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DETECTION OF MOULDS AND MYCOTOXINS IN SOME COMMON MANUFACTURED INFANT FOODS IN EGYPTIAN MARKETS

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ABSTRACT

One hundred and thirty five samples of certain manufactured infant foods were collected form five Governorates of Cairo, Giza, Alexandria, Monofia and Fayiom. Samples were examined for the natural occurrence of moulds and aflatoxins to determine their hygienic conditions. Some isolated strains of different types of moulds were examined for their ability to produce mycotoxins. Eighty four percent of examined samples contained different types of moulds such as *Aspergillus sydwii, A. niger, A. flavus, A. furmigatus, A. versicolor, A. candidus ,Fusarium rosum, F. compartum, F. oxysporum, F. chlamydosporium, Pencillium digitatum, P. funiculosum and Alternaria* sp., while only 2% were positive for the presence of aflatoxin B1. Different types of mycotoxins were produced by 50 % of grown isolated moulds. These results demonstrate the need of applying quality control techniques in the manufacture of infant foods.
Keywords: infant foods, mycotoxins and fungi.

INTRODUCTION

After 4 to 6 months of age, the quantity of breast feeding milk supplied by the mothers is insufficient to complete the requirements of the growing infants. Special foods are rarely available for these children. In Egypt, malnutrition was noticed during infancy and childhood as a result of insufficient supplies of required good healthy nutrition meet the relatively rapid growth of infants at this age. The conditions of infant foods production (i.e. cultivation, processes and storage) may lead to the contamination with many different types of fungi which produce mycotoxins. These metabolites show toxic and carcinogenic properties (Mourean, 1971). There are more than 300 known mycotoxins produced by different moulds (Bhata and Vasanthi, 1999). In Egypt, as in many areas of the world, the production of milk is not sufficient to supply the needs of the population, for this reason many efforts had been carried out to produce protein rich food mixtures suitable for infants with addition of some cereals (wheat, rice, oat, barley, sorghum and soybeans), fruits, vegetables, and other sources alone or mixed (Emam, 1996).

Penicillium spp., *Cladosporium* spp., *Aspergillus monilia* and *Alternaria* spp. were isolated form 50 samples of infant foods (Cutuli and Saurez,1983). In studies on 85 samples of dried formulas for infants, moulds were isolated from 47 samples; aflatoxinB1 was detected in 24 smaples (Trigo *et al.*, 1981).

Milk used in the manufacture of products for infants in Istriskii (Russia) was found to be contaminated by moulds with average counts of $1.2x10^2$ to $1.2x10^3$cfu/ml (Shamanova *et al.*, 1992).

High levels of fumonisin (12.2-75.2 µg/g) were detected in all maize samples used in baby foods in Burundi (Munimbazi and Bullerman, 1996).

The purpose of the present study was to evaluate the hygienic practices of the occurrence of fungi in infant foods including determination of fungal counts and identification of isolated fungi as well as the presence of mycotoxins. The effect of various storage conditions, that may occur at home before using these foods on the ability of isolated fungi to produce mycotoxins was studied as well.

MATERIALS AND METHODS

The present work was carried out in the Central Laboratory for Food and Feed, Agric. Res. Center ,Giza .

Samples collection

One hundred and thirty five samples of commercial infant formulas were collected from different markets (pharmacies) in Egypt (Cairo, Giza , Alexandria, Monofia and Fayioum)for detection the presence of fungi and aflatoxins content (B_1,B_2, G_1 and G_2).The collected samples were representative of two leading brands :

1-***Powdered infant formulas*** : powder milk and cereals, dried vegetables and fruits in cartoon boxes.

2-***Ready to feed infant formulas***: mixtures of vegetables, fruits and vegetables with beef or turkey in glass jars.

Care was taken to ensure that contamination of infant formulas from the outside did not occur by swabbing of the outer package with 70% alcohol.

