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Fungal Contamination of White Cheese at the Stage of Consumption in Saudi Arabia

Laila A. Nasser
Girl's College of Education, Riyadh, Saudi Arabia

Abstract: Thirteen species appertaining to 4 genera of molds and yeast were isolated from twenty cheese samples using malt extract and 10% NaCl-Czapek's gar media at 25°C. The results revealed that the white cheese under examination were less polluted and most of the samples tested, were free of fungi comparing with other findings. The total fungal counts in cheese samples were relatively low and ranged from 95 and 125 colonies / 160 cheese pieces on the two isolation media, respectively. Members of *Penicillium* (*P. brevicompactum, P. chrysogenum, P. paxilli* and *P. waksmanii*) and *Aspergillus* (*A. flavus, A. niger* and *A. oryzae*) were the most prevalent fungi on cheese samples. Also, some unknown yeasts were encountered, in moderate occurrence. The preliminary examination, using 70 isolates, was done to test their ability to produce protease enzyme. The results indicated that 59 fungal isolates showed their ability to hydrolyze milk protein. Among the positive isolates 24 could produce the enzyme in high amount, while 20 produce moderate, and remaining 15 produce weak amounts.

Key words: Cheese, fungal contamination, *Aspergillus, Penicillium*

Introduction

Cheese is considered as one of the most important foodstuffs consumed by human and it contains a source of high quality animal protein having all the essential amino acids (El-Shrief, 2000). It is also a rich source of calcium, phosphorus and many other micronutrients. Cheese can easily become molded by a large number of species of fungi and a group of unicellular fungal organisms (yeast) during ripening, after cutting and slicing and during storage in shops or at home (Carter & Cole, 1990). Fungi are undesirable organisms which can grow, at a wide range, causing spoilage as well as are responsible for flavour defects, discoloration and poor appearance of the product (Walker, 1977). Furthermore, they produce toxic metabolites (mycotoxins) which may penetrate the cheese and polishing its surface might not sufficiently remove them and effect the health of the consumers (Abdel Sater *et al.*, 1995; Lund *et al.*, 1995; El-Sharief, 2000).

Proteases are one of the most important groups of enzymes constituting two thirds of the total industrial enzymes marketed (Gehartz, 1990; Singh *et al.*, 1994). They play an important role in the food processing industry (Wu & Hong, 1998). These enzymes have been isolated and characterized from mammals, plants, bacteria, yeasts and fungi (Nielsen, 1991; Beuchat & Pitt, 1992).

The main objective of this work was to establish the distribution of fungi in different types of cheese commonly consumed in Saudi Arabia. Also, evaluation of proteolytic activity by several strains of fungi was detected.

Materials and Methods

Twenty different cheese samples were collected from various markets in Riyadh-City, Saudi Arabia, (Table 1). The samples were transferred to the laboratory and kept in a refrigerator (6-8°C) till fungal analysis.

Estimation of fungi: The plating technique method was employed for this experiment. Four pieces (about 0.5 g) from each sample were scattered on the surface of plates containing solidified malt extract agar (g/L: malt extract, 20; peptone, 5; agar, 15) and 10% NaCl-Czapek's agar media (g/L; glucose, 10; $NaNO_3$ 3; KH_2PO_4. $7H_2O$; $MgSO_4$, 0.5; KCl^-, 0.5; agar, 15). Four plates for each sample (2 for each medium) were used. The plates were incubated at 25°C for 1-2 weaks and the developing fungi were counted, identified and calculated per 8 pieces for each sample.

Table 1: Number of sample and kind of cheese under examination

No.	Type of cheese
1	Pinar
2	Kraft light (Phildelphia)
3	Danish feta (Danland)
4	La Vache qui rit (cream)
5	Danish feta (Almarai)
6	Kraft (Milky Cheese spread)
7	Franch feta (Valbreso)
8	Danish white (The three cows)
9	La vache qui rit (Savoury)
10	Strong cow
11	Cream cheese (Almarai)
12	Kraft (cream Spread)
13	Mozzarella
14	Al Fallaha Labneh
15	Danish (Mozzarella
16	Egyptian cheese
17	Saudi Arabian Cheese
18	Syrian haloom Cheese
19	Turkish cheese
20	Czech cheese

Proteolytic activity: The prevalent species, in the current study, were screened for their ability to produce protease enzyme using the procedure of El-Gendy (1966). The medium of the following composition (g/L) was used. Peptone 10, agar 20, pH 6.8. After autoclaving the medium, sterile skim milk (10% solution of powder deffated milk in water was sterilized separately) was added at the rate of 5 ml per 100 ml medium before pouring in plates. Test fungi were inoculated onto the center of the plates and incubated at 28°C for 10 days. Complete degradation of milk protein was seen as clear zone around the colonies. The clear zones were recorded.

