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PREVALENCE OF MOULD AND YEAST IN SOME DAIRY PRODUCTS SOLD IN MENOFIA GOVERNORATE

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SUMMARY

A total of one hundred samples (25 each) of milk powder, yogurt, cheddar cheese and Ras cheese were collected from dairy shops and super markets in Menofia Governorate. Each sample was divided into two parts, one part for mycological studies and the other part for aflatoxins . Our results revealed that, the mould and yeast could be detected in all examined samples of milk powder, yoghurt, cheddar cheese and Ras cheese with mean count values of $8.3x\ 10^2 \pm 0.2x10^2$, $6.9x10 \pm 0.4x\ 10$, $5.3x\ 10^3 \pm 2.3x10^2$ and $4.1x10^3 \pm 1.6x10^2$ for mould and $0.5x\ 10^2 \pm 1.1x\ 10$, $1.4x10^2 \pm 0.5x\ 10^2$, $6.5x10^4 0 \pm .4x10^4$ and $5.4x\ 10^3 1 \pm .1x10^3$ for yeasts respectively .

Mould and yeast were isolated at varying percentages from all examined samples.The mould genera were, Asprigallus spp., Alternaria spp., Penicillium spp., Cladosporium spp., Mucor spp. and Rhizopus spp. While yeast genera were Candida spp, Rhodotorula spp, Saccharomyces spp and Torulopsis.

Aflatoxin M1 residues were detected in 16%, 16%, 32% and 44% of examined milk powder, yoghurt, cheddar cheese and Ras cheese samples respectively. Aflatoxin B1 residues were detected in 12%, 52% and 48% of examined milk powder, cheddar cheese and Ras cheese samples respectively. On the other hand, Aflatoxin B2 were detected in 12%, 24% and 12% of examined milk powder, cheddar cheese and Ras cheese samples respectively.

From the present investigation it could be concluded that, feeding of dairy animals with aflatoxins free feedstuff, using high quality raw milk , strict hygienic measures during manufacture, packing and distribution , proper personal hygiene, adequate cleaning and sanitation of dairy equipments and proper refrigeration are vital elements in the production of high quality dairy products.

INTRODUCTION

Milk and its products are adequate substrate for yeasts and moulds growth under suitable conditions of temperature and moisture contents (Barrois et al . 1997) The contamination of these products may occur from the raw material or during manufacturing , storage and distribution(Kure et al , 2004). These microorganisms influence the biochemical characters and flavour of these substrate as well as their appearance rendering them commercially undesirable and often resulting in down grading of the product (Demarigny et al ,1997 and Muir and Banks,2000).Some moulds can also adversly affect human and animal health as they can produce mycotoxins which are fungal secondary metabolites formed by consecutive series of enzyme- catalyzed reactions from a few biochemically simple intermediates of primary metabolism , these mycotoxin can enter the human and animal food chain by direct or indirect contamination (Bohra and Purohit, 2003) and they are related to a range of pathologies ranged from gastroenteritis to cancer as these mycotoxins are highly toxic, mutogenic,teratogenic and carcinogenic(Adams and Moss, 2000 and Li et al., 2000 and Hussein and Brasel,2001).

Mycotoxins are not destroyed by normal industrial processing or cooking since they are heat stable (Park,2002).The real hazard of mycotoxins to human health might be due to the cumulative effect of repeated exposure to very small or even undetectable doses of toxins in diet for comparatively long periods (Hendbrickse, 1991).So that the objective of this study was to characterize the fungal contamination in some dairy products during preparation ,storage or handling to safe guard consumers from being affected and to obtain a finished product of good keeping quality.

MATERIAL AND METHODS

1-Collection of samples.

