MOULDS AND YEASTS INCIDANCE IN SAMPLES FROM RAS CHEESE SURFACE AND IN THEIR PRODUCTION AREAS AIR

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ABSTRACT

Moulds isolated from visibly moulded Ras cheese collected from different rural and urban localities at Kafr- El-Sheikh, Tanta and Damietta were identified. Out of 360 isolates, fungi and yeasts constituted 90.3% and 9.7%, respectively. The genera of yeasts found were *Saccharomyces* (5.56%), *Trichosporon* (2.78%) and *Debaryomyces* (1.38%). However the fungi genera were *Penicillium* (41.7%), *Aspergillus* (37.5%), *Cladosporium* (4.17%), *Fusarium* (2.78%), *Alternaria* (1.38%), *Geotrichum* (1.38%) and *Steamphylium* (1.38%). The air microbial pollution in the production areas reflected its hygienic conditions and strongly correlated with the identified moulds on cheese surfaces.

INTRODUCTION

Ras cheese is a popular dairy product in Egypt. It has two common public names, Roumy or Turkey cheese. As any hard cheese, Ras cheese usually ripened for several months in relatively low temperature and high humidity rooms. Under such conditions moulds may grow on the cheese surface and may penetrate the cheese producing off flavors leading to sever economic losses. In addition, the contaminated cheese with moulds is a health hazard because some moulds are capable of producing toxic metabolites in cheese (Pugazhenthi et al., 2000; Taniwaki and Vandender, 1992 and Engel, 1978). Level of mould contamination depends on the hygienic quality of the environment during cheese production, ripening and storage. The moulds are widely spread in the air, walls and shelves surfaces of cheese ripening rooms and cause spoilage of Ras cheese, especially when general good cheese manufacturing practices are not fully followed. Therefore, prevention of moulds growth on cheese surface is of great importance. The present study was carried out to identify the moulds that grown on the surface of Ras cheese samples collected from dairy factories at rural and urban areas. The microbial pollution of air in the investigated dairy factories was also investigated.

MATERIALS AND METHODS

1) Cheese samples

Ras cheese samples were collected from shops at Kafr El-Sheikh city, and from the ripening rooms of Ras cheese pilot in rural areas at Abo Mandor and Qtoor, modern dairy products company (Misr Milk and Food Company, Dameitta) and from our department dairy pilot.

2) Isolation and identification of moulds

Yeasts and fungi were isolated on a potato dextrose agar (PDA) medium (Difco, 1974) amended with 25mg/ml streptomycin sulfate to eliminate bacterial contamination. Fungi and yeasts plates were incubated at 21ºC ± 2 for 7 days and 48 h, respectively.

The isolated fungi and yeasts were purified by hyphal tip or single spore technique. The isolated colonies were identified to genus level and sometimes to species level as described by Barnett and Hunter 1979 and Booth 1971. Isolates of each strain transferred to PDA slants and kept in a refrigerator at 4ºC as a stock culture.

3) Counting of total bacteria and moulds in air of the production areas

Total bacterial and fungi counts were carried out using Tryptone Glucose Extract Agar and PDA media (Difco, 1974). In brief, 5 petri dishes containing the media for each count were opened for 3 minutes in every investigated location at the above-mentioned locations. The investigated locations were the production halls, ripening rooms (22-25°C) and storage rooms (7-10 °C) except for Abo Mandor pilot, which has no storage room.

RESULTS AND DISCUSSION

Identification of moulds and yeasts isolated from Ras cheese collected from different regions.

As shown in Table 1, among 360 isolates from visibly moulded Ras cheese samples collected from different sources, fungi and yeasts represented 88.9% and 11.1%, respectively. Morphological and microscopial examinations showed that the isolated fungi were of the genera*, Aspergillus, Penicillium, Fusarium, Cladosporium, Steamphylium and Alternaria*, and the isolated yeasts were of the genera *Saccharomyces*, *Trichosporon Debaryomyces* and *Geotrichum*. The predominant fungi strains on Ras cheese surface were *Penicillium spp*. (46.8%) and *Aspergillus flavus* (35.9%). On the other hand, *Saccharomyces cerevisiae* represented 50% of the isolated yeasts. Abdel-Rahman and El-bassiony (1985) found that *Penicillium spp* was the predominant fungi of hard cheese collected from markets in Assiut city. Also, El-Essawy *et al*.(1984) collected 50 random samples of Ras cheese from shops in different localities in Cairo and Giza cities. They found the most predominant yeasts species were belonged to the genera *Candida, Saccharomyces, Torulopsis, Rhodotorula* and *Pichia*. However *Penicillium, Aspergillus, Neurospora, Rhizopus, Cephalosporium, Geotrichum, Mucor, Scopulariopsis, Putrichus, Cladosporium* and *Palliomyces* were the predominant fungi genera in the cheese samples.

