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OCCURRENCE OF MYCOBIOTA AND AFLATOXIN M_1 IN SOME DRIED DAIRY PRODUCTS

(With 4 Tables)

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تواجد الفطريات والأفلاتوكسين م1 في بعض منتجات الألبان الجافة

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أجريت هذه الدراسة على مائة وعشرة عينة عشوائية من منتجات الألبان الجافة بواقع 50 عينة من اللبن الجاف جمعت من محلات البقالة والسوبر ماركت وثلاثون عينة من كل من الكازين الجاف والشرش الجاف جمعت من المصانع الكبيرة في مدينة الإسكندرية. وأجريت هذه الدراسة لمعرفة مدى تواجد الفطريات والأفلاتوكسين م1 في العينات المفحوصة. وقد تبين من الفحص أن 40 ، 36.67 ، 43.33 % من عينات اللبن الجاف والكازين الجاف والشرش الجاف ملوثة بالفطريات بمتوسط قدره $2.2 X10^3$ ، $4.2 X10^2$ ، $2.1 X10^2$ لكل جرام على التوالي. وقد تبين عند عزل وتصنيف الفطريات المعزولة أن فطر الأسبرجلس هو الأكثر عزلاً يليه فطر البنسيليوم. وعند قياس قدرة العترات المعزولة من فطر الأسبرجلس فلافس على إفراز الأفلاتوكسين ب1 وجد أن خمس عترات لها القدرة على إفراز الأفلاتوكسين ب1 بتركيزات مختلفة . وقد تبين عند الكشف ع لى الأفلاتوكسين م1 باستخدام الإليزا فـي العينات المفحوصة وجوده بنسب وتركيزات مختلفة وعند مقارنتها بالمواصفات القياسية المصرية (1990) تبين أن كل العينات الإيجابية تخطت الح دود المسموح بها. وقد تم مناقشة الخطورة الصحية والأهمية الاقتصادية للفطريات المعزولة والأفلاتوكسين م1.

SUMMARY

A total of 110 samples of dried dairy products represented as full cream milk powder (50 sachets) were randomly collected from groceries & supermarkets and dried casein (30) and dried whey (30) in sterile polyethylene bags from large factories in Alexandria Governorate. All samples were still valid for consumption as shelf -life is at least to be one year from production time. The collected samples were transmitted to the laboratory for preparation. The samples were examined to determine the levels of fungal contamination and the possible presence of aflatoxin M_1. The obtained data pointed out that 40, 36.67 and 43.33% of the examined samples of milk powder, dried casein and dried whey were contaminated with molds with average counts of 2.2 X10^3, 4.2 X 10^2, and 2.1 X 10^2 /g, respectively. Aspergillus was the prevalent genus encountered in the examined samples followed by genus Penicillium. 5 out of 14 isolated strains of *A. flavus* (35.71%) proved to be aflatoxin B_1 (AFB_1) producers with different concentrations. Aflatoxins M_1 could be detected in the examined milk powder, dried casein and dried whey samples, using ELISA, with concentration levels of 0.07-0.6, 0.02-0.06 and 0.03-0.08 µg|kg, respectively. All positive samples were not complied with Egyptian Standards (1990) which stated that milk and milk products should be free from any aflatoxins. So, efforts have to be made to prevent mold growth and aflatoxins production along the entire food chain specially food related to children.

Key words: *Milk powder, dried casein, dried whey, mycobiota and aflatoxin.*

INTRODUCTION

Milk and dairy products have made a major contribution to the human diet nearly all countries allover the world. The drying of milk has become increasingly significant as the level of production within the world increased. Drying extends the shelf life of the milk, simultaneously reducing the weight and the volume, and consequently lowers the cost of transporting and storing of the product (Ranken and Baker, 1999).

The quality of milk powder depends not only on the condition of the raw milk, but also on the changes which may take place during manufacture, storage and distribution. Milk powder may be subjected to contamination with different species of molds, as they are widely distributed in nature as environmental contaminates of air, water, dust, etc.

