MYCOLOGICAL QUALITY OF YOGHURT WITH SPECIAL REFERENCE TO AFLATOXIGENIC MOULDS

BY

*El-Bagoury, A.M. and **Mosaad, A. A.
* Dept. of Food Hygiene and Control, Fac.Vet. Med., Sadat City, Minoufiya Univ.
** Dept. of Microbiology, Fac.Vet. Med., Sadat City, Minoufiya Univ.

SUMMARY

Seventy five random samples of yoghurt, 25 from each of plain, fruit and drinking types, were collected from various supermarkets and groceries at different localities in Minoufiya Governorate. The collected samples were mycologically examined for the presence and counts of moulds and yeasts and for detection of aflatoxigenic moulds and their related aflatoxins. Yeasts could be detected in 48%, 64% and 28% of the examined plain, fruit and drinking types of yoghurt, with mean count values of $5.5 \times 10^3 + 3.1 \times 10^3$, $4.6 \times 10^4 + 2.6 \times 10^4$ and $9.4 \times 10^2 + 5.2 \times 10^2$, respectively. While 52%, 80% and 44% of the examined plain, fruit and drinking types of yoghurt proved to be contaminated with moulds with mean count values of $9.7 \times 10^2 + 5.4 \times 10^2$, $6.3 \times 10^3 + 4.2 \times 10^3$ and $4.4 \times 10^2 + 1.6 \times 10^2$, respectively. Yeasts as Candida albicans, C. krusei, Saccharomyces cervisiae and Rhodotorula glutinis and Moulds as Aspergillus niger, A. fumigatus, A.terreus, A. candidus, A. flavus, alternaria species, rhizopus species, fusarium and mucor species could be identified after being isolated from the examined samples of the three types of yoghurt at various percentages. The isolated strains of A. flavus proved to be aflatoxin B_1-producing strains. The economic and public health significance of the isolated yeasts and moulds as well as suggested control and preventive measures to improve the quality of yoghurt and to safeguard the consumer were dicussed.

INTRODUCTION

Yoghurt and other fermented milks are identified, since early times, as an important food items for peoples of all ages due to their unique nutritional and therapeutic attributes. They are excellent source of high quality proteins, vitamins and minerals. They could be supply many lactic acid bacteria that may induce changes in the equilibrium and metabolism of the intestinal microflora and thus excert a healthful influence on the host (*Corinne, 1998; Weinsier and*

Krumdieck,2000 and Piaia,2001). Moreover, yoghurt exhibits significant antimutagenic activities against colon carcinogens, benificial effects against Osteoperosis, impaired lactose indigestion and positive effect on immune defence mechanism and cardiovascular diseases *(Robinson, 1993; Nadathur et al., 1995 and Cross et al.,2001).*

Although yoghurt has unique nutritional and therapeutic values, yet it constitutes a highly selective environment favouring growth of yeasts and moulds, propably due to its low pH value and high lactic acid concentration. It may be exposed to fungal contamination during the chain of production that may results in economic losses through rejection of the contaminated products. Moreover, contamination of yoghurt with aflatoxin- producing moulds constitutes a public health hazard, especially when able to produce their related aflatoxins during storage *(Mossel, 1982 and Varnam and Sutherland, 1994).*

Concerning fungal spoilage of yoghurt, yeasts are the most important spoilage organisms. Yeasts are most commonly associated with fermentation of lactose leading to gas production that is readily recognized by " doming" of foil led or even burst containers. Although moulds are usually of secondary important to yeasts, yet mould growth at the yoghurt/ air interface in the form of " mats " or "buttons" is a more significant problems in yoghurt held for extended period at low temperature before being transferred to retail display *(Varnam and Sutherland, 1994).*

Various types of yoghurt are widely exposed to sale in local markets and constitute important food items in the diet of consumers of all ages. As the fungal contamination of yoghurt constitutes economic and public health importance, therefore the present study was planned to investigate the rate of fungal contamination and aflatoxin- producing moulds in various types of yoghurt, including plain, fruit and drinking types which are sold in Minoufiya Governorate, Egypt.

MATERIAL AND METHODS

Seventy five random samples of yoghurt, 25 from each of plain, fruit and drinking types were collected from different localities in Minoufiya Governorate, Egypt. The collected samples were immediately transferred to the laboratory for mycological examination.

1-Preparation of samples for mycological examination:

Each sample was thoroughly mixed before being examined . Eleven grams were thoroughly mixed with 99 ml of sterile saline to make a dilution of 1/10, from which 10- fold serial dilutions were prepared as described by *AOAC (1990).*

2-Mycological examination:

Yeast and mould counts were determined according to the technique recommended by APHA (1985), where duplicate plates of sabouraud's dextrose agar (containing 0.05 mg of chloramphinicol / ml) were inoculated with one ml from

each serial dilution before being incubated at 25+2°C for 5 days. The counts of yeasts and moulds were calculated and recorded.