Samples were divided into four groups as follow: (1) mixed vegetables, (2) mixed fruits, (3) rice, (4) honey, wheat and milk.

Determination of total counts of fungi

Total counts of fungi were determined on potato dextrose agar (PDA) medium **(Christenssen, 1957)**. Plates were incubated at 25°C for 7 days and the counts of fungi (cfu/g) were determined as described in American Public Health, Association (1981) and Oxoid Manual(2000).

Isolation and identification of fungi

Developed colonies on PDA medium were transferred to PDA slants and purified using the single spore technique (Hansen, 1926) and/or hyphal tip technique (Riker and Riker, 1936). Purified isolates were identified according to their morphological and microscopical characters as described by Jenes *et al*. (1991) and confirmed by Dept., Plant Pathology Institute ARC, Egypt.

Mycotoxins analysis

All standards of mycotoxins were purchased from Sigma company, USA. All chemicals and solvents used were of ACS grade. Thin layer chromatography (TLC) was performed using 20 x 20 cm TLC alumonium plates recoated with 0.25 mm silica gel 60 (Merk). Aflatoxins, zearalenon, and fumonisin were extracted by B.F. method as described in AOAC (1998). Extracts were dissolved in 200µL chloroform and vortexed, 20µL aliquot and 10µL of the standards were spoted on TLC plates and developed in dark room with ethyl ether: methanol: water (96:3:1). After drying, the spots were examined with u.v at a wave length of 365 nm (AOAC 1998).

Efficiency of isolated fungi to produce the mycotoxins

All isolated strains were studied for theire efficiency to produce the myocotoxins such as aflatoxins (B_1,B_2,G_1 and G_2), zearalenone and fumonisins according to Jens *et al.* (1991). The amounts of the mycotoxins were determined as described by AOAC (1998).

RESULTS AND DISCUSSION

Samples of baby foods were purchased during 2005. Samples were representative of many factories in Egypt. The occurrence of isolated fungi strains varied from one variety to another as shown in Tables (1a,b,c and d). The highest number of isolated fungi was obtained from mixed vegetables recording total counts of $11x10^3$ cfu/g. Isolated fungi were identified as *Aspergillus sydowii, Aspergillus* Spp. and *Fusarium chlamydosporum*. The determined total counts of fungi in the mixed fruits was 10 $x10^3$ cfu/g. In this group of fruits, isolated fungi were identified as *Altemaria* sp, *A. fumigatus, A. candidus, A. niger, Fusarium rosum, F. compactum, Fusarium* Spp. *Penicillium funiculosum, and P. digitatum*. In the rice group, (Flasks rice with vitamins) the fungal counts were $8x10^3$ cfu/g with *A. flavus, A. sydowii, A. versicolor, A.niger, F. oxysporum, F. chlamydosporum* and *P. citrium* being the most frequently isolated species.

Table (1-A): Isolated fungi from mixed vegetable group as well as total counts of fungi (cfu/g) and total aflatoxins content (µg/kg).

Test Samples	Isolated fungi	Total counts of fungi (x 10^2)	Total aflatoxin content (µg/kg)
Mixed vegetables	*Alternaria tenuis*, sp., *Alternaria* sp., *Aspergillus sydowii*, *Fusarium* spp.	200	0.0
Mixed vegetables	*Fusarium chlamydosporum*, *Aspergillus sydowii*, *A.*spp	110	1.0
Mixed vegetable	*Aspergillus flavus*, *Aspergillus niger*, *Aspergillus sydowii*	50	16.0
Vegetables and chicken	*Aspergillus niger* spp. *Penicillium citreonigrum*	1.0	0.0
Vegetables and chicken	-	0.0	0.0
Rice and milk	*Asperiglius versicolor*	1.1	0.0
Rice, milk with vegetables	*Aspergillus sydowii*, *Aspergillus versicolor*, *Aspergillus* spp.,*F. oxysporum*	1.2	1.0
Rice and milk with vegetable	*Aspergillu sydowii*, *Fusarium oxysporum*, *Pencillium* spp.	1.5	0.0
Rice and milk with vegetable	*Aspergillus* spp.	2.0	0.0
Vegetables, wheat with milk	*Aspergillus fumigatus*, *Aspergillus niger*, *penicillium atramentosum*	2.0	0.0
Vegetables, wheat with milk	*Aspergillus candidus*, *Aspergilus flavus*, *Aspergillum spp.*, *penicillium*, *Atrmentosum*, *P.digitatum*,*P. funiculosum*	30.0	2.0
Vegetables wheat with milk	*Fusarium spp.*, *penicillium digitatum*, *P.* spp., *Rhizopus nigercons*	10.0	1.0

