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**ASSOCIATION OF OSMOPHILIC MOLDS
AND YEASTS WITH SOME SWEETENED
DAIRY PRODUCTS SOLD IN ASSIUT CITY**
(With 2 Tables)

By

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مصاحبة الفطريات والخمائر المحبة للسكر لمنتجات الألبان المحلاة والمباعة
في مدينة أسيوط

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تم جمع عدد ٨٠ عينة عشوائية من منتجات الألبان المحلاة والمتداولة في مدينة أسيوط وهي الأيس كريم (٣٠ عينة) ، الأرز باللبن (٢٥ عينة) ، المهلبية (١٥ عينة) واللبن المركز المحلى (١٠ عينات) وذلك من السوبر ماركت ومحلات الألبان والمطاعم وذلك لفحصها ميكولوجيا لمعرفة مدى تلوثها بالفطريات والخمائر المحبة للسكر وكذلك الفلورا الفطرية (أعفان وخمائر). وقد تمت الدراسة باستخدام وسطين للعزل والعد وهما مستخلص مولت (Malt extract) و ١٥% سكروز شابكس أجار والتحصين عند درجة ٢٥ °م ولقد تبين من الفحص الميكروبي ان عينات الأيس كريم والأرز باللبن هي أكثر الأنواع تلوثا بالفطريات عن مثيلتها في المهلبية واللبن المركز وجد أن متوسط العدد الكلى للفطريات في الجرام الواحد على وسطي العزل تتراوح بين ٥٤-٥٨٨ مستعمرة ، ٣٩-٣٠٩ مستعمرة على التوالي. تم عزل وتعريف العديد من الفطريات الوسطية على الاوساط المستخدمة وكان أكثرها انتشارا وتعددا هي الأنواع التي تنتمي إلى اجناس اسبيرجيلس ، كلانوسبوريم ، بنسليوم ، الريزوبس. أيضا وجد أن العينات ملوثة بواسطة الخمائر بنسب ١٠-٤٣.٣% ، ٤٠-٦٦.٦% من العينات المختبرة على وسطي العزل المستخدمين والتي تمثل ٢٢.٣ ، ٢٤.٧ ، ٣٠.٥ ، ١١.١ & ٤٥.٣ ، ٢٤.٤% من المجموع الكلى للفطريات المعزولة.

SUMMARY

The mycological analysis of some sweetend milk products commonly consumed in Egypt, was evaluated on two isolation media. The results indicated that the ice cream and rice with milk were highly polluted than

the other two products (mehallabia and condensed milk). The average counts of mesophilic fungi on malt extract and 15% sucrose-Czapek's agar were fluctuated between 54-588 and 39-309 colonies/g, respectively. Moreover, various species of mesophilic molds were recovered on the isolation media. Members of *Aspergillus*, *Cladosporium*, *Penicillium* and *Rhizopus* were the most prevalent species in all samples examined. Also, yeast were found to be present in 10-43.3% and 40-66.6% of the samples on the two type of media used, respectively. The total yeast count represented 22.3, 24.7, 30.5 and 11.1% and 45.3, 0.0 & 24.4% of total colony count of all fungi recovered respectively from the examined products on both Malt extract and Czapek's 15% sucrose agar media. The public health importance and the preventive measures were discussed.

Key words: *Osmophilic, Molds, Yeast, Dairy products.*

INTRODUCTION

Milk and milk products are adequate substrate for yeast and mold growth under suitable conditions of temperature and moisture content (Scott, 1989 and Barrios *et al.*, 1997). The contamination of these products may occur from the raw materials, or during manufacturing, storage and distribution (Mossel, 1975; Jay, 1978, and Nakoe and Yoneya, 1978). These microorganisms influence the biochemical characters and flavor of these substrates as well as their appearance rendering them commercially undesirable and often resulting in down grading of the products (Bouton and Grappin, 1995; Beuvier *et al.*, 1997 and Demarigny *et al.*, 1997). Also a large number of molds species have some strains which able to produce mycotoxins rendering them unpalatable and unsafe for consumption (Munimbazi and Bullerman, 1996).

More attention should be focused on the population of microorganism specially those which cause considerable economic losses through spoilage, discoloration and public health hazard. So that, the objective of this study was to characterize the fungal composition that contaminate some sweetened dairy products during preparation, storage or handling to safe guard consumers from being affected and to obtain a finished product of good keeping quality.

MATERIALS and METHODS

1- Collection of samples:

Eighty random samples of ice cream (30 samples); rice with milk (25 samples), mehallabeia (15 samples) and condensed milk (10 samples) were collected from different shops, restaurants and supermarkets in Assiut City. Each sample was obtained in its container and dispatched to the laboratory directly with a minimum of delay in ice-box where they were prepared and examined.

2-Preparation of samples:

Ice cream samples were left to melt in a thermostatically controlled water-bath at 44°C for 15 min., and mixed thoroughly by a sterile spoon. Cans of condensed milk were left in a water-bath at 44°C for 30 min., then they were cleaned thoroughly. The surface want to be opened was cleaned, flamed and then opened by a sterile opener. A sterile pipette was introduced and the content is mixed thoroughly (A.P.H.A., 1978).

3- Preparation of serial dilutions:

Eleven grams from each product were weighed after mixing the sample, and transferred into sterile flask containing 99 ml of sterile one tenth peptone water to obtain a dilution 1/10 (A.P.H.A, 1978).

A- Total yeast and mold counts:

was carried out according to Harrigan and Margaret (1976).

B- Osmophilic yeasts and mold counts:

Was performed according to Raper and Fennell (1965) using Czapek's agar containing 15% sucrose.