Determination of total bacterial and fungi counts in air of Ras cheese factories.

Table 2 shows average of total and fungi counts of air of the investigated Ras cheese factories. Total bacterial and mould counts increased in air of the factories in the rural areas (Qtoor and Abomandor) than in the faculty pilot and Dameitta factory. Table 2 also shows that ripening rooms were the most contaminated locations with moulds, however storage cold rooms were less contaminated. Level of moulds contamination of food products depends on the hygienic quality of the environment during manufacture, storage, duration of exposure of products to the environment and the nature of the product itself (Ottaviani and Franceschetti, 1982). Ras cheese is a hard cheese which is characteristic with a closed texture and compact body that prevent the presence of air in it. These conditions prevent the growth of moulds inside the cheese even in case of contamination with moulds spores. However, inside ripening and storage cold rooms the growth of moulds on cheese surface may occur and cause a great damage of cheese. The relatively lack of the aseptic conditions in the cheese factories in the rural areas increased the contamination inside it and therefore increased the risk for moulds growth on surface of cheese as shown in Table 2.

Table 1. Strains and sources of moulds and yeasts isolated from Ras cheese.

Strain name	Source					Number of isolates	% of moulds or yeasts	% of total isolates
	Kafr El-Sheikh	Damitta	AboMandor	Qtoor	Faculty Plant			
Fungi								
Penicillium Spp.	15	50	35	40	10	150	46.8	41.7
Aspergillus flavus	15	25	50	20	5	115	35.9	31.9
Aspergillus parasiticus	1	5	5	4	-	15	4.68	4.17
Cladosporium herbarum	2	5	5	3	-	15	4.68	4.17
Fusarium roseom	1	2	3	4	-	10	3.12	2.78
Alternaria Tenius	-	2	1	2	-	5	1.56	1.38
Aspergillus candidus	1	2	1	1	-	5	1.56	1.38
Steamphylium spp.	-	2	1	2	-	5	1.56	1.38
Total	35	93	101	76	15	320	100	
Yeast								
Saccharomyces cerevisiae	3	7	4	4	2	20	50.0	5.56
Trichosporon barassicae	1	4	2	3	-	10	25.0	2.78
Debaryomyces hansenii	-	2	1	1	1	5	12.5	1.38
Geotrichum candidum	1	1	2	1	-	5	12.5	1.38
Total	5	14	9	9	3	40	100	100

Table 2. Average of total (T. C) and moulds (M. C) counts (CFU/dish) in air of the investigated dairy factories

Place of count	Faculty		Dameitta		Qtoor		AboMandoor	
	T.C	M.C	T.C	M.C	T.C	M.C	T.C	M.C
Production halls	46.0	20.7	14.3	4.30	128	59.7	258	146
Ripening rooms	29.7	19.0	81.7	70.0	161	114	211	172
Storage cold rooms	17.0	10.7	10.7	8.30	75.3	119	-	-

Average of 5 petri dishes per place.

Lund *et al*. 1995, examined 371 different factories samples of cheese, with and without surface smear, from various European countries and USA for yeasts and fungi. They found that 91%of the isolates were belonged to *Penicillium spp*. and demonstrated that controlling of cheese smear was important in preventing fungal growth on cheese surface.

Taking in account that cheese samples used in this study were collected from cheese ripening rooms. The identified moulds strains from the air of the ripening rooms in the investigated dairy factories were in consistent with the strains isolated from the corresponding cheese surface. The presented data also show that cheese production halls and cold storage rooms seems to participate to some extent in the contamination of cheese surface.

Moulds and yeasts strains in air of the faculty dairy pilot.

As shown in Table 3, *Penicillium spp. Aspergillus flavus* and *Saccharomyces spp* were the predominant moulds in air of the ripening room in the faculty dairy pilot and accounted for 87.7% of the isolated strains. On the other hand these strains were predominant on cheese surface (83.3%) of the corresponding cheese samples (Table 1).

Moulds and yeasts strains in air of Dameitta factory.

Table 4 shows data for air in Dameitta factory. The predominant mould strains were *Penicillium spp.*, *Aspergillus flavu*s, *Aspergillus parasiticus, Cladosporium spp. and Saccharomyces spp.* These strains are in consistent with that obtained from the collected cheese samples as shown in Table 1.

Moulds and yeasts strains in air of Abo Mandoor Ras cheese pilot.