A total of one hundred samples (25 each) of milk powder , yogurt , cheddar cheese and Ras cheese were collected from dairy shops and supermarkets in Menofia Governorate , each samples were transported in sterile air tight jars to the laboratory with a minimum of delay to be prepared and examined . Each sample was divided into two parts, one part for mycological studies and the other part for aflatoxin analysis

2- Enumeration, Isolation and Identification of fungi :

The yeast and mould counts of examined prepared samples were determined according to the technique recommended by A.P.H.A (1985). Mould isolates were identified according to Koneman and Roberts(1985),while yeast isolates were identified according to Looder and Kreger Van RiJ (1970).

3- Detection of mycotoxin:

Aflatoxin in the examined samples were extract-

ed, purified and determined by using thin layer chromatography as described by A.O.A.C (1984).

RESULTS

Table (1): Total mould count/ ml of examined samples:

Type of samples	No. of samples	Positive sample		Count/ml		
		No.	%	Min	Max	Mean ±S.E
Milk powder	25	22	88	1.2 x 10	$1.2x10^3$	$8.3x10^2 \pm 0.2x10^2$
Yogurt	25	21	84	1.0 x 10	$2.7x10^2$	6.9x10±0.4x10
Cheddar cheese	25	18	72	1.0x10	$3.0x10^4$	$5.3x10^3 \pm 2.3x10^3$
Ras cheese	25	20	80	8.2x10	$9.6x10^3$	$4.1x10^3 \pm 1.6x10^2$

Table(2):Total yeast count/ ml of examined sample:

Type of samples	No of samples	Positive samples		Count/ ml		
		No	%	Min	Max	Mean ± S.E
Milk powder	25	16	64	2.0 x10	$3.9x10^2$	$0.5x10^2 1 \pm .1x10$
Yogurt	25	20	80	3.0 x10	$7.2x10^2$	$1.4x10^2 0 \pm .5x10^2$
Cheddar cheese	25	25	100	9.0 x10	$3.5x10^5$	$6.5x10^4 \pm 0.4x10^4$
Ras cheese	25	19	76	6.0 x10	$1.1x10^3$	$5.4x10^3 1 \pm .1x\ 10^3$

Table (3): Prevalence of individual members of fungi in examined samples of milk products

Genera and species	Milk powder		Yogurt		Cheddar cheese		Ras cheese	
	No	%	No	%	No	%	No	%
A. flavus	16	64	1	4	4	16	3	12
A. niger	13	52	2	8	19	76	7	28
A.Fumigatus	5	20	5	20	3	12	2	8
A.ochracus	6	24	3	12	8	32	2	8
A.nidulans	-	-	-	-	6	24	-	-
A.candidus	2	8	-	-	-	-	-	-
A.Tserus	1	4	-	-	-	-	1	4
Alternaria spp.	-	-	3	12	-	-	-	-
Pencillum spp.	8	32	7	28	3	12	8	32
Fusarium spp	1	4	4	16	3	12	4	16
Cladosporium spp.	-	-	5	20	3	12	6	24
Mucor spp.	6	20	2	8	2	8	-	-
Rhizopus spp.	2	8	1	4	2	8	-	-
Candida spp.	-	-	4	16	13	52	5	20
Rhodotorula spp.	-	-	2	8	4	16	2	8
Saccharomyces spp	-	-	-	-	6	24	-	-
Trulopsis spp	-	-	1	4	-	-	-	-

Table (4): Incidence and quantitative estimation of aflatoxins in examined samples (ug/kg)

Types of samples	M1					B1					B2				
	+ve samples		Min	Max	Mean	+ve samples		Min	Max	Mean	+ve samples		Min	Max	Mean
	No	%				No	%				No	%			
Milk powder	4	16	0.6	1.5	0.9±0.06	3	12	0.5	1.9	0.8±0.01	3	12	0.9	2.1	1.9±0.1
Yogurt	4	16	2.0	3.0	2.3±0.5	-	-	-	-	-	-	-	-	-	-
Cheddar	8	32	3 1	4.0	3.5±0.8	13	52	3.1	7.0	5.4±0.6	6	2.4	2.1	3.2	3.8±0.2
Ras	11	44	1.9	5.3	3.5±0.02	12	48	2.1	4.3	3.9±0.4	3	12	1.6	3.3	2.9±0.04