Results and Discussion

The mycological analysis of the most consumed white cheese in Saudi Arabia, revealed that the samples were less contaminated and most of the samples tested were free from fungi comparing with other findings (Table 2). Thirteen species representing 4 genera of moulds and yeast were isolated from the twenty cheese samples tested using plate-bating technique on malt extract (8 species + 3 genera) and 10% NaCl-Czapek's agar (12 and 4) at 25°C. The fungal total counts in cheese samples were relatively low and ranged from 95 to 125 colonies/160 cheese pieces on the two isolation media, respectively. The lowest incidence of fungal propagules in tested cheese signifying the accurate methods of processing, pickling and preservation techniques of mainly cheeses. Also, due to continuous sampling of different food products to be analyzed for microbiology and get rid of positive lots and the severe restrictions and rigorous laws made by Saudi government on export food and foodstuffs. The present results are in contrast with other reports on milk and milk products in many parts of the world. For instance, the counts of moulds in Swiss cheese, as reported by Bullerman (1976), ranged from 10-750 colonies/g. Kivance (1990) examined 25 random samples of fresh Van herby and pickled white Turkish cheeses for moulds and aflatoxins. He observed that the mean values of the total moulds were $2.5x10^5$ and $4.95x10^4$ colonies/g, respectively. Also, the same author (1992) tested hard cheese (surface and core) for fungal contamination and found that the mean total mould counts were $3.02x10^{10}$ and $3.02x10^3$ colonies/g, respectively. Lund *et al.* (1995) identified 371 fungal isolates from hard, semi hard and semi soft cheeses from Denmark, France and other countries. But, in spainian cheese, Arizcum *et al.* (1996) found that all mould counts were <1000 colonies/g and they could identify 9 genera from the samples examined. In Egyptian cheese, El-Bassiony *et al.* (1980) isolated 11 genera of moulds. El-Sawi *et al.* (1994) examined buffaloes milk cottage and isolated 35 species belonging to 9 genera. Also, in (1995) Abdel-Sater *et al.* and Saad & Hemida isolated respectively 26 and 29 species belonging to 16 and 8 genera from four types of local Egyptian and roqueforti cheeses. The previous authors indicated that these cheeses were heavily polluted with micro-organisms with counts of 3500-11000 colonies/g cheese. More recently, El-Shrief, in Egypt (2000) examined 260 samples representing 5 kinds of cheese locally manufactured.

The results in Table 2 indicated that members of *Penicillium* and *Aspergillus* were the mainly contaminated moulds of all the tested samples. These results were greatly identical to those obtained by Polonelli *et al.* (1984), Pitt & Hocking (1985), Scott (1989), Abdel-Sater *et al.* (1995), Taniwaki & Dender (1992); Arizcum *et al.* (1996), El-Shrief (2000). They indicated that the majority of moulds isolated from cheeses and other foodstuffs consisted of *Penicillium* and *Aspergillus*.

Penicillium was the first common genus in tested samples. It was occurred in 56 and 59% of the samples contributing 56.8 and 66.7% of total fungi on malt extract and 10% NaCl-Czapek's agar, respectively. From this genus, 7 species were identified of which *P. brevicompactum*, *P. chrysogenum*, *P. paxillii* and *P. waksmanii* were the most prevalent. They were present in 25-45% and 20-55% of the samples on the two isolation media. The remaining three *Penicillium* species were isolated in rare occurrence at least on one medium and not on the other (*P. corylophillum* and *P. frequentans* on 10% NaCl, but *P. roquefortii* on malt extract) at 25°C (Table 2). The above species and other *Penicillium* species were also, isolated, with variable incidences, from different cheese samples all over the world (El-Malt, 1993; Hassanin, 1993; El-Sawi *et al.*, 1994; Abdel-Sater *et al.*, 1995; Lund *et al.*,1995, Larsen, 1997; El-Sharief, 2000).

