دراسة عن الفطريات الموجوده فى الجبن

ت . البسيونى ، م . عطيه ، ف . أبوالخير

تم فحص ٨٠ عينه من أنواع الجبن المختلفهجمعت من أسواق مدينة أسيوط للتعرف على أنواع الفطريات المختلفة المحتمل وجودها . وقد تم عزل عدد كبير من الفطريات المختلفة وأثبتت النتائج أن بعضها لـه القدرة على أفراز السموم (الافلاتوكسين) .

وقد وجد أن الجرعة المميته عند حقن الفئران فى الغشاء للبريتونى بمستخلص الافلاتنكسين كانت ٧٥ر٠ سم لكل ١٠٠ جرام من وزن الحيوان .

أن وجود الفطريات المعزولة بكميات كبيرة فى الجبن دليل على أهمال الاشتراطات الصحية الواجب توافرها أثناء تصنيع وتوزيع هذا المنتج .

وقد تم مناقشة الأهمية الصحية لوجود هذه الفطريات وسمومها على الصحة العامة .

Dept. of Hygiene and Food Control,
Faculty of Vet. Med., Assiut University,
Head of Dept. Prof. Dr. Ali Lotfi.

SEARCH FOR THE PREDOMINANCE OF FUNGI SPECIES IN CHEESE

(With 5 Tables and 3 Figures)

By

T.A. EL-BASSIONY, M. ATIA and F. ABOUL KHIER

(Received at 3/2/1980)

SUMMARY

A total of 80 samples of Damietta, Kareish, hard and processed cheese were collected from Assiut City Markets. 11 genera of molds have been isolated in different percentages. Aspergillus species were the most prevalent. On the other hand, candida species specially C.albicans were more prevalent in examined cheese samples rather than other thpes of yeasts.

From the isolated fungi, one strain of Asp, flavus was found to be a toxin producer. Regarding the mortality dose responses, the results revealed that 0.75 ml of the extract/100 grams body weight was the minimum dose that killed 8.3% of the injected rats in 24 hours. The public health significance of each isolate was discussed.

INTRODUCTION

Pathogenic fungi can infect udder tissues and then excreted in large numbers in milk, some of the species implicated in cases of mastitis, in dairy animals, may induce pathological conditions in man, hence their presence in milk and dairy products constitutes a public health hazard.

The molds capable of forming toxins (mycotoxins) causing mycotoxicosis in man and animals are distributed allover the world. They grow and produce toxins on a wide variety of foods over wide ranges of temperature and humidity.

Yeasts and molds are undesirable organisms in most dairy products because they affect flavour, producing musty odour and bitter taste. Moreover, some species are of special interest as they may cause different diseases in man and animals.

DIMITROV and KHADZHIMISTSEV (1960) isolated 5 strains of Penicillium mold and one of Asperigillus flavus form cheese during storage at 4-7°C, while GHONEIM (1963) examined 200 samples of Damietta cheese and found that yeasts were present in all samples. Candida species were predominated than Torulopsis, Cryptococcus and Trichosporon species. On the other hand, molds were detected in 91% of samples. Penicillium and Aspergillus species were the most prevalent, while Rhizopus, Cephalosporium and Mucor species were the least. Different species of yeasts and molds were also isolated from cheese by BULLERMAN and OLIVIGNI (1974), TILBURY et al., (1974), MEHRAN et al., (1975) and Bullerman, (1976).

OLDHAM et al. (1971) inoculated the surface of cheddar cheese samples with Asp. flavus and found that aflatoxins were detected after 12 days in the samples incubated at 25°C, but not in refrigerated samples kept at 4.4°C or 7.2°C. SHIH and MARTH (1972) found that Asp, flavus produced aflatoxins in cheese stored at 29°C after 14 weeks, while Asp. parasiticus after only one week.

Realizing that cheese is an excellent medium for many species of fungi and in-turn is regarded as a potential danger to man, therefore, this work was planned to secure the occurrence of fungi in different types of cheese.

MATERIAL AND METHODS

80 random samoles of Damietta, Kareish, hard and processed cheese (20 each) were collected from Assiut City markets. With a minimum time of delay each sample was thoroughly mashed in a sterile morter, where a part was blended in a sterile 2% sodium citrate solution for plating.