The isolated yeasts were purified and identified according to *Looder and kreger Van-Rij (1970),* while the isolated moulds were identified according to *Samson et al.(1981); Larone ,(1987); Refai, (1988) and Deak and Beuchate (1996).*

3-Screening for aflatoxin producing strains of Aspergillus flavus:

The isolated strains of *A. flavus* were screened for aflatoxin production using the technique recommened by *Davis et al. (1987).*

4- Qualitative determination of aflatoxins:

Qualitative estimation of aflatoxins produced by the aflatoxigenic strains of *A. flavus* isolated from the examined plain and fruit types of yoghurt samples, was done according the technique recommended by *Shin and Marth, (1969).*

5- Quantitative determination of aflatoxin B_1 :

Quantitative estimation of aflatoxin B1 produced by the aflatoxigenic strains of *A. flavus* was performed according the technique recommended by Park et al., (1990).

RESULTS AND DISCUSSION

The results given in table (1) point out that fruit yoghurt shows the highest incidence (64%) and mean count $4.6x10^4 + 2.6x10^4$ of yeasts, followed by plain yoghurt (48%) with mean count of $5.5x10^3 + 3.1x10^3$ Drinking yoghurt shows the lowest incidence (28%) and mean count value $9.4x10^2 + 5.3x10^2$. Comparatively higher counts were reported by *Saad et al., (1987); Zin El-Din and El Sawah (1997) and Al Ganzoury (1994).* While relatively lower incidence and counts were recorded by *Varabioff (1983); Jordano Salinas (1984) and Nogueria et al., (1998).* The obtained results agree to some extent with those reported by *Ibrahim et al. (1989) and Manal (1994).*

The results recoded in table (2) reveal that 52%, 80% and 44% of the examined samples of plain , fruit and drinking type of yoghurt proved to be contaminated with moulds. The mean count values were $9.7x10^2 + 5.4x10^2$, $6.3x10^3 + 4.2x10^3$ and $4.4x10^2 + 1.6x10^2$ respectively.

The obtained results substantiated what have been reported by *Nagueria et al., (1998) and Mohamed (1999).* Relatively higher counts were reported by *Rodriguez- Ferri et al. (1978) and Saad et al.(1987)* while comparatively lower findings was reported by *Mansour et al., (1986).*

From the forementioned results it is evident that fruit yoghurt shows the highest incidence and counts of yeasts and moulds as compared to the other two types of yoghurt. This may attributed to the use of inferior quality fruits or fruit juices and / or inadequate sanitizing method in production, improper packaging or faulty

storage of the final product *(Foster et al. 1983; Varnam and Sutherland, 1994 and Jakobson and Nervhus, 1996)*

In general, the yeast and mould counts are used as an index for the state of sanitation and quality control of certain dairy products including yoghurt *(Jay, 1986).* Moreover, presence of large number of yeasts and moulds in yoghurt may induce undesirable changes that rendering the product unmarketable or even unfit for human consumption *(Robinson, 1990).*

The results recorded in table (3) reveals that the isolated yeasts from the examined samples of plain, fruit and drinking types of yoghurt were identified as *Candida albicans* (28%, 48% and 20%), *C. krusei* (20%, 24% and 8%), *Saccharomyces cervisiae* (12%, 20% and 0%) and *Rhodotorula glutinis* (8%, 0% and 0%) . Similar species of yeasts were isolated from yoghurt by *Rohm et al. (1992) and Al- Ganzoury (1993).*

Saccharomyces species are most commonly involved in fermentative spoilage of yoghurt. Oxidative yeasts as species of candida and rhodotorula are also important spoilage organisms in yoghurt, where their growth is limited by availability of oxygen and may be restricted to the air/ yoghurt interface in the form of moist, flat colonies or as a film of growth (Varnam and Sutherland, 1994).

Reviewing the results in table (3), the predominating moulds isolated from the examined plain, fruit and drinking types of yoghurt were *Aspergillus niger* (48%, 60% and 32%); *A. flavus* (4%, 4% and 0%); *A. fumigatus* (8%, 15% and 0%); *A. terreus* (4%, 8% and 0%); *A. candidus* (4%, 4% and 0%); Alternaria species (24%, 32% and 24%); Rhizopus species (12%, 20% and 12%); Fusarium species (8%, 12% and 8%) and Mucor species (8%, 4% and 4%), respectively.