Table (1-B): Isolated fungi from mixed fruits group as well as total counts of fungi (cfu/g) and total aflatoxins content (µg/kg).

Test Samples	Isolated fungi	Total counts of fungi (x 10^2)	Total aflatoxin content (µg/kg)
Fruit dessert	*Alternaria* spp., *Aspergillus sydowii, Fusarium* spp.	10 .0	0.0
Cocktail fruit	*Alternaria* spp., *Aspergillus niger, Aspergillus sydowii, Fusarium* spp.	5.0	2.0
Fruits, wheat with milk	*Aletemaria* spp., *Aspergillus fumigatus, Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., *Rhizopus nigercons*	2 .0	2.0
Fruits, wheat with milk	*Aspergillus candidus, Aspergillus niger, Eurotium repens, Rhizopus nigercons*	2 .0	0.0
Fruits wheat with milk	*Alternaria* sp, *Aspergillus* spp., *Fusarium resum, Fusarium* spp., *penicillium funiculosum, penicillium digitatum, Rhizopus nigrcons*	10 0.0	0.0
Fruits wheat with milk	*Aspergillus* spp., *Penicillium digitatum. Penicillium funiculosum*	10 .0	0.0
Apple sauce	*Aspergillus niger, Aspergillus sydowii, Fusarium chlamydosporum, Penicillium digitatum*	30.0	0.0
Fruit cocktail and rice	*Aspergillus niger, Aspergillus* spp., *Fusarium* spp., *Penicillium citrinum*	17.0	0.0
Fruit and rice with milk	-	0.0	0.0
Fruit and rice with milk	*Aspergillus niger, Aspergillus sydowii, Aspergillus versicolor*	31 .0	0.0
Apricots	-	0.0	0.0

Table (1-C): Isolated fungi from rice group as well as total counts of fungi (cfu/g) and total aflatoxin content (µg/kg).

Test Samples	Isolated fungi	Total counts of fungi (x 10^2)	Total aflatoxin content (µg/kg)
Turky rice	*Alternaria* spp., *Aspergillus niger, Aspergillus* spp., *Fusarium oxysporum, F.* spp.	2.0	0.0
Turky rice	-	0.0	0.0
Rice and milk with protein	*Aspergillus flavus, Aspergillus* spp., *Fusarium chlamydosporum*	25.0	0.0
Rice and milk with protein	*Aspergillus* spp.	3.0	0.0
Flakes rice with vitamins	*Aspergillus flavus, Aspergillus niger, Aspergillus sydowii, Aspergillus versicolor, Fusarium oxysporum, Fusarium chlamydosportium, Penicillium citrinum*	80.0	2.0
Flakes rice with vitamins	-	0.0	0.0
Orange and rice with milk	*Aspergillus* spp., *Penicillium citrinum*	11.0	0.0
Rice and chocolate with milk	*Aspergillus* spp.	2.0	0.0

Table (1-D): Isolated fungi from wheat with honey group and wheat with milk group as well as total counts of fungi (cfu/g) and total aflatoxin content (µg/kg).

