Dept of Food Control,
Fac. of Vet. Med., Zagazig Univ., Egypt.

OCCURRENCE OF FUNGI AND AFLATOXINS IN YOGHURT MARKETED IN ZAGAZIG CITY

(With 6 Tables)

By

*A.A. BAHOUT and A.H. MOUSTAFA**

* Animal Health Research Institute, Mansoura, Egypt.

(Received at 25/12/2002)

تواجد الفطريات والأفلاتوكسينات فى الزبادى المعروض للبيع بمدينة الزقازيق

على أحمد على بحوت ، عادل حسانين محمود مصطفى

أجرى هذا البحث على ٨٠ عينة من الزبادى الطبيعى والزبادى بالفواكه (٤٠ من كل منتج) جمعت عشوائياً من محلات بيع الألبان والسوبر ماركت بمدينة الزقازيق لمعرفة مدى تواجد الفطريات والأفلاتوكسينات بها. وقد أوضحت النتائج أن ٢٢,٥% ، ٣٠% من العينات كانت ملوثة بالخمائر بمتوسط قدره ١,٢٦ × ١٠^{٢} ، ١,٩٤ × ١٠^{٢}خلية / ملى . بينما تواجد العفن فى ٨٥% ، ١٠٠% من العينات بمتوسط قدره ٤٨,٥ ، ٥٦,٣ خلية / ملى. ولقد أمكن عزل أنواع عديدة من الخمائر تمثلت فى جنس الكانديدا ، جنس الكلافيروميسس ، جنس البيكيا ، جنس الرودوتوريولا ، جنس التريولوميسس ، جنس السكاروميسس وأيضا أنواع من العفن مثل جنس البنسيليوم ، جنس الكلادسبوريوم ، وجنس الجيوتريكم ، وجنس المونيليا ، جنس الأسبرجيلس ، وجنس الميكور بنسب مختلفة. ومن ناحية أخرى أثبتت التحليلات الكروماتوجرافية بالفصل على الألواح الرقيقة للكشف عن الأفلاتوكسينات على وجود الأفلاتوكسين م١ فى عينات الزبادى الطبيعى والزبادى بالفواكه بنسبة ٥% ، ٢,٥% على التوالى . بينما تواجد الأفلاتوكسين ب١ فى عينات الزبادى بالفواكه فقط بنسبة ٥%. هذا وقد تمت مناقشة النتائج من الناحية الصحية والاقتصادية وكذلك اقتراح الإجراءات الصحية التى يجب اتباعها لتحسين جودة المنتج للحفاظ على صحة المستهلك.

SUMMARY

Eighty random samples of plain and fruit yoghurt (40 of each) were collected from dairy shops and supermarkets in Zagazig City, Egypt, during summer 2002 and examined for the presence of fungi and aflatoxins. Yeasts could be detected in 22.5% and 30% of examined plain and fruit yoghurt samples respectively, with a mean value of 1.26 x 10^2 and 1.94 x 10^2/ml respectively. While molds were isolated from 85%

and 100% of examined plain and fruit yoghurt samples respectively, with a mean count of 48.5 and 56.3 /ml respectively. The isolated yeasts were identified as Candida spp., Kluyveromyces spp., Pichia spp., Rhodotorula spp., Torulopsis spp. and Saccharomyces spp., while the recovered molds were recognized as Penicillium spp., Cladosporium spp., Geotrichum spp., Monilia spp., Aspergillus spp. and Mucor spp. Aflatoxin M_1 residues were detected in 5.0%, 2.5% of plain and fruit yoghurt samples respectively. Aflatoxin B_1 residues were detected in 5.0% of fruit yoghurt samples. The public health importance and economic significance of isolated fungi and aflatoxins as well as control measures for improving the quality of yoghurt and to safeguard the consumers were discussed.

Key words: Fungi, Aflatoxins, Yoghurt.

INTRODUCTION

Natural or plain yoghurt is the traditional type of yoghurt with a sharp acidic taste, while fruit yoghurt is made by the addition of fruits and sweeting agents to natural yoghurt (Potter and Hotchkiss, 1995).

Yoghurt is highly nutritious food and of a useful therapeutic value as it prevents the intestinal putrefaction resulting from anaerobic decomposition, prevents the gastrointestinal disorders, prevents the coronary heart diseases, reduces the risk of colon cancer, exerts a hypocholesterolaemic effect, produces antibiotics as acidophilin, lactocidin, nicin, and lactoline that inhibit the growth of many pathogens (Robinson, 1991).

Yoghurt has a high water activity, low pH and contains good quality protein, so it considered an excellent medium for growth of many species of fungi (Marth and Steele, 2001).

Yeasts and molds can enter foods through inadequately sanitized equipments or as air-borne contaminants, moreover, can tolerate the psychotrophic temperature. Therefore, they become a problem in dairy products (Ray, 1996).