DISCUSSION

Results reported in Table (1) revealed that, the examined samples of milk powder, yogurt, cheddar cheese and Ras cheese were contaminated with moulds at the percentages of 88,84,72 and 80% respectively with mean values of 8.3x 102± 0.2x102,6.9x100±.4x 10, 5.3x 103 ±2.3x102and 4.1x103 1±.6x102 respectively. Nearly similar results were reported by Abou Zeid (1996) , Hassan and Hammad(2001), Ali et al., (2004) and Amany et al.,(2005).

As shown in Table (2) samples of milk powder, yogurt, cheddar and Ras cheese were contaminated with yeast at the percentages of 64,80,100 and 76% respectively with mean values of $0.5x\ 10^2$ $\pm 1.1x\ 10$, $1.4x10^2\ \pm 0.5x\ 10^2$, $6.5x10^40\pm.4x10^4$ and $5.4x\ 10^31\pm.1x10^3$ respectively. These results were similar to these obtained by Bullerman(1981); Ismail and Saad (1995); Zin El-din and Elsawah (1997), and Dermarigny et al. (1997).

Although some yeasts and moulds may be used in the manufacture of many dairy products and milk beverages to attain specific flavour or aiding in ripening of certain type of cheese(Fadda et al,, 2004).Yet many species of yeast and moulds are implicated in many defects in milk and its products, as off flavour, colour defects and spoilage of such food(Filtenborg etal., 1996 and Sarias et al., 1996).

our results in Table(3) show that in milk powder samples species of Asperigillus as A.Flavus, A niger, A. fumigatus, A. Ochracus, A. Candidus and A. Tserus isolated in percentages of 64, 52, 20, 24, 8 and 4% respectively. While penicillium Spp was present in high percentage of 32%. Fusarium Spp,Mucor Spp and Rhizopus Spp were detected in 4%, 20% and 8% respectively. Similar results were obtained by Hassan and Hammad (2001). Yeasts and moulds are widely distributed as environmental contaminants of air, water, soil and dust, so the presence of moulds and yeasts in milk products may be attributed to poor sanitary practices during manufacturing, packing and distribution or the use of bad quality raw ingredients (Ray, 1996).

In samples of yogurt, A.Flavus, A. niger, A. fumigatus, and A. Ochracus, were present in percentages of 4, 8, 20 and 12% respectively. Fusarium Spp and Candida spp were present in percentags of 16% . Also Rhizopus Spp and Torulopsis spp were present in the same percentage of 4%. Moreover, Alternata Spp and Rhodo Torula Spp were present in the percentage of 8 and 12 % respectively. Similar results were obtained by Eman and Abdel Satar (2003). While lower results were obtained by Hassan (1999). Yogurt is considered as an excellent medium for growth of many fungi because of its high water, low pH and its good quality protein(Marth and Steel, 2001). For samples of cheddar cheese, Asperigallus spp

were present as A. Flavus,A. niger, A. fumigatus, A. ochracus and A. nidulans in percentages of 16 ,76,12, 32 and 24% respectively. Candida spp were present in high percentags of 52% followed by Sacchromyces in a percentages of 24% and Rhodotorula spp in a percentages of 16%

Penicillium spp, Fusarium spp and Cladosporium spp were present in the same percentage of 12% also Mucor spp and Rhizopus spp were present in percentage of 8%. Nearly similar results were reported by Lund et al (1995) and Fardos(2001).