Test Samples	Isolated fungi	Total counts of fungi (x 10^2)	Total aflatoxin content (µg/kg)
Honey and wheat with milk	*Aspergillus candidus, Aspergillus fumigatus, , Aspergillus lucknowensis, Fusarium* spp., *penicillium atramentosum*	20.0	1.0
Wheat and milk	*Aspergillus candidus, Aspergilus flavus, Aspergillus niger, Fusarium* spp.	12.0	0.0
Honey and wheat with milk	*Aspergillus flavus, Aspergillus niger, Altemaria* spp., *Penicillium funiculosum*	30.0	0.0
Honey and wheat with milk	*Aspergillus candidus, Aspergillus niger, Penicillium digitatum, Penicillium* spp., *Microphomina* spp.	13.0	0.0
Cereal with milk	*Aspergillus* spp.	20.0	0.0
Cereal with milk	*Aspergillus* .spp	1.0	0.0
Wheat and milk	*Alternaria* spp., *Aspergillus* spp., *Penicillium digitatum*	2.0	0.0
Wheat and milk	*Alternaria tenuis , Penicillium capsulatum, P. digitatum, P.* spp., *Rhizopus* spp.	1.0	0.0
Wheat and milk	*Aspergillus niger, A. fumigatus*	7.0	0.0
Wheat and milk	*Aspergillus* spp.	20.0	0.0

These findings are in harmony with the previously recorded results of Munimbazi and Bullerman (1996). They isolated many types of fungi from various baby foods in Burundi such as corn sorghum meal, polished rice, millet and millet meal. The isolated fungi were *Fusarium moniliforme F. semitectum, F. equiseti, F. pallidoroseum, Aspergillus* ocharaceus, *A. wenttii, A. falvus, A.niger, A. sydowii, A. parasiticus, A .oryzae, A. tamari, Penicillium citrium, P.corylophilum,* and *P. chrysogenum.* On the other hand, El-Prince and Korashy (2003) examined 90 samples of dried milk foods in Assiout City, Egypt during 2001-2002. The examined samples contained aerobic bacteria, anaerobes, yeasts and moulds. Ostry *et al.* (2004) found that the fungi contamination of apples of the Gloster variety (used in processing baby foods) was *Penicillium expansum.* Shamanova *et al.*(1992), Bhatt *and vasanthi* (1999),Garrido *et al.*(1992) isolated 31 kinds of fungi species were isolated from baby food samples collected from market in Spain. In this respect, many authors verified the findings such as Sayed (2004) who showed that total numbers of different types of microorganisms were low in baby foods. These results are in harmony with Singh *et al.*(1992) who found that, 14 species of *Penicillium* were isolated from dairy products and dairy environments in India. Also, the given data in the present study showed negative results to fungi detection for many samples (mixed vegetables and chicken, cocktail, fruit, rice and milk with fruit, apricots, turkey rice and flakes rice with vitamins). These results are in agreement with Egyptian Organization for Standardization and Quality Control for infant foods.

In this respect , ***Aspergillus flavus*** was found only in mixed vegetable samples, rice and milk with protein, flakes rice with vitamins, wheat with milk and wheat and milk with honey .Meanwhile, the highest amounts of aflatoxin B_1 was 16 ppb in mixed vegetables samples, but the other samples contained amounts of aflatoxins ranging from 1 to 2 ppb. These results are in the same trend with Aksit *et al.* (1997) who demonstrated that aflatoxin B_1 was detected in infant foods and mother breast milk. On the other hand, Turek and Gregarova (1982) examined 870 samples of foods and isolated 182 strains of ***Aspergillus flavus*** and 23% of these strains were capale of forming aflatoxins.

Meanwhile, Trigo *et al.* (1981) found that aflatoxin B_1 was detected in 24 infant food samples from 85 collected samples.