Aspergillus (5 species) was the second dominant genus, emerging in 53% and 58% of the samples comprising 41.1 and 34.4% of total fungal isolates on malt extract and 10% NaCl-Czapek's agar, respectively. Among the 5 species isolated from the genus, *A. flavus* and *A. niger* were the most common. They were isolated from 50 and 53 and 46 and 50% of the samples on the two isolation media, respectively. *A. oryzae*, *A. sydowii* and *A. terreus* were less frequent and isolated each from 15 or 20% of the samples (Table 2). In this respect, several species of *Aspergillus* were isolated from raw milk or milk products in many parts of the world. Bullerman (1980) isolated *A. flavus* and *A. ochraceus* from domestic and imported retail samples of cheese. Aran & Eke (1987) could isolate *A. versicolor* and *Eurotium* species from Turkish Kasar cheese. Hassanin (1993) could isolate *A. flavus* and *A. niger* from 16 samples of cheese collected randomly from several locations in Cairo and Giza, Egypt. Lund *et al.* (1995) isolated *A. versicolor* from hard, semi-hard and semi-soft cheese from Denmark, France and other countries. Also, Saad & Hemida (1995) examined 40 samples of Roquefort cheese and could isolate *A. flavus* and *A. niger*. Barrios *et al.* (1997) examined 52 commercial cheese produced in southern Spain and could detect *A.glaucus*, *A. niger*,*A. terreus* and *A. flavus* in most of the examined samples. Abdel-Sater *et al.* (1995) and could isolate *A. flavus*, *A. niger*, *A. sydowii*, *A. tamarii*, *A. terreus*, *A. ochraceus* and *A. scolerotiorum* from the *Egyptian cheese*. More recently, El-Sharief (2000) examined cheese samples and found *A. flavus*, *A. flavipes*, *A. niger* and *A. terreus* among the microorganisms contaminating the samples.

Cladosporium cladosporioides was isolated only on 10% NaCl-Czapek's agar, occurring in 15% of the samples. This specie and other *Cladosporium* species were very common with a world-wide distribution and occurring on foodstuffs (including cheese), soil, air, textiles, seeds, plant materials, stored fruits, cereal grains and other organic matter (Samson *et al.*, 1995). Also, *Cladosporium* species were isolated from different types of cheese such as kariech (El-Bassiony *et al.*, 1977); hard (Abdel-Rahman & El-Bassiony 1984); Teleme (Zerfiridis, 1985); Spanish (Diaz *et al.*, 1995); Soft (Sympayo *et al.*, 1995) and Egyptian cheeses (El-Sharief, 2000). The results recorded in Table 2 show that the total counts of yeasts fluctuated between 11-15 colonies/160 pieces in all samples. They occurred in 30 and 45% of the samples contributing 11.6 and 12.0% of total fungal isolates. Yeasts were also, isolated from food or food products as indicated by several workers in many parts of the world (Mercado & Rivas, 1986; Guven *et al.*, 1995; Abdel-Sater *et al.*, 1994; Barakat & Abdel-Sater, 1999; El-Sharief, 2000).

Table 2: Total counts (TC per 160 pieces in all samples) and percent frequency (F%) of mycoflora isolated from examined cheese on malt extract and 10% NaCl-Czapek's agar at 25°C.

Organisms	Malt extract		10% NaCl	
	TC	F%	TC	F%
Aspergillus	39	53	43	58
A. flavus Link	14	50	10	53
A. niger Van Tieghem	21	46	19	50
A. oryzae (hlb.) Cohn.	-	-	5	15
A. sydowii (Bain.&Sart.)Thom & Church	4	15	5	20
A. terreus Thom	-	-	4	15
Cladosporium cladosporioides (Fres.) de Vries	-	-	2	5
Penicillium	54	56	80	59
P. brevicompactum Dierckx	7	30	10	20
P. chrysogenum Thom	18	45	17	35
P. corylophilum Dierckx	-	-	3	5
P. frequentans Westing	-	-	2	5
P. paxilli Bain.	3	25	11	25
P. roquefortii Thom	14	20	-	-
P. waksmanii Zaleski	12	40	37	55
Yeasts	11	30	15	45
Total counts	**95**		**125**	
Number of genera = 4	3		4	
Number of speices = 13	8		12	

Testing the ability of different fungal isolates, recovered in current study, to produce protease enzymes indicated, that the most fungal isolates (59 out of 70) were able to produce these enzymes (Table 3). It was observed that not only the species of a single genus differed in the production of enzymes but also, the different isolates within the same species. Of 70 isolates tested 48.3 % could be realized as producers of protease enzymes. Among the positive isolates, 24 (40.7%) belonging to *Aspergillus niger*, *Penicillium roquefortii* and *P. waksmanii* achieved high activity and

Table 3: Protease production by common fungal isolates tested.