Nowadays, the growing use of the dried milk has made its microbial quality of primary concern due to the high susceptibility of consumers to food – borne diseases. These microorganisms gain entrance to the milk powder either from the used milk, air contamination or utensils during stages of production, processing, transport or storage (Cross, 1997). Moreover, growth of commonly occurring mycobiota in food may result in production of mycotoxins, which can cause a variety of ill effects in human, from allergic responses to immunosuppression and cancer.

Unfortunately, the absence of visual fungal growth does not mean that they are not present but their numbers may be low or their growth may be internal. By growth and metabolic activity, these microorganisms may cause lipolytic and proteolytic spoilage as discoloration and off-flavor of foods *(*Besancon *et al.,* 1992, Jakobson and Narvuhus, 1996).

Aflatoxin M1 (AFM_1) is an important mycotoxin frequently found in milk and dairy products. Milk products may be contaminated by aflatoxin M1 when dairy cattle have fed with Aflatoxin B1-contaminated feeds (Pietri *et al.,* 1997). AFM_1 is a hydroxylated metabolite of aflatoxin B_1, toxic metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus,* its parent molecule has been categorized as Class 1 human carcinogen (IARC, 1993). The presence of AFM_1 in milk and milk products is considered undesirable due to toxic and carcinogenic properties (Prado *et al.,* 2000).

The rising cost of skim milk powder leads to the increased demand for whey protein as a substitute. Whey protein products could be used in improvement of bakery quality and as egg white replacer in confectionary and nutritional enhancer in dairy products. While, casein powder could be used in cheese as protein base; yoghurt as stabilizer; cheese spreads as flavor enhancer and emulsifier, and confectionary as texture improver (Varnam and Sutherland, 2006).

The magnitude of the risk that the presence of some mold species and their metabolites in dried dairy products may pose to their health. So, this study was carried out to determine the levels of fungal contamination and the possible presence of aflatoxin M_1 in such products.

MATERIALS and METHODS

1. Collection of samples:

A total of 110 samples of dried dairy products represented as full cream milk powder (50 sachets) were randomly collected from groceries and supermarkets while, dried casein (30) and dried whey (30) in sterile polyethylene bags from large factories in Alexandria Governorate. All samples were still valid for consumption as shelf -life is at least to be one year from production time. The collected samples were transmitted to the laboratory for preparation and examination.

2. Preparation of samples (APHA, 1985):

Eleven grams from the thoroughly mixed sachet and polyethylene bags contents were transferred to a sterile beaker containing 99 ml of sterile 0.1% peptone water to make a dilution of 1: 10 from which 10-fold serial dilutions were prepared for mycological examination.

3. Mycobiota count (Mislivec *et al.*, 1992).

4. Identification of molds according to Samson *et al.* (1995).

5. Screening of *Aspergillus flavus* Link isolated from dried dairy products for aflatoxins B1 production (Munimbazi and Bullerman, 1996).

6. Determination of aflatoxin M1 by direct Enzyme Linked Immuno-Sorbent Assay method (ELISA) according to Riedel De-Haen (1997).

7. Calculation of extrapolated values of AFB_1 concentration in feeds:

The values of AFB_1 in cattle feeds were extrapolated from back calculation of the values of AFM_1 obtained from analysis of milk powder samples. The calculation was based on the assumption that only 1.6% of ingested AFB_1 is converted to AFM_1 by dairy cattle. The formula used for calculation was AFB_1 (µg|kg) = AFM_1 x 100 /1.6 (Forbisch *et al.,* 1986; Rastogi *et al.,* 2004).

RESULTS

Table 1: Statistical analytical results of mycobiota recovered from the examined samples of dried dairy products.

Dried products	No. of examined samples	Positive samples		Counts / gram		
		No.	%	Minimum	Maximum	Average
Milk powder	50	20	40	1.2 X10^3	3.1 X10^4	2.2 X10^3
Dried casein	30	11	36.67	1.1 X 10^2	2.1 X 10^3	4.2 X 10^2
Dried whey	30	13	43.33	0.9 X 10^2	1.0 X 10^3	2.1 X 10^2

Table 2: Incidence of different types of mycobiota recovered from the examined samples of dried dairy products.