Similar isolation rate of moulds from yoghurt were reported by *Sharma et al. (1993)* and *Mohamed (1999).* Relatively higher incidence of *A. flavus* in yoghurt was reported by *Jordano et al. (1989).*

Although moulds are usually of secondary importance to yeasts. Mould growth is sometimes a more significant problem in yoghurts held for extended peroids at law temperatures before transfere to retail display *(Varnam and Sutherland, 1994).*

Recorded results in table (4) reveal that two strains of *Aspergillus flavus* were isolated from two samples only, one from each of plain and fruit yoghurt and proved to be aflatoxigenic. Aflatoxins B_1 was produced by the two isolated strains in a concentration of 14 mg/ litre medium for the strain isolated from plain yoghurt and 16 mg/ litre medium for the strain isolated from fruit yoghurt samples.

Relatively higher incidence of aflatoxigenic strains of *A. flavus* was reported by *Jodral et al. (1991).*

Presence of aflatoxigenic strains of *A. flavus* in yoghurt constitutes a public health hazard, especially when they able to produce their aflatoxins in the products. Aflatoxins have been reported to cause acute hepatic necrosis and subacute liver

cirrhosis in childern *(Amla et al., 1971)* and hepatocarcinogens in man *(Marth and Calanog, 1976; Egmond and Wagstoffe, 1984 and Wood, 1992).*

In conclusion, one can realize from the results obtained in this work that the various types of yoghurts locally manufactured are still harbour undesirable fungi that affect their quality and safety. Therefore, strict hygienic measures should be imposed during the chain of production and storage. Using fruits of good quality or sterilized juices in fruit yoghurt is essential to improve the product quality and to minimize the public health risk of the consumers

REFERENCES

Al-Ganzoury, H.M. (1994): Occurrence of yeasts in milk and some dairy products. M.V.Sc. Thesis, Food Control Dept., Fac. Vet.Med., Zagazig Univ.

American Public Health Association ((APHA)) (1985): Standered methods for examination of dairy products. 15thEd., Washington, D.C., USA.

Amla, I.; Kamala, C.S.; Gopala Karishna, G.S.; Jayarai, A.P.; Sreenivasamurthy, V. and Parpia, H.A.(1971): Cirrhosis in children from Peanut meal contaminated by aflatoxin. American J. Clin. Nutr., 24 : 609-614.

Association of Official Analytical Chemists "AOAC" (1990): Official Methods of Analysis. 15th Ed., Arlington, VA, USA.

Corinne, G.D.; Claire, C.; Christophe, P.; Francoise, P.; Piere, V.; Christine, B.; Odette, S. and Cloude, A. (1998): Milk fermented with yoghurt cultures and Lactobacills casei compared with yoghurt and gelled milk : Influence on intestinal microflora in healthy infants. Am.J. Clin.Nutr., 67: 111-117.

Cross, M.L.; Stevenson, L.M. and Gill, H.S. (2001): Antiallergy properties of fermented foods : An important immunoregulatory mechanism of lactic acid bacteria. Int.Immunopharmacol., 1 (5): 891-901.

Davis, N.D.; Lyer, S.K. and Diener, U.L. (1987): Improved method of screening for aflatoxin with coconut agar medium. Appl.Environ. Microbiol., 53 : 1593-1599.

Deak, T. and Beuchate, L.R. (1996): Handbook of Food Spoilage yeasts. CRC Press, Inc., USA.

Egmond, H.P. and Wagstoffe, P.J. (1984): The certification of aflatoxin M_1 in milk powder samples. Commission of the European Communities. 10412, 6 pp. 248-285.

Foster, G.M.; Nelson, F.E.; Speck, M.L.; Doetsch, R.N. and Olson, J.C.(1983): Dairy microbiology, 3rd Ed., Ridgriew Publ. Co., California, USA.

Ibrahim, M.R.; El-Batawy, M.A. and Elein, S.G. (1989): Evaluation of yoghurt on the Cairo market. Egypt.J.of Dairy Sci., 17: 125-136.

Jakobson, M.and Narrhus, J.(1996): Yeasts and their possible benificial and negative effects on the quality of dairy products. Intern. Dairy J. 6: 755-768.

Jay, J.M. (1986): Modern Food Microbiology. 3rd Ed. Van Nosrand, Reinhold Co., New York.

Jodral, M.; Salmeron, J.; Fernadez, C.; Garriedo, M. and Jordano, R. (1991): Influence of evaluation of the pH on contaminating microflora of heat treated fermented milk. Scienzae-Tecnica- Lattiero-Gasearia, Italy 42 (3): 161-170.