Isolation and identification of fungi:

Loopfulls from cheese dilution were streaked on Sabourauds dextrose, as well as Litman oxgal agar plates (MOSS and Mc QUEWN, 1969) with and without penicillin and streptomycin. Inoculated plates were incubated at 37°C for 48 hours, then left at room temperature (20-25°C) for 7 days before bieng examined. Suspected colonies of isolated fungi were recognized by the morphological and physiological characters as well as microscopically according to LODDER and KREGAR (1967) and RIPPON (1975).

Detection of Aflatoxins:

6 strains of Asp. Flavus and 9 Asp, niger were isolated from different types of cheese were tested for aflatoxin production. The selected strains were morphologically apparent to be aflatoxins producer (MUROKAMI and SUZUKI, 1968).

For preparation of toxins, each strain of Aspergillus was cultured on Sabouraud glucose broth and modified Czapek liquid media(DAVIS et al. 1966). The cultures were incubated at 25°C for 4 weeks. The fungal mycelium was exposed to reflux extraction with either and the culture broth was filtered by EK Seitz fiter. Both the extracts and culture filtrates were tested for the occurrence of toxins by:

1. Ring test (SHESTAK, 1960).
2. Dermal test in rabbit (FORGACS and CARLL, 1962).
3. Absorption of U.V. rays (WOGAN, 1966).

Estimation of pathogenicity of toxins to rats:

For estimation of the mortality dose response (BLISS, 1962), 9 groups, 8 rats each, were injected intrapertitinially with increassing doses (0.50, 0.65, 0.75, 0.85, 1.1, 1.4, 1.8, 2.0 2.5) of Asp, flavus extract. A. control group (non injected) of rats was included. The animals that survive were sloughtered after 14 days where the Livers and Kidneys were examined histopathologically.

RESULTS AND DISCUSSION

All results obtained are recorded in Tables (1-5) and Fig. (1-3).

The analytical results of isolated yeast species given in Tables (1& 2) show that candida species specially C. albicans were more prevalent in examined cheese samples rather than other types of yeasts. Similar findings were reported by GHONEIM (1963) and TILBURY et al. (1974).

Yeasts may gain access to cheese from animals suffering from mycotic mastitis or during processing. In this respect, C. albicans, C. parapsilosis and C. pseudotropicalis were isolated by REFAI (1963) from milk in case of mastitis. The higher incidence of yeasts in cheese, may be attributed to the wide use of antibiotics practised nowadays (REDAELLI, 1957, KREJAKOVIC and STOJANOVIC, 1960 and MEHNERT; 1962).

Table (1): Occurence of different types of yeast in cheese:

Types of cheese	Damietta		Dareish		Hard		Processed	
Types of yeasts	No. of samples +ve	%	No. of samples +ve	%	No. of samples +ve	%	No. of samples +ve	%
Candida sp.	7	35	9	45	4	20	6	30
Cryptococcus sp.	-	-	7	35	2	10	-	-
Geotrichum candidum	3	15	2	10	-	-	2	10
Rhodotorula glutinis	-	-	2	10	2	10	-	-
Torulopsis	1	5	-	-	-	-	-	-

Most of the isolated yeasts are saprophytes which contaminate cheese. Their presence ultimately leads to cheese spoiloge by breadking down its component and liberating different acids and gases with subsequent change of its odour and flavour.

Tables (2&3) show that 11 gnera of molds have been isolated from examined samples in different percentages. Aspergillus species were the most prevalent. Similar results have been reproted by GHONEIM (1963),

MEHRAN et al. (1975) and BULLERMAN (1976).

Table (2): Frequency distribution of Candida species in cheese.

Types of cheese	Damietta		Kareish		Hard		Processed	
Candida species	No.of samples +ve	%	No. of samples +ve	%	No. of samples +ve	%	No. of samples +ve	%
C. albicans.	3	42.80	6	66.66	3	75	2	33.33
C. tropicalis	2	28.60	-	-	-	-	-	-
C. pseudetropicalis	2	28.60	-	-	-	-	2	33.33
C. parapsilosis	-	-	3	33.33	1	25	2	33.33
Total	7	100	9	100	4	100	6	100

Molds may grow over an extremely wide range of temperature therefore, molds can be present on practically all food at almost any temparature under which foods are held, Among the important mold species usually responsible for food spoilage are Aspergillus, penicillium,Mucor and Rhizopus. Moreover, molds are capable of forming toxins and causing mycotoxicosis in man and animals.