Aflatoxins are secondary metabolites that are produced by certain species of molds as *Aspergillus flavus* and *Aspergillus parasiticus* under certain conditions of temperature and relative humidity. The major aflatoxins of concern are B_1, B_2, G_1, G_2 and M_1. Aflatoxin B_1 is the predominant toxin in amount and toxicity, while aflatoxin M_1 is a toxic metabolite of B_1 and it is excreted in milk of dairy cattle that have consumed aflatoxin contaminated feed (Kisza and Domagala, 1994).

Dairy products can be contaminated directly with mycotoxins by molds that grow on them and produce the toxins or indirectly by the carryover of mycotoxins into milk as a result of dairy cows consuming mycotoxin-contaminated feeds (Van Egmond, 1989).

In Egypt, the importance of yoghurt can be seen by its rate of consumption by large number of people and as a weaning food for babies. Recently, good attention was paid to prevent the existence of aflatoxins in food. Therefore, this study was planned to investigate the occurrence of fungi and aflatoxins in yoghurt marketed in Zagazig City, Egypt.

MATERIAL and METHODS

Eighty random samples of plain and fruit yoghurt (40 of each) were collected in their original containers from dairy shops and supermarkets in Zagazig City, Egypt during summer 2002. The collected samples were transferred immediately to the laboratory for mycological examination and estimation of aflatoxins M_1 and B_1 residues.

Enumeration, isolation and identification of yeasts and molds:

The yeast and mold counts of examined yoghurt samples were determined according to the technique recommended by A.P.H.A. (1985). Yeast isolates were identified according to Looder and Kreger Van Rij (1970). While, isolated molds were identified according to Koneman and Roberts (1985).

Estimation of aflatoxins B_1 and M_1 residues in examined yoghurt samples:

Aflatoxins B_1 and M_1 in the yoghurt samples were extracted, purified and determined by using thin layer chromatography as described by A.O.A.C. (1984).

RESULTS

Table 1: Total yeast count/ml of examined yoghurt samples.

Type of samples	No. of samples	Positive samples		Count / ml			
		No.	%	Min.	Max.	Mean	S.E.M. ±
Plain yoghurt	40	9	22.5	20	300	$1.26x10^2$	0.4×10^2
Fruit yoghurt	40	12	30.0	100	500	$1.94x10^2$	$0.3x10^2$

Table 2: Total mold count/ml of examined yoghurt samples.

Type of samples	No. of samples	Positive samples		Count / ml			
		No.	%	Min.	Max.	Mean	S.E.M. ±
Plain yoghurt	40	34	85.0	10	230	48.5	8.3
Fruit yoghurt	40	40	100.0	30	260	56.3	6.6

Table 3: Frequency distribution of total yeast and mold count/ml of examined yoghurt samples.

Intervals	Frequency							
	Plain yoghurt				Fruit yoghurt			
	Yeast count		Mold count		Yeast count		Mold count	
	No. of samples	%	No. of samples	%	No. of samples	%	No. of samples	%
0-100	4	44.4	31	91.2	--	--	30	75.0
100-200	3	33.4	3	8.8	8	66.7	10	25.0
200-300	2	22.2			2	26.7		
300-400					1	8.3		
400-500					--	--		
500-600					1	8.3		
Total	9	100.0	34	100.0	12	100.0	40	100.0

Table (4): Incidence of isolated fungi in the examined yoghurt samples.

Isolates	Plain yoghurt		Fruit yoghurt	
	No. of samples	%	No. of samples	%
Isolated mold species:				
Penicillium spp.	18	45.0	20	50.0
Cladosporium spp.	13	32.5	12	30.0
Geotrichum spp.	9	22.5	10	25.0
Monilia spp.	7	17.5	10	25.0
Aspergillus spp.	6	15.0	8	20.0
Mucor spp.	4	10.0	6	15.0
Isolated yeast species:				
Candida spp.	7	17.5	8	20.0
Kluyveromyces spp.	4	10.0	5	12.5
Pichia spp.	4	10.0	4	10.0
Rhodotorula spp.	2	5.0	3	7.5
Torulopsis spp.	2	5.0	2	5.5
Saccharomyces spp.	1	2.5	4	10.0

Table 5: Concentration of aflatoxins M_1 and B_1 residues in examined yoghurt samples

Type of samples	No. of examined samples	Aflatoxin M_1 (µg/Kg)				Aflatoxin B_1 (µg/Kg)			
		Positive samples		Range	Mean	Positive samples		Range	Mean
		No.	%			No.	%		
Plain yoghurt	40	2	5.0	0.2-0.3	0.25	0	0	-	-
Fruit yoghurt	40	1	2.5	-	0.42	2	5.0	0.32-0.5	0.41