In samples of Ras cheese, Asperigallus spp were represented as A. flavus, A.niger, A. fumigatus, A. ochracus and A.tsresus in percentages of 12, 28, 8, and 4% respectively. Meanwhile Penicillum spp, Fusarium, Cladosporium spp were present in percentage of 32, 16 and 24% respectivelly. Candida and Rhodtorula spp were present in the percentage of 20% and 8%. Nearly similar results were obtained by Salwa(1999) and Corbo et al (2001)

Kure et al (2004) reported that , the major source of cheese contaminants were found in samples of air, equipment and plastic film. He added that air is considered as the major source of cheese contaminants so high quality air with low number of contaminants in production room especially the wrapping room is important in order to reduce mould contamination .

As shown in [illegible] Cladosporium and candida spp were the most common genera recovered from the four types of examined milk products.Similar results were recorded by Sampayo etal(1995), Elprince and Ismail (1998) and El- Sherif(2000) and Montagna et al.,(2004). Growth of moulds as mucor, Alternaria, Penicillium and Geotricum on the surface of dairy products lead to off flavour while growth of Penicillium, Cladosporium, Asperigillus and Mucor may responsible for bitterness and rancidity of cheese. Penicillium species may lead to softness of the surface of cheese(Minervini et al., 2001). Some species of Asperigallus, Cladosporium, Penicillium and Fusarium were responsible for kerato-conjunctivitis in man while Asperigellus niger casue otomycosis and allergic condition, some species of penicillium have been associated with pulmonary infections, urinary tract infections and yellow rice disease causing several deathes in man(Nilesen et al., 1998).

From the results reported in Table (4), regarding the milk powder samples, Aflatoxin M1, B1 and B2 can be detected in 16%, 12% and 12% of examined samples respectively with average concentrations of 0.9 ±0.06, 0.8 ± 0.01 and1.90 ±.1ug/kg respectively. These findings agreed with that reported by Olivera et al(1997) and Chia et al (2005).Penicillium species mycotoxins was failed to be detected as that reported by Hamilton(1975) who stated that milk powder are substrates which have low carbohydrate and high protein

content which combine with toxins of penicillium species.

Neither storage or processing determine a reduction of aflatoxin content in milk powder (Deveci and Emel, 2006).

For samples of yogurt, 16% of the examined samples proved to be contain aflatoxin M1 at an average concentration of 2.3 0 ±. 5µg/ kg. A nearly similar results were recorded by Farag(2002) and Martins and Martins (2004). A highly reduction of AFM1 in yoghurt is due to degradation of AFM1, caused by enzymatic, microbial and particularly acid coagulation of manufactured yoghurt. Moreover, the lactic acid produced in yoghurt processed a detoxification action as the toxic form of AFB1 into non toxic form"B2a" (Galvano et al ., 1996)

Aflatoxins M1, B1 and B2 can be detected in 32%, 52% and 24% of the examined samples of Ras cheeses with mean values of 3.5 0 ±.8, 5.4 ±1.6 and 3.8 0 ±. 2 µ/ kg. Nearly similar results were reported by EL Gerbi et al (2004) and Manal et al (2006).Aflatoxins in cheese resulted from the presence of it in raw milk as a consequence of carry over of aflatoxins M1 from contaminated cow food to milk synthesis of aflatoxins (B1, B2,G1 and G2) by A. flavus and A. parasiticus growing in cheese and the presence of these toxins in the dried milk used to enriched the milk used to make cheese or other dairy products(Kaya et al, 1998 and Aycicek et al., 2002).

For samples of Ras cheese, aflatoxins M1, B1 and B2 can be detected in 44%, 48% and 12% of the examined samples with mean values of 3.5 ±0. 02, 3.9 0 ±.4 and 2.9 ±0.04 µ/ kg. Nearly , similar results were reported by Abou Zeid et al(1996) and Manetta et al (2005).

Milk contained mycotoxins considered as a vehicle for transferring such toxins into cheese where it appears to be associated with casein, the cheese is considered as a curdle from milk protein so, it may contain 3.5- 5 times more toxins than the level present in milk from which it was made (Chapman and Sharpe, 1990).. Mycotoxin cause alteration in carbohydrate and lipid metabolism, toxic damage of the liver disturbance in blood clotting mechanism and with immune response. Aflatoxins were classified as group1 carcinogens in 1993 by the WHO international agency for research on cancer (IARC) (Pestka, 1995).