The pothogenic and conditionally pathogenic species of Aspergillus induce various clinical forms of aspergillosis which is most prevalent in birds,but may also occure in mammals including man. Moreover, certain Aspergillus species can produce flatoxin in cheese (OLDHAM et al. 1971; SHIN and MARTH, 1972; GRANT and CARLSON 1974 and JACQUET and TEHERANI, 1976).

The results obtained in Table (5)revelaed that all strains of Asp. niger isolated from cheese samples failed to produce toxins, while one strain (16.7%) of Asp. flavus was found to be a toxin producer. The pressence of toxins in the mycelial extract and cultural filtrate was confirmed by the formation of a white ring at the junction of ether and the mixture of extract or filtrate with 2 N sodium hydroxide solution. Also, both the filtrate and extract shwoed strong fluorscence when exposed to

Table (3): Occurence of different types of moulds in cheese:

Types of moulds	Demietta		Kareish		Hard		Processed	
	No. of Samples +ve	%	No. of samples +ve	%	No. of samples +ve	%	No. of samples +ve	%
Aspergillus sp.	9	45	6	30	4	20	7	35
Penicillium sp.	2	10	5	25	6	30	2	10
Muccor sp.	-	-	3	15	4	20	-	-
Cladosporium werneckii	-	-	2	10	-	-	-	-
Cephalosporium acremonium	2	10	-	-	1	5	1	5
Alternaria sp.	4	20	-	-	-	-	-	-
Fusarium	-	-	1	5	-	-	-	-
Streptomyces	-	-	-	-	2	10	-	-
Allescheria boydii	-	-	-	-	1	5	-	-
Botrytes cenaria	4	20	-	-	-	-	4	20
Dematiaceae	1	5	-	-	2	10	4	20

Table (4) Frequency distribution of Asperigillus species in cheese:

Type of cheese / Aspergillus sp.	Damietta		Kareish		Hard		Processed	
	No. of samples +ve	%	No. of samples +ve	%	No. of samples +ve	%	No. of samples +ve	%
Asp. flavus	-	-	2	33.33	2	50	2	28.60
" niger	2	22.22	4	66.66	-	-	3	42.80
" fumigatus	3	33.33	-	-	2	50	-	-
" terreus	2	22.22	-	-	-	-	2	28.60
" minor	2	22.22	-	-	-	-	-	-
Total	9	100	6	100	4	100	7	100

Table (5): Aflatoxin production from Asp. flavus and Asp. niger.

Types of molds	No. of strains isolated	Aflatoxin production			
		+ve		-ve	
		No. of strains	%	No. of strains	%
Asp. flavus	6	1	16.7	5	83.3
Asp. niger	9	0	0	9	100

to U.V. light. In oddition, the application of the mycelial extract in oil to the skin of rabbit caused acute dermatitis.

Regarding the mortality dose respones,it was noticed that 0.75 ml. of the extract/100 grams body weight was the minimum dose that killed 8.3% of the injected rats in 24 hours. The mortality rate increased in accordance with the inoculated dose. When 2-2.5 ml./100grams body weight were injected all animals died.

Moreover, the dead rats showed cyanosis of the nose, limbs, mucous membranes, liver, spleen and kidneys. Histopathological investigation revealed marked toxic tissue alteration. The change in the liver varied from cloudy swelling with acidophilic and granular cellular degeneration to fatty metamorphosis (Fig. 1 & 2), while the kidneys showed interstitial nephritis (Fig. 3).

The yeast and mold counts are used as an index of the proper sanitation and quality control products for certain dairy products (FOSTER et al., 1958).

From the above achieved results it is worthmentioning that cheese is liable to contamination from different sources during its processing and handling. Moreover, the contaminants whether pathogenic or deteriorating foungi, find opportunities to thrive in the product constituting a

public health hazard.