Table 6: Grading of examined yoghurt samples according to the permissible limits in Egyptian standards (1990) and FDA (Wood, 1992)

Type of samples	No. of examined samples	AFM_1 contaminated samples				AFB_1 contaminated samples			
		>E.S. limit (zero)		>FDA limit (0.5 µg/kg)		>E.S. limit (zero)		>FDA limit (0.5 µg/kg)	
		No.	%	No.	%	No.	%	No.	%
Plain yoghurt	40	2	5.0	0	0.0	0	0.0	0	0.0
Fruit yoghurt	40	1	2.5	0	0.0	2	5.0	1	2.5

DISCUSSION

The results reported in Table (1) revealed that the examined plain and fruit yoghurt samples were contaminated with yeasts, at the percentages of 22.5 and 30 respectively, with a mean values of $1.26 \times 10^2 \pm 0.4 \times 10^2$ and $1.94 \times 10^2 \pm 0.3 \times 10^2$/ml respectively. Most of the examined samples within the range of 100-300 (Table, 3).

Nearly similar results were reported by Garcia and Fernandez (1984), while, lower results were reported by Rodriguez Alvarez *et al.* (1990).

Table (2) revealed that the molds were recognized in 34 (85%) and 40 (100%) of plain and fruit yoghurt respectively, with the mean value was 48.5 ± 8.3 and 56.3 ± 6.6/ ml respectively. Most of examined samples within the range of 0-100 (Table, 3).

These results were similar to those obtained by Saad *et al.* (1987) and El-Badry (1998), while lower results were reported by Jordano Salinas (1986) and Green and Ibe (1987).

Yeasts and molds are widely distributed in the environmental contaminants of air, water, soil and dust. The presence of yeasts and molds in yoghurt may be attributed to poor sanitary practices during manufacturing, packing and distribution or the use of bad quality raw ingredients as milk and fruits (Ray, 1996).

The isolated molds from the examined plain and fruit yoghurt samples were identified as Penicillium spp. (45% and 50%), Cladosporium spp., (32.5% and 30%), Geotrichum spp. (22.5% and 25%), Monilia spp. (17.5% and 25%), Aspergillus spp. (15% and 20%) and Mucor spp. (10% and 15%) respectively (Table, 4).

Similar mold genera were isolated from yoghurt by Mansour *et al.* (1986), El-Shinawy (1987), Hui (1993) and Bahout and El-Shawaf (1999).

Candida spp., Kluyveromyces spp., Pichia spp., Rhodotorula spp., Torulopsis spp. and Saccharomyces spp. could be isolated from the plain and fruit yoghurt samples in percentages ranged from 2.5% to 17.5 and 5 to 20% respectively (Table, 4).

Similar species of yeasts were isolated from yoghurt by Fleet and Main (1987) and Hui (1993).

Yeasts are very active biochemically and can grow over a wide range of pH and temperature. The presence of yeasts in yoghurt may cause undesirable changes, off flavour and which render the product of inferior quality (Robinson, 1990).

Aflatoxin residues in yoghurt samples in Table (5) showed that aflatoxin M_1 residue was recognized in 2 (5%) of plain yoghurt by 0.20 and 0.30 µg/kg., and 1 (2.5%) of fruit yoghurt by 0.42 µg/kg. While aflatoxin B_1 residue wasn't detected in any samples of plain yoghurt, but present in 2 (5%) of fruit yoghurt by 0.32 and 0.50 µg/kg.

Approximately similar levels of both AFM_1 and AFB_1 were determined in yoghurt by Choudhary *et al.* (1997) and Farag (2002). While lower levels were estimated by Galvano *et al.* (1998), and Srivastava *et al.* (2001).

Table (6) compares between the obtained aflatoxin M_1 and B_1 levels with those allowable limits of both toxins set by Egyptian and FDA regulations and reveals that two and one sample of plain and fruit yoghurt were contaminated with aflatoxin M_1 and two samples of fruit yoghurt were contaminated with aflatoxin B_1 and exceeded the permissible Egyptian limit (zero for each aflatoxin). While only one sample of fruit yoghurt was contaminated with aflatoxin B_1 and exceeded the FDA limit (0.5 µg/Kg).

Aflatoxins are highly toxic, mutagenic, teratogenic and carcinogenic compounds. They are responsible for serious public health hazards (Van Egmond, 1989).

In conclusion, feeding of dairy animals with aflatoxin free feedstuffs, using high quality raw milk and fruits, 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 yoghurt.

REFERENCES

American Public Health Association" A.P.H.A." (1985): Standard Methods for the Examination of Dairy Products. 15th ed., Washington, DC.

Association of Official Analytical Chemists "A.O.A.C." (1984): Official Methods of Analysis. 14th ed., Washington, DC.