Organisms	NIT	NIP	Degree of production		
			+	++	+++
Aspergillus flavus	10	10	1	2	7
A. niger	8	8	2	3	3
A. sydowii	9	7	3	2	2
Penicillium brevicombactum	6	5	1	3	1
P. chrysogenum	11	9	2	4	3
P. paxilli	4	2	1	1	-
P. roquefortii	15	12	3	4	5
P. waksmanii	7	6	2	1	3
Total isolates	70	59	15	20	24

NIT= number of isolates tested; NIP= number of isolates positive; +: weak producers; ++: moderate producers; +++: high producers.

these . Isolates of *P. brevicompactum* and *P. chrysogenum* proved to be moderate producers whereas, isolates apperataining to *A. sydowii* and *P. paxilli* could produce the enzyme with weak amounts (Table 3). These results were in agreement with those of Moharram and El- Zayat (1989). They noticed that most of isolates recovered from *Tilapia* fish scales could produce the protease enzymes. More recently Omar *et al.* (1999) reported 72 out of 96 isolates to be proteolytic including 28 isolates highly proteolytic, 21 moderately degrading milk protein and the remaining isolates were non proteolytic. Also, protease enzymes were isolated and characterized from differrent fungal species as reported by several workers (Desphpande, 1992; Singh *et al.*, 1994; Simpanya and Baxter, 1996; Staszczak *et al.*, 1996; Lopez - Diaz *et al.*, 1996; Wu and Hang, 1998).

In conclusion, most cheese samples were free from fungal species, since the government makes severe restrictions and rigorous laws on foodstuff products. Also, good processing and preservation techniques of different products lessen to great extent any contamination with microorganisms. For these and other reasons cheese samples examined in the present study were less conaminated with fungi.

References

Abdel-Rahman, H.A. and T.A. El-Bassiony, 1984. Psychotrophic mold in some food products. First Sci. Cong., Fac. Vet. Med., Assiut Univ., 17-19.

Abdel-Sater, M.A., A.H.A. Ahmed, N.A. Saad and M.L. El-Malt, 1995. Mycological evaluation of some Egyptian cheeses at the stage of consumption. Assiut Vet. Med. J., 32: 164-172.

Aran, N. and D. Eke, 1987. Mold mycoflora of kasar cheese at the stage of consumption. Food Microbiol., 4: 101-104.

Arevalo, M.P., C. Rodriguezaverez, A. Arias and A. Sierra, 1996. Occurrence of molds in fresh cheese. J. Food Quality, 19: 251.

Arizcum, C., M. Itulatin, J. Salmeron and P. Rorre, 1996. Study of roncal and idiazabal cheeses with demonination of origin, manufactured in Navarra. Acta Alimentaria, 34: 274.

Barakat, A. and M.A. Abdel-Sater, 1999. Preliminary characterization and lipolytic activity of moulds associated with raw butter. Bull. Fac. Sci., Assiut Univ., 28: 109-122.

Barrios, M.J., L.M. Medina, M.G. Cordoba and R. Jordano, 1997. Aflatoxin producing strain of *Aspergillus flavus* isolated from cheese. J. Food Prot., 60: 192.

Beuchat, L.R. and J.I. Pitt, 1992. Detection and enumeration of heat-resistant molds. In Compendium of Methods for the Microbiological Examination of Foods. ed. Vanderzant C. & Splettstoesser D.F. pp: 252-263. Washington, D.C.: American Public Health Association.

Bullerman, L.B., 1980. Incidence of mycotoxin molds in domestic and imported cheeses. J. Food Safety, 2: 47.

Carter G.R. and J.R. Cole, 1990. Diagnostic procedures in Vet. Bacteriology and mycology. 4th ed. (1984), Copyright (1990), Academic Press, P: 405.

Deshpande, M. V., 1992. Proteinases in fungal morphogensis. World J. Microbiol. Biotech., 8: 242- 250.

El-Bassiony, T.A., A. Atia and F. Abou-El-Khier, 1980. Search for the predominance of fungi species in cheese. Assiut Vet. Med. J., 11: 124.

El-Gendy, S.M., 1966. Microbial assay of blue-veined cheese. Roqueforti type. Bull. Sci. Technol. (Univ. of Assiut, Egypt) 9: 367-384.