Moulds strains isolated from air of Abo Mandoor Ras cheese pilot are presented in Table 5. The predominant isolated moulds were *Penicillium spp.*, *Aspergillus flavus*, *Aspergillus parasiticus, Cladosporium spp. and Saccharomyces spp.* These species were also the main moulds strains isolated from the surface of cheese samples obtained from the pilot (Table 1).

Moulds and yeasts strains in air of Qtoor Ras cheese pilot.

Table 6 shows the moulds isolated from air of Qtoor Ras cheese pilot. The predominant moulds strains were *Penicillium spp.*, *Aspergillus flavus*, *Alternaria spp*., *Aspergillus parasiticus, Cladosporium spp*., *Aspergillus niger*, *Geotricum candidum and Saccharomyces spp*. These results are in consistent with that of cheese samples as shown in Table 1.

In summary, air of the investigated cheese factories especially that of the rural areas are highly contaminated with moulds. The predominant fungi strains isolated from air of the dairy factories were *Penicillium spp.*, *Aspergillus spp.*, *Alternaria spp.* and *Cladosporium spp.* However the predominant yeasts strains were *Saccharomyces spp*. and *Trichosporon spp*. In conclusion, aseptic and hygienic attention should be done to minimize the microbial air pollution in Ras cheese factories by controlling the possible sources for contamination, e.g. ventilation inlets, factory walls, factory ground, utensils, factory staff, ……etc.

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Table 3. Moulds strains isolated from air of the faculty dairy pilot.

Place	Mould name	Count	%
Production halls	*Candida spp.*	14	22.6
	Fusarium spp.	13	21.0
	Alternaria spp.	8	12.9
	Aspergillus flavus	6	9.7
	Penicillium spp.	6	9.7
	Actinomyces spp.	4	6.5
	Aspergillus niger	4	6.5
	Cladosporium spp.	3	4.8
	Epicocum spp.	1	1.6
	Monlinia spp.	1	1.6
	Saccharomyces spp.	1	1.6
	Steaphilum spp.	1	1.6
Total		62	100
Ripening rooms	*Penicillium spp.*	33	57.9
	Aspergillus flavus	17	29.8
	Alternaria spp.	2	3.5
	Cladosporium spp.	1	1.8
	Fusarium spp.	1	1.8
	Actinomyces spp.	1	1.8
	Aspergillus niger	1	1.8
	Saccharomyces spp	1	1.8
Total		57	100
Storage cold rooms	*Penicillium spp.*	12	37.5
	Cladosporium spp.	7	21.9
	Fusarium spp.	7	21.9
	Aspergillus flavus	3	9.4
	Alternaria spp.	2	6.3
	Saccharomyces spp.	1	3.1
Total		32	100

Table 4. Moulds strains isolated from air of Dameitta dairy factory.

Place	Mould name	Count	%
Production halls	*Aspergillus niger*	5	38.5
	Alternaria spp.	3	23.1
	Penicillium spp.	2	15.4
	Saccharomyces spp.	2	15.4
	Aspergillus flavus	1	7.7
Total		13	100
Ripening rooms	*Penicillium spp.*	101	48.1
	Aspergillus flavus	38	18.1
	Geotrichum candidum	11	5.2
	Aspergillus parasiticus	10	4.8
	Saccharomyces spp.	9	4.3
	Cladosporium spp.	8	3.8
	Alternaria spp.	7	3.3
	Aspergillus candidus	6	2.9
	Trichosporon barassicae	6	2.9
	Steamphylium spp.	5	2.4
	Debaryomyces hansenii	4	1.9
	Fusarium spp.	3	1.4
	Aspergillus niger	2	1.0
Total		210	100
Storage cold rooms	*Penicillium spp.*	13	52
	Aspergillus flavus	4	16
	Saccharomyces spp.	3	12
	Aspergillus parasiticus	2	8
	Cladosporium spp.	1	4
	Mucor spp.	1	4
	Trichosporon barassicae	1	4
Total		25	100

Table 5. Moulds strains isolated from air of AboMandoor Ras cheese pilot.