Jordano-Salinas, R.(1984): Yoghurt contamination with yeasts and moulds. Archivos Zootecia, 33(125): 97-103. Dairy Sci. Abst., 47, 2 (1985).

Jordano, R.; Jodral, M.; Martinez, P.; Salmeron, J. and Pozo, R. (1989): Aflatoxin-producing strains of Aspergillus flavus in yoghurt. J.Food Prot., 5 (11): 823-824.

Larone, D.H. (1987): Medically important fungi. A guide to identification. 2nd Ed., Elsever Science Publ.Comp.

Looder, J. and Kreger Van Rij, N.J.W. (1970): The yeasts. A taxonomic study. 2ndEd.,North Holand Publishing Co., Amesterdam.

Manal, E.I.H. (1994): Assessment of microbial quality of Egyptian dairy Desserts. Zagazig Vet.J. 22(1): 15-17.

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

Marth, E.H. and Calanog , B.G. (1976): Toxigenic fungi. pp.210-256. In M.P. Defigueridо and D.F. Splittoesser (eds) Food Microbiology : Public health and spoilage aspects. AVT Publishing Co.

Mohamed, A. Salwa (1999): Studies on Mycotoxins in milk and some dairy products. Ph.D. Thesis, Food Control Dept., Fac.Vet.Med. Cairo Univ.

Mossel, D.A. (1982): Microbiology of food.3rd Ed. The Univ. of Ultrech., The Netherlands.

Nadathur, SR.; Gould SJ. And Bakalinsky, AT. (1995): Antimutagenicity of an acetone extract of yoghurt. Mutate Res., 334 (4) : 213-221.

Nogueria, C.; Albano, H.; Gibbs, P. and Teixeira, P. (1998): Microbiological quality of Portuguese yoghurts. J. Of Indust.Microbiol. and Biotechnol., 21 (1/2):19-21.

Park, D.L.; Nesheim, S.; Trucksess, W.M.; Stack, M.E. and Newell, F.R. (1990): Liquid chromatographic method for determination of aflatoxin B1, B2, G1 and G2 in corn and peanut products. Collaborative study. J. Ass. Off. Anal. Chem., 73 (2): 260-266.

Piaia, M. (2001): Fermented milk and successful aging. Danone World Newsletter No. 22, 1-14.

Refai, M.K. (1988): Isolation and identification of fungi.
Published by Fac.of Vet.Med.Cairo Univ.

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

Robinson,R.K. (1993): Modern dairy technology, Vol.2: Advances in milk products 2nd Ed., Elsevier Science Publishers Ltd., London and New York.

Rodriguez-Ferri.; Diazferndez, E, and Alvarez, F.E. (1978): Hygienic quality of yoghurt. Alimentaria, 91, 35-39. Dairy Sci. Abst., 40, 10 : 626.

Rohm, H.; Eliskases-Lechner, F. and Brauer, M. (1992): Diversity of yeasts in selected dairy products. J.Appl. Bact. 72(5): 370-376.

Saad, N.M.; Moustafa, M.K. and Ahmed, A.A. (1987): Microbiological quality of yoghurt produced in Assuit City. Assuit Vet.Med.J. 19: 37-87.

Samson, R.A. ; Hoeksta, E.S. and Van Ooerschot, C.A.N. (1981): Introduction to food fungi. Centraalbureau voor Schimme lcultures, Baarn, The Netherlands.

Sharma, S.D.; Gill, J.P.; Joshi, D.V. and Kwatra, M.S. (1993): Microflora of indian fermented milk product. Indian J. Dairy Sci., 46 (2): 85-87.

Shin, C.N. and Marth, E.H. (1969): Improved procedures for measurement of aflatoxin with thin layer chromatogrphy and flometery.
J.Milk Food Technol., 32: 213-217.

Varabioff, Y. (1983): Spoilage organisms in yoghurt. Dairy Products J. (2): 8-12. Dairy Sci. Abst., 46(9): 115 (1984).
Varnam, A.H. and Sutherland, J.P. (1994): Milk and milk products. Technology, Chemistry and Microbiology. 1st Ed. Chapman and Hall Inc., New York, USA.
Weinsier, R.L. and Krumdieck, C.L. (2000): Dairy foods and bone health: Examination of the evidence. Am.J. Clin.Nutr., 72 (3): 681-689.
Wood, G.E. (1992): Mycotoxins in food and feeds in the United States. J. Animal Sci., 70 (12): 3941-3949.
Zin El-Din, M and E-Sawah, M. (1997): Evaluation of yoghurt manufactured and cosumed in Mansura City. Agric. Sci.J. 22 (12) : 4427-4442.