Ahmed and Singh(1991) reported that mycotoxins were detected in chickpeas stored in jute bags (up to 205 µg/kg) and metal pins(up to 130 µg/kg) in Bihar, India. Five mycotoxins were produced by these isolates: citrinin, ochratoxins, patulin, penicillic acid and cyclopiazonic acid. These mycotoxins were detected from dairy products and a dairy environment in India Singh *et al.*(1992). Shipra *et al.* (2004)showed that the incidence of contamination of aflatoxin M_1 in cereal weaning foods was magnitude of 87.3%, higher in infant milk products (65-1012 ng/L) than liquid milk (28-164 ng/L)while Aflatoxin B_1 in dairy cattle feedstuffs indicate that the contamination rang from 1.4 to 63.6µg /kg. Lin *et al.*(2004) found aflatoxin B_1 in dairy milk powder and fresh milk in Taiwan.

Concerning the efficiency of isolated fungi to produce the mycotoxins such as aflatoxin, zearalenone and fumonisins (Table 2), the highest amount of total aflatoxins was 1760 ppb in the group of flakes rice with vitamins. The fungi isolated from the other 3 food group produced mycotoxins ranged from 6 to 200 ppb. The obtained results pointed to the ability of some isolated fungi such as *Fusarium* spp., *F.oxysporum*, and *F. Chlamydosporum* to produce zearalenon toxin. The amounts of produced toxin ranged from 0.01 to 0.42 ppm. Rice and fruit with milk, rice and vegetables and with protein were contaminated by zearelenon -produced fungi.

The presented data showed the presence of fumonisin toxin produced by some fungi strains isolated from creal with milk, rice and milk with vegetables and rice with milk and protein. The levels of produced frumonsin toxin ranged from 1.0 to 4.0 ppm.

Similar results were obtained by Castro *et al.*(2004) who analyzed 196 samples of corn based infant foods from 13 cities of Sao Paulo Brazil. They found that most samples were free of fumonisin contamination, but the samples of corn meal contained fumonisins in the levels of 2242 µg/kg.

These results are in harmony with Munimbazi and Bullerman (1996), who tested the efficiency of fungi such as *Aspergilus falvus* and *A. parasiticus* isolated from 50 infant Musalac food samples for production of aflatoxins. Sixty seven of 95 isolates of *A. flavus* strains produced cyclopiazonic acid (CPA) and all aflatoxin, and 4 isolates of *F. proliferatum* produced fumonisins . High levels of funonisin B_1 (12.2-75.2 µg/g) were detected in all 6 samples of maize and 1 sample of sorghum meal. Neither aflatoxin nor CPA were found in any of the tested foods.

Table (2): Efficiency of isolated fungi to produce mycotoxis .

No. of Samples	Samples	Test fungi	Aflatoxin (ppb)				Zearalenone (ppm)	Fumonisins (ppm)
			B_1	B_2	M_1	Total ppb		
4	Vegetables and chicken	*Aspergillus* spp.	0.0	0.0	0.0	0.0	0.0	0.0
7	Apple sause	*Fusarium chlamydosporum, A. falvus*	12	4	0.0	16	0.0	1.0
12	Turkey rice	*Fusarium oxysporum, F.* spp.	0.0	0.0	0.0	0.0	0.0	0.0
13	Mixed vegetable	*Aspergillus flavus,F. oxysporum*	6.0	0.0	0.0	6.0	0.0	0.0
17	Cereal with milk	*Aspergillus* spp.	0.0	0.0	0.0	0.0	0.0	2.0
18	Rice and with milk fruit	*Fusarium* spp. , *Aspergillus* spp.	0.0	0.0	0.0	0.0	0.03	0.0
22	Rice and milk with vegetables	*Fusarium oxysporum, Aspergillus versicolor*	0.0	0.0	0.0	0.0	0.01	1.0
24	Flakes rice with vitamins	*Aspergillus falvus, F. oxysporum, F. clamydosporum*	1610	150	0.0	1760	0.0	0.0
27	Rice and milk with protein	*A. flavus, F. chlamydosporum*	170	30	0.0	200.0	0.042	4.0
28	Protein plus and rice with milk	*Aspergillus* spp., *Fusarium* spp.	20.0	8.0	0.0	28.0	0.2	2.1
30	Rice and milk with chocolate	*Aspergillus* spp.	0.0	0.0	0.0	0.0	0.0	0.0
32	Fruits wheat and milk	*A.flavus, Fusarium.* spp.	10	5	10	25	0.0	0.0
37	Vegetables wheat and milk	*A. falvus*	0.0	0.0	10.0	10.0	0.0	0.0
41	Wheat and milk	*A. flavus, Fusarium.* spp.	0.0	0.0	4.0	4.0	0.0	0.0
42	Honey wheat and milk	*A. flavus*	8.0	0.0	0.0	0.8	0.0	0.0