Isolated mycobiota	Milk powder		Dried casein		Dried whey	
	No./50	%	No./30	%	No./30	%
• Alternaria alternaria Fr. Keissler	3	6	4	13.33	2	6.67
• Aspergillus species:						
A.flavus Link	10	20	6	20	10	33.33
A. fumigatus Fresenius	5	10	3	10	6	20
A. niger Van Tieghem	2	4	1	3.33	2	6.67
• Cladosporium cladosporoides (fres)Veries	3	6	2	6.67	2	6.67
• Mucor spp	2	4	-	-	1	3.33
• Penicillium spp:	3	6	2	6.67	1	3.33
P. chrysogenum Thom	6	12	3	10	4	13.33
P. cyclopium Thom	3	6	1	3.33	1	3.33
P. funiculosum Thom	1	2	2	6.67	3	10
• Rhizopus spp	2	4	-	-	-	-
	3	6	2	6.67	2	6.67
Total	27	54	17	56.67	20	66.67

Table 3: Determination of aflatoxin B_1 (ppb) produced by *Aspergillus flavus* Link isolated from samples of dried dairy products.

Dried products	No. of isolated strains	Toxigenic strains		Counts of Aflatoxin B_1 /ppb		
		No.	%	Min.	Max.	Mean ± SEM
Milk powder	5	2	40	13	26	19.50 ± 4.04
Dried casein	3	1	33.33	10	10	10 ± 0.0
Dried whey	6	2	33.33	14	27	20.50 ± 3.12

Table 4: Determination of aflatoxin M_1 in the examined samples of dried dairy products using ELISA.

Dried products	Positive samples of Aflatoxin M_1					Extrapolated values of AFB_1 (µg|kg)
	Above PL*		Within PL*		Concentration range of AFM_1 (µg|kg)	
	No.	%	No.	%		
Milk powder	5	10	0	0	0.07-0.6	4.38-37.50
Dried casein	3	10	0	0	0.02-0.06	1.25-3.75
Dried whey	2	6.67	0	0	0.03-0.08	1.88- 5.00

*PL = Permissible limit of AFM1 = 0 µg|kg (ES, 1990)

DISCUSSION

The presence of mycobiota in dairy products is objectionable as they grow at a wide range of temperature and pH values resulting in spoilage of the products. They may reach the products from different sources as well as unhygienic methods of manufactures.

The data presented in Table 1 pointed out that 40, 36.67 and 43.33% of the examined samples of milk powder, dried casein and dried whey were contaminated with molds with average counts of 2.2 X10^3, 4.2 X 10^2 and 2.1 X 10^2 /g, respectively. These findings substantiated what has been reported by Ismail and Saad (1995) and Abdel-Mohsen and El-Prince (2002), while higher counts were recorded by Abdel-Hakiem (1996) in the examined samples of milk powder.

The contamination of milk powder by fungi could be resulted from different sources including the milk used, air, water and equipment as well as through persons taking part in manufacturing or handling the product. The invading organisms may find the opportunity to grow and multiply in the product inducing undesirable changes, rendering the product unmarketable. Likewise, some species of isolated mycobiota (Penicillium) have a lipolytic activity and were found to be causative agents of rancidity in full cream milk powder.

The microbiology of milk protein products has received relatively little attention, although the hygiene problems associated with membrane techniques are well known. To control these problems, heat treatment of the starting materials at a level equivalent to pasteurization, prevention of the growth of microorganisms during processing stages such as evaporation, reverse osmosis, and prevention of re-contamination either the products steam or the end products. The increasing sophistication of processing potentially creates more opportunities for microbial contamination or growth especially where the product is intended for incorporation in infant's foods.

The growth of microorganisms during production of high protein whey powder by ultrafilteration at 50^{o}C may lead to not only high count in the finished powder, but also an adverse effect on functionability and off flavor during end use in ice cream processing (Varnam and Sutherland, 2006).

