)** using monoclonal antibody columns (Vicam Science Technology, Watertown, MA.USA).

Aflatoxin identification was performed by a modification of HPLC – Af latest procedure (Agillent 1200 equipment with Florence detector and c_{18}, Lichrospler 100 RP-18, 5 µm x 25cm). The mobile phase consisted with water : methanol : acetonitrile (54:29:17, V/V/V) at flow rate of 1ml/min. The excitation and emission wavelengths of all aflatoxins were 362 and 460 nm. Standard for aflatoxins were obtained from Sigma Chemical Co. (St.

Louis, MO, USA). Samples have been analyzed in duplicate and the mean was calculated.

Isolation and identification of fungi associated with peanut seeds:

Peanut seeds were disinfected by immersing in 5% sodium hypochlorite solution for 3 minutes-washed thoroughly in three changes of sterilized water and dried between sterilized filter paper. Seeds were aseptically transferred to ready plates of potato dextrose agar (P. D. A) (**Christensen, 1957**). Plates were then incubated at 25°C. observations were daily recorded up to the 7 day. The emerged fungi were counted, isolated on P. D. A. plates and purified using the single spore technique and/or the hyphal tip technique (**Esia *et al.*, 1996**). Blotter paper technique (BPT) applied according to **Ito *et al.* (1992).** Identification of the associated fungi was carried out in Plant Pathology Institute, Agricultural Research Center.

Processing of peanut butter:

After roasting peanut in a 350° F oven for 7 minutes, peanuts were poured into blender for chopping. The blender was stop periodically to scrape the peanut butter from around the blades until all the peanuts have been ground into peanut butter. Add, if necessary; oil was added to help the grinding process (**Rubenstein & Dumbledor, 2012**). Each one of peanut butter has been stored after processing for while, at both room temperature (25° ± 3° C) and at refrigerator temperature (6-8° C)

Results and Discussion:

The mean results for aflatoxin content in different samples of peanut products & peanut butter are shown in Table (1). Peanut products have shown negative aflatoxin content and on other hand peanut butter samples present positive aflatoxin content with relatively high concentration, much more than 10 ppb. Whereas, according to Codex Committee, the maximum levels for total aflatoxins are 15 µg/kg for raw peanuts and 10 µg/kg for processed peanuts (**Codex, 1995**). The negative aflatoxin content results of some samples causing to the little peanut content in the product. Whereas, these products contains about (3- 4g) of peanut and the total weight of product is about (40- 50g). So, peanut content presents only about (8-10%)

of the product. Therefore, even if the used peanut contains aflatoxin, the whole product would contain negligible amount of aflatoxin. While in peanut butter, peanut present almost the whole product. This agrees with **Oliveira *et al.*, 2009** who investigated the aflatoxin levels in peanut products traded in Brazil. Whereas, Peanut paste candies showed the highest number of samples positive for aflatoxin.

Table (1): Total aflatoxin content in peanut products & peanut butter samples

Sample	Total aflatoxin content (ppb)	± SD
Candy chocolate with peanut (1)	-ve*	-
Candy chocolate with peanut (2)	-ve*	-
hocolate with peanut (3)	-ve*	-
Chocolate with peanut (1)	-ve*	-
Chocolate with peanut (2)	-ve*	-
Chocolate with peanut (3)	-ve*	-
Halva with peanut (1)	-ve*	-
Halva with peanut (2)	-ve*	-
Nougat with peanut (1)	-ve*	-
Nougat with peanut (2)	-ve*	-
Peanut butter (1)	33	0.04
Peanut butter (2)	60	0.05
Peanut butter (3)	49	0.04
Peanut butter (4)	14	0.03
Peanut butter (5)	42	0.04
Peanut butter (6)	57	0.05
Peanut butter (7)	28	0.04
Peanut butter (8)	17	0.03

* : Free aflatoxins

Table (2): Occurrence and frequency isolated fungi from peanut seeds

Isolated fungi	BPT			PDA		
	Giza 6	Gregorea	Valencea	Giza 6	Gregorea	Valencea
Aspergillus niger	26	20	22	40	28	28
A. ochraceous	5	8	10	18	12	15
A. flavus	25	10	12	35	15	32
A.fumigatus	6	0	0	3	0	0
A. Terreus	0	0	0	10	0	0
A.nidulans	2	0	0	5	0	0
A.tamarii	3	0	0	1	0	0
A.versicolor	2	8	10	7	15	12
A.ssp	10	6	6	2	4	2
Fusarium rosum	0	1.0	2	5	5	0
F.chlamydosporium	0	0	0	8	0	0
F.oxysporum	8	2	3	2	10	8
F.semitectum	3	0	0	1	0	0
F.solani	5	6	0	0	10	2
F.spp	0	0	2	3	2	7
Penicillium puberulum	3	0	0	2	0	0
Chaetomium sp.	2	0	0	0	0	8
Rhizoctonia solani	0	0	0	2	8	0
Stemphylium sp.	7	0	0	0	0	0
Rhizopus sp.	8	0	6	2	0	10
Alternaria alternata	2	0	2	9	6	12
Total.	117	61	75	155	115	136

Fungi associated with peanut seeds:

Results of occurrence and frequency of the associated fungi are presented in Table (2). Identification trials showed that, the isolated fungi belong to 8 genera and more than 20 species. *Aspergillus niger and Aspergillus flavus* showed the higher occurrence in all three varieties, but *Aspergillus Terreus, Aspergillus tamarii, Chaetomium sp. Alternaria alternate* were showed lower occurrence and frequency of the associated fungi in the same three varieties. Generally, in this study, the most dominant fungi associated with peanut seeds could be arranged descendingly, according to the occurrence, as follows: *Aspergillus niger, Aspergillus flavus* , *Aspergillus* ssp., *F. oxysporum* ,by used blotter paper technique (BPT) in variety Giza 6, also arranged descentingly for using varieties as follow Giza 6, Valencea and Gregorea by using two methods blotter paper technique and potato dextrose agar

Aflatoxin in peanut butter:

The total aflatoxin concentrations of the three peanut cultivars were –ve, 4, 8 ppb for Gregorea, Valeancea and Giza 6 respectively. These levels were below the Codex recommended safe level. These three peanut cultivars have been used individually for peanut butter processing. Each one of peanut butter has been analyzed after processing and after storing for while, at both room temperature ($25^{\circ} \pm 3^{\circ}$ C) and at refrigerator temperature (6-8° C), once a month.

1 Kg of peanut gave about 450 gm of butter, so aflatoxin level may increase in butter than in raw peanut. Roasting alone did not provide sufficient control of aflatoxins in some cases in particular when temperature and time combination during roasting are not adequate **(Bankole *et al.*, 2004)**. Besides, studies in Brazil involving dry roasting of peanut in the laboratory and industry exerted negligible reduction of aflatoxins by roasting (**Siwela *et al.*, 2011)**.

Table (3): Aflatoxin content (ppb) in peanut butter processed by the three peanut cultivars during storage for 3 months

Storage	Gregorea peanut		Valeancea peanut		Giza 6 peanut	
	At room temperature	At 6-8°	At room temperature	At 6-8°	At room temperature	At 6-8°
one day	-ve	-ve	5	5	8	8
one month	-ve	-ve	7	6	11	10
two months	-ve	-ve	9	7	14	11
three months	-ve	-ve	10	9	17	13

Aflatoxins content in butter after processing and after storing at both temperatures are shown in Table (3). It was noticed that, aflatoxins content in butter which were made from Valeancea (4 ppb) and Giza 6 (8 ppb) peanut increased after storing at both temperatures. It was found that, aflatoxin content in butter stored in cool place was increasing in rate less than that stored at room temperature. Aflatoxin reached 10 ppb level, Codex safe level for processed peanuts, for Giza 6 peanut after one month at both temperatures. While for Valeancea, aflatoxin reached this level after three months at room temperature.

On the other hand, aflatoxin in Gregorea still –ve in butter after processing and after storing among the three months. Then, even at low concentration of aflatoxin in raw peanut, butter may contain high concentrations of aflatoxin after storing for some while.

CONCLUSION:

Some peanut product such as peanut butter may be a health risk for containing some levels of aflatoxin. There is danger from long-term exposure (even low levels) to aflatoxin, especially among children. To avoid this, the peanut used in peanut butter manufacture should be absolutely free of aflatoxin. The finished product should be stored under controlled conditions, i.e., below 5° and less than 70% humidity. Aflatoxin test should be checked after production, before and after marketing periodically.

Consumer must keep peanut butter in refrigerator for preventing as possible any fungus from multiplying.

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تأثير تصنيع وتخزين منتجات الفول السوداني على تركيز الأفلاتوكسين بها

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الملخص العربي

يعتبر الفول السوداني واحد من أهم المحاصيل التي يتم تصديرها في مصر ومع ذلك فان نسبة التصدير تراجعت في السنوات الأخيرة بسبب إصابة القرون بالعفن والبذور بالأفلاتوكسين. البذور التي تحتوي على الأفلاتوكسين يمكن أن تنقل الأفلاتوكسين الي منتجات الفول السوداني، لذلك فهذا يمكن أن يمثل مشكلة في الفول السوداني المصنع مثل زبدة الفول السوداني . في هذه الدراسة تم تقدير مستويات الأفلاتوكسين في بعض منتجات الفول السوداني في مصر .

تم عزل وتعريف الفطريات الملوثة للبذور وكانت كالتالى: العزلات تنتمي إلى 8 أجناس و21 نوع من الفطريات وهي: أسبرجلس نيجر وأسبرجلس فلافس الذى سجل أعلى تواجدا فى كل الاصناف المختبرة وباقى الفطريات مثل أسبرجلس تيريس وكيتوميم والترناريا النرناتا سجلت أقل تواجدا فى طريقتى العزل وأيضا البيئتين المستخدمتين ولم يظهر فطر أسبرجلس تمارا الا في صنف جيزة 6 .

كما تم دراسة محتوى الأفلاتوكسين في العينات الموجبة أثناء عملية تصنيعها ، ووجد أنه اذا كان الفول السوداني الخام يحتوي على تركيز قليل من الافلاتوكسين حتى ولو كان أقل من الحد الآمن للكوديكس فإن الزبدة المصنعة منه يمكن أن تحتوي على نسبة عالية من الأفلاتوكسين بعد تخزينها لبعض الوقت، لذلك يجب العناية فحص مستوى الأفلاتوكسين قبل تصنيعها فورا ، لذلك يجب العناية فحص مستوى الأفلاتوكسين قبل تصنيعها