Therefore, to safe-guard consumers from bieng infected and to safe a lot of the product from being deteriorated, hygienic measures and strict regulations should be imposed for production and handling of the product.

REFERENCES

Bliss,R. (1962): Cited by SolimanM.A. (1964): Thesis, Fac. of Vet. Med. Giza.

Bullerman,L.B. (1976): Examination of swiss cheese for incidence of mycotoxin producng molds. J. of Food Sci., 41, 1.

Bullerman, L.B. and Olivigni, F.I. (1974): Mycotoxin producing potential of molds isolated from cheddar cheese. J. of Food Sci. 39, 6: 116-1168.

Davis,N., Dinner, U and Kldridge,D. (1966): Production of Aflatoxin BI and GI by Asp. flavus in synthetic media. Appl. Microbiol., 14,378.

Dimitrove,M. and Khadzhimistsev,P.(1960): Normal microflore of kachkaval cheese and its effect on its composition, Sborn. Trud. Nauchnoizsl. Zan-Khigien. Inst. 6. 137-140, Dairy Sci. Abs. 25 : 7 (1963).

Forgacs,J. and Carll,T. (1962): Vet. Sci., 7 : 213.

Foster.,E.M., Nelson, F.E., Speck, M.L., Duetsch, R.N. and Olson, J.C., (1958): Dairy Microbiology. Macmillan and Co. LTD. London.

Ghoneim,N. (1963): Studies on the microflora of Damietta cheese M.D.Vet. Thesis, Cairo University.

Grant,D.W. and Carlson,F.W. (1974): Partitioning behaviour of Aflatoxin M.in dairy products.Review of Medical and Veterinary Mycology, Vol. 9. No. 3.

Jacquet,J. and Teherani,M.(1976): Unusuall presence of aflatoxin in certain products of animal origin. Review of Medical and Vet. Mycology Vol. II. No. 1.

Krejakovic and Stogjanovic (1960): Cited by Osman et al. (1970).

Lodder,J. and Kregar,V.N. (1967): The yeasts a taxonemic study. 2nd Fd. No. 2th Holland Publishing company Amesterdam, Holland.

Mehnert (1962): Cited by Osman et al. (1970): The yest flore of Egyptian butter. Mykosen 13 (12): 97-10.

Mehran,M., Behboodi,M. and Rouhbekhsh-Kh,A, (1075): Microbial contamination of Iranian white cheese produced from rawmilk. J. Dairy Sci. 58 (5) 784.

Moss, E.S. and Mcquewn, A.L. (1969): Atlas of medical mycology. Williams and Wilkins Co. Baltimore.

Murokami, H. and Suzaki, M. (1968): Mycological differences between the producer and non producer of Aflatoxin of Aspergillus. Processing. ISt U.S. Gapan onference on toxic microorganisms.

Oldham,L.S., Oheechme,F.W. and Kelley,D.C. (1971): Production of aflatoxin in pre-packaged lunchoon meat and cheese at refrigerator temperature. J. Milk Food. Technol., 34, 7 549-351.

Redaelli (1957): Cited by Osman et al. (1970).

Refai (1963): Cited by Ghoniem and Refai (1968): Incidence of candida species in Damietta cheese. Mykeson 11 (4): 295-298.

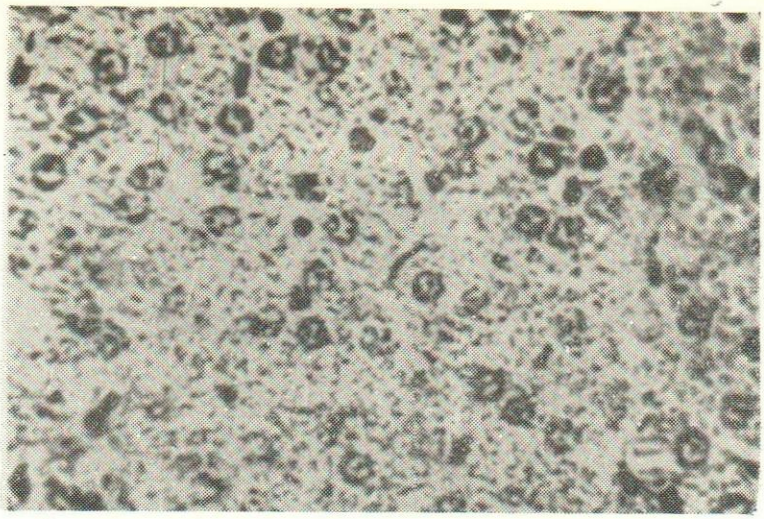
Rippon, J.W. (1974): Medical mycology. The pathogenic fungi and the pothogenic actinomycetes. 1st ed. W.B. Saunders S. Philadelphia London Ioronto.