Table (1) Statistical analytical results of yeast count/g. of examined yoghurt samples.

Type of samples	No. of samples	positive Samples		Minimum	Maximum	Mean+	SEM
		No.	%				
Plain yoghurt	25	12	48	$2x10^2$	$4x10^4$	$5.5x10^3$	$3.1x10^3$
Fruit yoghut	25	16	64	$5x10^2$	$3.5x10^5$	$4.6x10^4$	$2.6x10^4$
Drinking yoghurt	25	7	28	6x10	$7.3x10^3$	$9.4x10^2$	$5.3x10^2$

Table (2) Statistical analytical results of moulds count/g. of examined yoghurt samples.

Type of samples	No. of samples	positive Samples		Minimum	Maximum	Mean+	SEM
		No.	%				
Plain yoghurt	25	13	52	3x10	$4x10^3$	$9.7x10^2$	$5.4x10^2$
Fruit yoghut	25	20	80	$2x10^2$	$1.7x10^4$	$6.3x10^3$	$4.2x10^3$
Drinking yoghurt	25	11	44	2x10	$2x10^3$	$4.4x10^2$	$1.6x10^2$

Table (3): Incidence of isolated yeasts and moulds in the examined yoghurt samples.

Isolates	Plain Yoghurt		Fruit yoghurt		Drinking Yoghurt	
	No.	% *	No.	% *	No.	% *
Yeasts:						
Candida albicans	7	28	12	48	5	20
C. krusei	5	20	6	24	2	8
Saccharomyces cervisiae	3	12	5	20	0	0
Rhodotorula glutinis	2	8	0	0	0	0
Moulds:						
Aspergillus niger	12	48	15	60	8	32
A.flavus	1	4	1	4	0	0
A.fumigatus	2	8	3	15	0	0
A. terreus	1	4	2	8	0	0
A. candidus	1	4	1	4	0	0
Alternaria species	6	24	8	32	6	24
Rhizopus species	3	12	5	20	3	12
Mucor species	2	8	1	4	1	4
Fusarium species	2	8	3	12	2	8

* The percentages were calculated according to the total number of samples (25)

Table (4): Incidence of Aflatoxin-producing strains of Aspergillus flavus isolated from examined yoghurt samples and their aflatoxin produced.

Types of samples	No. of samples	%	No. of isolates	Type of aflatoxin	Concentration of aflatoxin
Plain yoghurt	1	4	1	B_1	14 mg/L.
Fruir yoghurt	1	4	1	B_1	16 mg/L.

الملخص العربى

الجودة الميكولوجية للزبادي مع الأشارة الخاصة للفطريات المفرزة للأفلاتوكسين

عبدالرحمن محمود الباجورى و عبد العزيز عبد الخالق مسعد

تم الفحص الميكولوجى لعدد ٧٥ عينة عشوائية من الزبادى ، ٢٥ عينة من كل من الزبادى الطبيعى؛ الزبادى بالفواكه ومشروب الزبادى والتى ثم تجميعها من السوبر ماركت ومحلات البقالة باماكن مختلفة من محافظة المنوفية وقد أسفرت النتائج من النسب والعدد الكلى للخمائر فى أنواع الزبادى والتى تم فحصها كانت بنسب ٤٨%، ٦٤% ، ٢٨% ، على التوالى وكان العدد الكلى ٥,٥ x ١٠ ^{٣} + ٣.١ x ١٠ ^{٣} ،٤,٦ x ١٠ ^{٤} +٢.٦ x ١٠ ^{٤} و٩.٤ x ١٠ ^{٢} + ٥و٢ x ١٠ ^{٢} على التوالى

بينما نسب تواجد الفطريات هى ٥٢ % ،٨٠ %، ٤٤ % على التوالى وبعدد كلى٧و٩ x ١٠ ^{٢} + ٤و٥ x ١٠ ^{٢} ،٣ و٦ x ١٠ ^{٣} +٢و٤x ١٠ ^{٣} و٤و٤x ١٠ ٢ + ٦و١ x ١٠ ^{٢} على التوالى كما تم عزل وتصنيف اصناف مختلفة من الخمائر والفطريات بنسب مختلفة.

وكذلك تم عزل وتصنيف فطر الأسبرجيلاس من عينة من كل من الزبادى الطبيعى والزبادى بالفواكة. وقد أثبتت التحاليل ان العترات المعزولة من النوع المفرز للأفلاتوكسين ب١ . وقد تم مناقشة الأهمية الصحيه والإقتصاديه للخمائر والفطريات المعزوله وكذلك الإقتراحات الخاصه لتحسين جودة المنتج وكذلك حماية المستهلك..