Inspection of results in Table 2 declared that various species of mycobiota were recovered from the examined dried dairy products samples. Aspergillus was the prevalent genus encountered in the examined samples followed by genus Penicillium. Higher percentage of *Aspergillus flavus* was recorded in milk powder samples examined by Abdel-Hakiem (1996) and Abdel-Mohsen and El-Prince (2002). This could be attributed to the fact that *A.flavus* is one of the common contaminants in foods and is soil organisms. Also, it is adapted to warmer environment such as tropical and subtropical regions (Dorner *et al.,* 1989). Furthermore, it was noted that many of the encountered molds are known to be mycotoxins-producers, notably *Aspergillus flavus* (Aflatoxins), *A.niger* (nigragillin), *Penicillium* species (citrinin and patulin) and *Alternaria alternaria* (Tenuazonic acid) which were implicated in human cases of food poisoning and liver cancer.

The results recorded in Table 3 revealed that 5 out of 14 isolated strains of *A. flavus* (35.71%) proved to be aflatoxin B_1 (AFB_1) producers. 2 out of 5 strains (40%) of *A. flavus* were isolated from milk powder samples with a mean AFB_1 concentration of 19.50 ± 4.04 ppb, 1 out of 3 strains (33.33%) were isolated from dried casein samples with a mean AFB_1 concentration of 10.0 ± 0.0 ppb, and 2 out of 6 strains of *A. flavus* Link were isolated from dried whey samples with a mean AFB_1 concentration of 20.50 ± 3.12 ppb.

The presence of *A. flavus* Link in dairy products does not automatically imply the presence of aflatoxins in these products as these strains are non-toxigenic strains. Growth of the fungus may not actively have occurred following contamination by spore, and growth may be at too early stage for toxin production to have started. Conversely, aflatoxin may be detected without being possible to isolate the mold. This may be due to the growth of fungus for a long period followed by disappearance after the production of toxin. The food may be treated by a process which kills the fungus without destroying the toxin or there is even a possibility that molds other than *A. flavus* were responsible for aflatoxin production.

Results recorded in Table 4 revealed that aflatoxin M_1 could be detected in the examined milk powder, dried casein and dried whey samples using ELISA with concentration levels of 0.07-0.6, 0.02-0.06 and 0.03-0.08 µg|kg, respectively. All positive samples of AFM_1 were not complied with Egyptian Standards (1990) which stated that milk and milk products should be free from any aflatoxins.

Several investigators (Saad and Zaky; 1995; Abdel-Hakiem, 1996; Aman, 1998) detected AFM1 in milk powder samples. Milk powder in Egypt is imported mostly from European countries which implement a strict maximum tolerance level of AFM_1 in milk (50 ppb). These obtained concentrations were very toxic to infants if compared with the permissible limits recorded by FDA as 0.5 ppb for liquid milk products due to its high risk to infants and young children and 20 ppb for other foods (George, 1989).

Aflatoxins are the most important mycotoxins, produced by toxigenic strains of *A. flavus* Link during its growth on dairy products under certain conditions of temperature and relative humidity (Kisza and Domagala, 1994).

Aspergillus flavus Link is a pathogenic fungus produces aflatoxin that renders food toxic to the consumer. Although *A. flavus* has a wide distribution range, various factors restrict the contamination with aflatoxins. The most important factors are moisture content of substrate, relative humidity and temperature which affect production of aflatoxin. The optimal growth of *A. flavus* occurs at 36 to 37 °C, while the maximum aflatoxin production occurs at 25 to 29 °C (Cross, 1997).

Aflatoxins are potent hepatoxins and carcinogens, their effects vary with dose, duration of exposure and nutritional status. The clinical signs of acute aflatoxicosis represented by lack of appetite, weight loss, jaundice, neurological abnormalities, ascitis and oedema of the lower extremities. Mortality was high and death occurred suddenly as a result of massive gastrointestinal hemorrhage (Hendrickse, 1997).

Therefore, prevalence of mycobiota in milk powder is indicative of unhygienic measures adopted during processing and handling of the products. So, efforts should be made to prevent mold growth and aflatoxins production along the entire food chain specially food related to children.

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