Bahout, A.A. and El-Shawaf, A.M. (1999): Evaluation of some chemical and microbiological aspects of commercial yoghurt in Sharkia province. J. Vet. Med. Res., 1 (1): 1-9.

Choudhary, P.L.; Sharma, R.S.; Borkhatriya, V.N.; Murthi, T.N. and Wadodkar, U.R. (1997): Survey on the levels of aflatoxin M_1 in raw and market milk in and around Anand town. Ind. J. of Dairy Sci., 50 (2): 156-158.

Egyptian Organization for Standardization and Quality Control (1990): Egyptian Standard (1875-1990) Maximum limits for Mycotoxins in Food. Part I. Aflatoxin.

El-Badry, S.A. (1998): Sanitary Quality of Yoghurt. M.V.Sc. Thesis, Fac. of Vet. Med., Zagazig Univ., Egypt.

El-Shinawy, S.H. (1987): Microbiological Studies on Fermented Milks. Ph. D. Thesis, Fac. of Vet. Med., Zagazig Univ., Egypt.

Farag, M. (2002): Occurrence of Aflatoxin in Fermented Milks. M.V.Sc. Thesis, Fac. of Vet. Med., Zagazig Univ., Egypt.

Fleet, G.H. and Main, M.A. (1987): The occurrence and growth of yeasts in dairy products. International Journal of Food Microbiology, 4: 145-155.

Galvano, F.; Galofare, V.; Angelis, A-de, Galvano, M.; Bognano, M. and Galvano, G. (1998): Survey of the occurrence of aflatoxin M_1 in dairy products marketed in Italy. J. of Food Prot., 61 (6): 738-741.

Garcia, A.M. and Fernandez, G.S (1984): Contaminating mycoflora in yoghurt: general aspects and special reference to the genus Penicillium. J. of Food Prot., 47 (8): 629-636.

Green, M.D. and Ibe, S.N. (1987): yeast as primary contaminants in yoghurts produced commercially in Lagos, Nigeria. J. of Food Prot., 50 (3): 193-198.

Hui, Y.H. (1993): Dairy Science and Technology Handbook. 2. Product Manufacturing. VCH Publishers, Inc., New York, USA.

Jordano Salinas, R. (1986): Hygiene quality of commercial yoghurt. Alimentaria 178: 27-30. Dairy Sci. Abst., 49, 7 (1987).

Kisza, J. and Domagala, J. (1994): Aflatoxins in feed and milk: Occurrence, assessment and methods of elimination. Medycyna of Weteryavyja. 50 (60): 250-254.

Koneman, E.W. and Roberts, G.D. (1985): Practical Laboratory Mycology. 3rd ed., Williams and Wilkins, Baltimore, London.

Looder, J. and Kreger Van Rij, N.J.W. (1970): The yeasts. A taxonomic study. 2nd ed., North Holland Publishing Co., Amsterdam.

Mansour, M.A.; Amer, I.H. and El-Sayed, M.S. (1986): Fungal contamination of yoghurt. Zagazig Vet. J., 13: 11-19.

Marth, E.H. and Steele, J.L. (2001): Applied Dairy Microbiology. 2nd ed., Marcal Dekker, Inc., New York.

Potter, N.A. and Hotchkiss, J.H. (1995): Food Science. 5th ed., Chapman and Hall, London, England.

Ray, B. (1996): Fundamental Food Microbiology. CRC Press, Inc., Tokyo, New York.

Robinson, R.K. (1990): Dairy Microbiology. 2nd ed., Chapman and Hall, London, New York.

Robinson, R.K. (1991): Therapeutic Properties of Fermented Milks. Elsevier Applied Science. London and New York.

Rodriguez Alvarez, C.; Pintor Diaz, M.C.; Gonzalez Padron, A.; Alvarez Marante, R. and Hardisson De La Torre, A. (1990): Microorganisms of food hygiene interest in commercial yoghurts in the canary Islands. Alimentaria, 212: 55-58.

Saad, N.M.; Khalil, M. and Abd El-Hamied, A. (1987): Microbiological quality of yoghurt produced in Assiut City. Assiut Vet. Med. J., 19 (37): 87-91.

Srivastava, V.P.; Bu-Abbas, A.; Basuny, A.A.; Al-Johar, W.; Al-Mufti, S. and Siddiqui, M.K.J. (2001): Aflatoxin M_1 contamination in commercial samples of milk and dairy products in Kuwait. Food Additives and Contaminants, 18 (11): 993-997.

Van Egmond, H.P. (1989): Mycotoxins in dairy products. Elsevier Applied Science, London.

Wood, G.E. (1992): Mycotoxins in food and feed in the United States. J. Anim. Sci.., 70: 3941-3949.