El-Malt, L.M., 1993. Search for micro-organisms of public health hazard in locally manufactured cheese. M.Sc., Thesis, Fac. Vet. Med. Assiut Univ.

El-Sawi, N.M., M.El-MagO, H.S. Mahran and M.A. Abo-Gharib, 1994. Abnormal contamination of cottage cheese in Egypt. J. Appl. Anim. Res., 6: 81.

El-Shrief, L.T.A., 2000. Incidence of mycoflora and some mycotoxins in localy manufactured cheese. M.Sc. Thesis, Fac. Vet. Med., Assiut University.

Gerhartz, W., 1990. Enzymes in Industry: Production and Applications. P. 38. Weinheim.

Guven, M., A. Konar and A. Kleeberger, 1995. Microbiological qualities of tulum cheese made from cow, ewe and goat milk and packaged and ripened in goat skin bags. Turkish J. Agric. Forest., 19: 293.

Hassanin, N.I., 1993. Detection of mycotoxinogenic fungi and bacteria in processed cheese in Egypt. Int. Biod. Biodeg., 31: 15-23.

Kivance, M., 1990. Mold growth and presence of aflatoxin in some Turkish cheese. J. Food Safety, 10: 287-294.

Kivance, M., 1992. Fungal contamination of kasar cheese in Turkey. Nahrung, 36: 578.

Larsen, T.O., 1997. Identification of cheese associated fungi using selected ion monitoring of volatile terpenes. Lett. Appl. Microbiol., 24: 463-466.

Lund, F., O. Filtenborg and J.C. Frisvad, 1995. Associated mycoflora of cheese. Food Microbiol., 12: 173.

Mercado, E.C. and M. Rivas, 1986. Microbiological specifications for processed cheese and processed cheese spread. Rivista Argentina de Microbiol., 18: 115.

Moharram, A. M. and S. A. El- Zayat, 1989. Lipase and protease production by fungi isolated from scales of *Tilapia nilotica* Bull. Fac. Sci.,Assiut Univ., 18 : 109- 117.

Nielsen, P.V., 1991. Preservative and temperature effect on growth of three varieties of the heat resistant mold, *Neosarotrya fischari*, as a measured by an impedimetric method. J. Food Sci., 56: 1735-1740.

Omar, S. A., A. Barakat and A. A. El- Shanawany, 1999. Microbial degradation of chicken feather by saprophytic fungi. Bull. Fac. Sci., Assiut Univ., 28: 123- 138 .

Pitt, J.I. and A.D. Hocking, 1985. Fungi and food spoilage. pp. 413. Sedney, Academic Press.

Polonelli, L., D. Orsini and G. Moraco, 1984. Toxin producing potential of fungi isolated from food. Ingiene Moderna, 81: 483.

Saad, N.M. and S.K. Hemida, 1995. Isolation and identification of molds present as contaminants in roquefort cheese. Assiut Vet. Med. J. 33: 116.

Samson, R. A., E. S. Hoekestra , J. C. Frisvad and O. Fiitenborg, 1995. Introduction to food–borne fungi.Centraalbureauvoor Schimmelcultures Baarn Delft.

Scott, P.M., 1989. Mycotoxigenic fungal contaminants of cheese and other dairy products. In Mycotoxins in dairy products (Egmond, H.P. ed.) London, UK Elsevier Applied Science Publishers, P. 193-211.

Simpanya, M. F. and M. C. Baxter, 1996. Multiple proteinases from two *Microsprum* species. J. Med. & Vet. Mycol., 34: 31- 36.

Singh, A., V.K. Ghosh and P. Ghosh, 1994. Production of thermostable acid protease by *Aspergillus niger*. Lett. Appl. Microbiol., 18: 177-180.

Staszczak, M., G. Nowak and K. Grzywnowicz, 1996. Proteolytic activities in cultures of selected white- rot fungi. J. Basic Microbiol., 36: 193 – 203.

Taniwaki, M.H. and A.G.F. Dender, 1992. Occurrence of toxigenic molds in Biazilian cheese. J. Food Protect., 55: 187.

Walker, H.W., 1977. Spoilage of food by yeast. Food Technol., 3: 57-65.

Wu, L.C. and Y.D. Hang, 1998. Purification and characterization of acid proteinase from *Neosartorya fischeri* var. *spinosa* IBT 4872. Lett. Appl. Microbiol. 27: 71-75.

Zerfiridis, G.K., 1985. Potential aflatoxin hazards to human health from direct mould growth on teleme cheese. J. Dairy Sci., 68: 2184-2188.