Naturally occurring toxicant contamination of foods with mycotoxins is unavoidable and unpredictable and posses a unique challenge to food safety, so practical decontamination procedures must include the following hygienic procedures: inactivate, destroy or remove the toxin, not produce or leave toxic residues in the food , retain the nutritive value of the food, not alter the ac-

ceptability or the technological properties of the product and if possible destroy fungal spores (Park,2002). Also there is a must to increase the intake of diet components such as vitamins , antioxidants and substances known to prevent carcinogenesis(Creppy, 2002). From the present investigation it could be concluded that, feeding of dairy animals with aflatoxins free feedstuff, using high quality raw milk , strict hygienic measures during manufacture, packing and distribution , proper personal hygiene, adequate cleaning and sanitation of dairy equipments and proper refrigeration are vital elements in the production of high quality dairy products

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مدي تواجد الفطريات والخمائر في بعض منتجات الالبان المباعة بمحافظه المنوفية

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أجرى هذا البحث علي مائة عينة من اللبن البودرة و الزبادي و الجبن الشيدر والجبن الرومي (٢٥) عينه من كل منتج تم تجميعها من محلات بيع الالبان والسوبر ماركت في محافظة المنوفية لمعرفة مدي تواجد الفطريات والا فلا توكسينات بها. وقد اوضحت النتائج ان العفن والخمائر قد تواجدت في كل عينات اللبن البودرة والزبادي والجبن الشيدر والرومي بمتوسط قدره ٣و٨×١٠^{٢} و٩و٦×١٠و ٣و٥×١٠^{٣}و ١و٤×١٠^{٣}علي التوالي بالنسبه للعفن و كانت بمتوسط قدره ٥و × ١٠^{٢} و ٦و٩ × ١٠و٣و٥ ×١٠و ٤و١× ١٠ على التوالى بالنسبة للعفن. وكانت بمتوسط قدره و٥ × ١٠^{٢}و ٤و١ × ١٠^{٢}و٥و٦ × ١٠^{٤} و٤و٥× ١٠^{٣} على التوالى بالنسبة للخمائر. ولقد أمكن عزل أنواع عديدة من العفن تمثلت فى جنس السبرجلس و جنس الألترناريا و جنس البنسليوم و جنس الكلادوسبوريم و جنس الميوكر، وجنس الريزوبس . أما الخمائر فتمثلت فى جنس الكانديدا وجنس الرودرتوريلا وجنس السكارومسيز وجنس التوريولوبوسس .

ومن ناحية اخرى أثبتت التحليلات الكروماتوجرافية بالفصل على الألواح الرقيقة بالكشف على الأفلاتوكسينات عن تواجد الأفلاتوكسين م ١ فى عينات لبن البودرة والزبادى والجبن الشيدر والجبن الرومى بنسب ١٦ % و ١٦%و ٣٢% على التوالى . اما بالنسبة الى الأفلاتوكسين ب ١ فقد تواجد فى عينات لبن البودرة والجبن الشيدر والجبن الرومى بنسب ١٢%و ٥٢%و ٤٨% على التوالى .اما بالنسبة الى الأفلاتوكسين ب ٢ فقد تواجد فى عينات لبن البودرة والجبن الشيدر والجبن الرومى بنسب ١٢%و ٢٤%و ١٢% على التوالى.

وقد تم استنتاج ان تغذية الحيوانات بعلائق خالية من السموم الفطرية وكذلك استخدام لبن عالى الجودة واتباع الأساليب الصحية أثناء الصناعة والتعبئة والتوزيع والتبريد الجيد كلها اسباب اساسية للحد من التلوث بالسموم الفطرية والحصول على منتج عالى الجودة.