Place	Mould name	Count	%
Production halls	*Aspergillus flavus*	88	20.0
	Cladosporium spp.	72	16.4
	Fusarium spp.	69	15.7
	Candida spp.	61	13.9
	Penicillium spp.	49	11.2
	Trichosporon barassicae	26	5.9
	Saccharomyces spp.	23	5.2
	Geotrichum candidum	12	2.7
	Aspergillus candidus	11	2.5
	Aspergillus parasiticus	10	2.3
	Rhizopus spp.	10	2.3
	Alternaria spp.	8	1.8
Total		439	100
Ripening rooms	*Aspergillus flavus*	161	31.1
	Penicillium spp.	92	17.8
	Aspergillus parasiticus	49	9.5
	Cladosporium spp.	47	9.1
	Saccharomyces spp.	29	5.6
	Geotrichum candidum	28	5.4
	Aspergillus candidus	23	4.4
	Candida spp.	17	3.3
	Fusarium spp.	17	3.3
	Aspergillus niger	14	2.7
	Alternaria spp.	12	2.3
	Debaryomyces hansenii	11	2.1
	Trichosporon barassicae	10	1.9
	Rhizopus spp.	7	1.4
Total		517	100

Table 6. Moulds strains isolated from air of Qtoor Ras cheese pilot

Place	Mould name	Count	%
Production halls	*Aspergillus flavus*	52	29.1
	Candida spp.	43	24.0
	Fusarium spp.	32	17.9
	Cladosporium spp.	18	10.1
	Saccharomyces spp.	16	8.9
	Penicillium spp.	10	5.6
	Alternaria spp.	4	2.2
	Aspergillus niger	4	2.2
Total		179	100
Ripening rooms	*Penicillium spp.*	107	31.1
	Aspergillus flavus	44	12.8
	Alternaria spp.	24	7.0
	Aspergillus parasiticus	22	6.4
	Cladosporium spp.	22	6.4
	Aspergillus niger	18	5.2
	Geotrichum candidum	16	4.7
	Saccharomyces spp.	16	4.7
	Rhizopus spp.	14	4.1
	Fusarium spp.	13	3.8
	Aspergillus candidus	12	3.5
	Trichosporon barassicae	12	3.5
	Steamphylium spp.	8	2.3
	Debaryomyces hansenii	7	2.0
	Actinomyces spp.	6	1.7
	Candida spp.	3	0.9
Total		344	100
Storage cold rooms	*Penicillium spp.*	99	27.6
	Aspergillus flavus	53	13.9
	Geotrichum candidum	50	14.5
	Saccharomyces spp.	44	12.3
	Fusarium spp.	22	6.1
	Candida spp.	20	5.6
	Aspergillus niger	19	5.3
	Trichosporon barassicae	13	3.6
	Cladosporium spp.	12	3.3
	Debaryomyces hansenii	10	2.8
	Alternaria spp.	8	2.2
	Aspergillus parasiticus	4	1.1
	Rhizopus spp.	3	0.8
	Actinomyces spp.	2	0.6
Total		359	100

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مدى تواجد الفطريات والخمائر فى عينات مأخوذة من سطح الجبن الراس وكذلك هواء المصانع المنتجه بها

محسن عبد العزيز زماره و مصطفى على راشد
كلية الزراعة بكفر الشيخ– جامعة طنطا

تم عزل الفطريات النامية على سطح عينات من الجبن الراس المأخوذة من ٥ مواقع مختلفه تشمل مناطق ريفية وأخرى حضرية. تشمل المناطق الريفية معمل لتصنيع الجبن الراس فى مدينة قطور (محافظة الغربية) وأخر فى قرية أبومندور (محافظة كفر الشيخ)، بينما تشمل المناطق الحضرية بعض المحلات فى مدينة كفر الشيخ ومعمل كلية الزراعة بكفر الشيخ ومصنع شركة مصر للألبان وألأغذية بمدينة دمياط.

أوضحت الدراسة أنه من بين ٣٦٠عزلة للفطريات والخمائر من على سطح الجبن مثلت الفطريات ٩٠,٣% بينما مثلت الخمائر٩,٧% من العزلات. كانت الفطريات المعزوله تتبع الأجناس *Penicillium spp* (٤١,٧ %)،*Aspergillus spp.* (٣٧,٥%)، *Cladosporium spp.* (٤,١٧%)، *Fusarium spp.* (٢,٧٨%)، *Alternaria spp.* (١,٣٨%)، *Cladosporium spp.*، (١,٣٨%) *Geotrichum spp.*، (١,٣٨%) ، *Steamphylium spp* (١,٣٨%). بينما كانت الخمائر المعزولة تتبع الأجناس *Saccharomyces spp.* (٥,٥٦%) ، *Trichosporon spp* (٢,٧٨%).*Debaryomyces spp.* (١,٣٨%).

عكس الفحص الميكروبى لهواء صالات التصنيع وغرف التسوية (المناشر) و ثلاجات تخزين الجبن بالمصـانع المـأخوذ مـنها عينات الجبن الظروف الصحية المتبعه بها وأوضح تلوثها الشديد بنفس الأجناس المعزولة من على سطح الجبن.