On the other hand, Schollenberger *et al.* (1999) showed that zearalemone was not found in 237 commercially available samples of cereal–based foods including bread, baby and infant foods. One hundred and ninety six of infant food samples were free of fumonisins contamination and all samples contained feminizing (Castro *et al.* 2004).

Conclusion

Many types of fungi were isolated from Egyptian food infant. These food should be free of fungi as described in Egyptian Organization for Standardization (1992), but these isolates were considered from raw material such as milk, cereals , vegetables and fruits or during processing and storage of final products. Foods contaminated with pathogens are major factor in the causc diarihoea L disease and associated malnutrition. Motajemi *et al.* (1994Considering the producers of these kinds of products, firms should pay more attention to raw materials quality with respect to fungal contamination as well as complete avoidance of the presence of mycotoxins. These foods should be consumed directly after preparation·and not retaired as left overs for futures use. Storage during this period should occur in main trained refrigerators .

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الكشف عن تواجد الفطريات وسمومها فى الأغذية التكميلية للأطفال الرضع الشائع استهلاكها داخل الأسواق المصرية

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أجريت هذه الدراسة بتجميع ١٣٥ عينة للأغذية التكميلية للأطفال بحيث تمثل هذه العينات ٥ محافظات هى القاهرة – الجيزة – الاسكندرية-المنوفية – الفيوم كل عينة على حدة لدراسة الآتى:
العدد الكلى للفطريات والعزل الفطرى وتعريف السلالات المتواجدة والسموم الفطرية (الأفلاتوكسين) المتواجدة داخل هذه العينات المختبرة وذلك للوقوف على سلامتها من الناحية الصحية للاستهلاك.

وقد أوضحت النتائج أن ٨٤% من العينات لمختبرة كانت ملوثة بالفطريات التى تختلف حسب نوع العينة المختبرة ومن هذه الفطريات أسبرجلس فلافس، أسبرجلس سيداوى، أسبرجلس نيجر، أسبرجلس فيومجاتس، أسبرجلس فيرسكلور، أسبرجلس كنديديس، وبعض الأجناس الأخرى من الأسبرجلس، وسلالات من الفيوزاريوم منها فيوزاريوم أوكسيسبور وفيوزاريوم كلاميدوسبوريم، فيوزاريوم، فيوزاريوم كمبكتم، وبعض الأجناس الأخرى من الفيوزاريوم، وسلالات من البنسليوم، منها بنسليوم فينكيولوزم، بنسليوم ديجاتتم وسلالات من فطر الالترناريا وحوالى ٢% من هذه العينات المختبرة يوجد بها الأفلاتوكسين. وتم تقدير قدرة هذه العزلات على انتاج السموم الفطرية مثل الأفلاتوكسين، الزيرالينون والفيومينسين . فأوضحت النتائج أن ٥٠% من هذه الفطريات قادرة على انتاج السموم الفطرية السابق ذكرها. وقد أثبتت هذه الدراسة الاحتياج إلى تطبيق أساليب الجودة الحديثة عند انتاج أغذية الأطفال بصورة أكثر أماناً من المتواجدة حالياً فى المصانع.