Shestak,S. (1960): Detection of Mycotoxins. Veterinariya., 8: 67.

Shin,C.N. and Marth,E.H. (1972): Experimental production of aflatoxin on birck cheese. J. Milk food Iechnol. 35, 10 : 585-587.

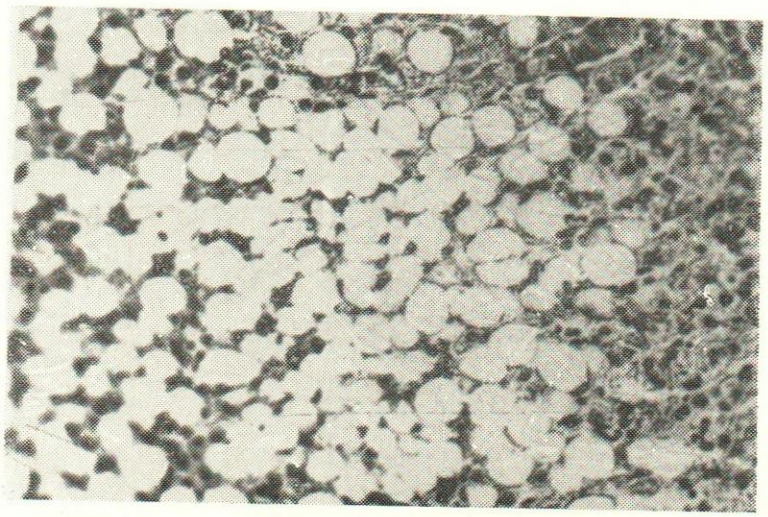
Tilbury,R.H., Davis,J.G., Rfrench,S., Imrie,F.K.E., Campbeli-lent,K. and Orbell, C. (1974): Taxonomy of yeasts in yoghourts and other dairy products. In proceeding of the fourth Internat. Symposium on yeast. Vienna, Austria, Inst. of food Technol. Univ. of Agricl. 265-266. Dairy Sci. Abs. 38, 3 (1976).

Wogan,G; (1966): Bact. Rev., 2 : 460.



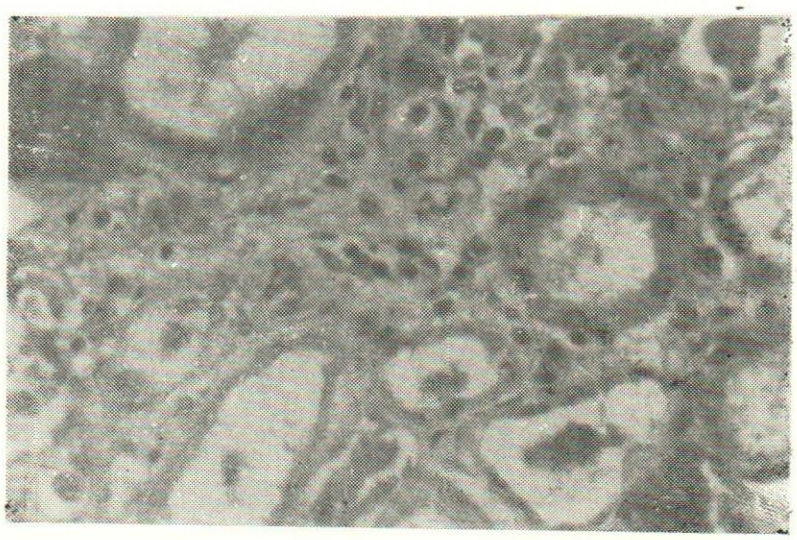
(Fig. 1)

Cloudy swelling in the liver



(Fig 2)

Fatty metamorphosis in the liver



(Fig. 3)

Nephritis in the kidney