C- Identification of isolated molds:

Pure cultures were prepared from different colonies of molds recovered on both malt extract and 15% sucrose-Czapek's agar, for identification. The methods for identification of fungal isolates were based on macro- and microscopic characteristics according to Raper and Fennell (1965); Ellis (1971); Pitt and Hocking (1985); Samson and Van Reenen-Hoekstra (1988).

RESULTS

The obtained results were recorded in Tables 1 and 2.

DISCUSSION

A- Fungi recovered on malt extract agar:

Thirty species belonging to 11 genera of fungi were recovered from the four types of sweetened dairy products examined, ice cream (7 genera and 16 species); rice with milk (11 genera and 22 species); mehallabeia (5 and 11), and condensed milk (4 and 11) using malt extract agar medium incubated at 25°C. The total count for each sample of dairy products under investigation was high in ice cream (19.6 colonies /samples), followed by rice with milk (12 colonies); mehallabeia (5.5 colonics) and condensed milk (4.5 colonics). The obtained results revealed that all of ice cream samples rice with milk and condensed milk were contaminated by mold and yeast (100%), whereas, mehallabeia was polluted by mold and yeast in percentage of 73.3% (Table 1). These results are basically similar to those recorded by Mossel (1975); Nakoe and Yoneya (1978); Aboul-Khier *et al.* (1985); Ahmed *et al.* (1988); Abdel-Haleem (1995); Abdel-Sater *et al.* (1995) El-Prince and Ismail (1998); Barakat and Abdel-Sater (1999) and El-Shrief (2000).

Aspergillus, *Cladosporium* and *Penicillium* as shown in Table 1 were the most common genera recovered from the four types of milk products examined. They occurred in 36.6-68, 23.3-88% and 20-100% of the total samples examined constituting 6.0 -22, 3.3-64.6 and 3.2 -37.7% of the total fungi, respectively. In this respect, Galikeev *et al.* (1971); Bullerman and Olivigni (1972); Leistner (1984); Pitt and Hocking (1985); Ibrahim (1987); Abdel Haleem (1995); Abdel-Sater *et al.* (1995); Sampayo *et al.* (1995); El-Prince and Ismail (1998) and El-Shrief (2000) have indicated that the majority of mold isolated from various food stuffs including milk products consisted of *Penicillium* and *Aspergillus* species. Aboul-Khier *et al.* (1985) reported that, the fungi isolated from dried ice cream mix with chocolate were *Aspergillus*, *Penicillium*, *Mucor*, *Cladosporium* and *Rhizopus* in the following percentages 33.33%, 16.66%, 16.66%, 25% and 25%, respectively. Also, Abdel-Sater *et al.* (1995) noticed that the most common molds in four types of cheescs were *Aspergillus* and *Penicillium* species. They occurred in 23 - 53 and 17-33% of the examined samples, comprising 26.1 - 65.7% and 17.1 - 31.8% of total molds, respectively. Whereas, the three genera were isolated in percentage of 24.99, 25.57 and 20.11% of the isolates, contaminated mozzarella cheese, respectively (El-Prince and Ismail, 1998).

Aspergillus falvus, *A. niger*; *Cladosporium cladosporioides*; *C. herbarum* and *Penicillium chrysogenum* were the most common species. They were encountered in 6.6 - 60% of the samples, matching 0.9-21.6% of total fungi (Table 1). Some species were encountered only in one or two products and not in the others such as *A. sydowii*; *C. sphaerospermum*; *P. corylophilum* and *P. frequentens* were isolated from three of the milk products examined and not from the fourth one. On the other hand, *A. fumigatus*; *A. ochraceus*; *A. terreus*; *A. versicolor*; *P. aurantiogriseum*; *P. canescens*; *P. duclauxii* and *P. funiculosum* were existed only in one or two types of milk products examined (Table 1). Numerous *Aspergillus* and *Penicillium* species were isolated in high occurrence from milk and milk products including cheese, ice cream, butter, examined by several investigators, in this instance, Sinha and Ranjam (1991) examined 19 samples of cheese and could isolate *A. ochraceus* (74%) and *P. citrinum* (88%). While Abdel-Haleem (1995) recorded that *Aspergillus* was the most prevalent genus comprising 69.2% of total molds recovered from the examined ice cream samples. *Aspergillus* was represented by *A. niger*; *A. flavus* var *columnaris*; *A. fumigatus* and *A. terreus*. He also stated that *Penicillium* ranked second in the numbers of cases of isolation and was represented by *P. aurantiogriseum*, *P. chrysogenum* and *P. duclauxii*. Leistner (1984) and Pitt and Hocking (1985) have indicated that the majority of *Penicillia* isolated from various food stuffs consisted of similar species. Also Barakat and Abdel-Sater (1999) could isolate *A. flavus*; *A. niger*; *A. carneus*; *A. ochraceus*; *A. oryzae*; *A. versicolor*; *A. wentii*; *P. roqueforti* and other species from butter samples. El-shrief (2000) could detect many species of molds in cheese samples, including members of *A. niger*; *A. flavus*; *A. ochraceus*; *P. citrinum*; *P. verrucosum*; *P. roqueforti*; *P. veridicatum* and *P. cyclopium* (= *P. aurantiogriseum*).

Rhizopus stolonifera was also common and recovered from 46.6, 24, 20 and 30% of the examined products, respectively (Table 1). This species was previously isolated from different food stuffs. Abdel-Haleem (1995) could isolate *Rhizopus* represented by *R. stolonifera* from examined ice cream samples. Also, this species was detected by Abdel-Sater et al. (1995); Sampayo et al. (1995); Barrios et al. (1997); El-Prince and Ismail (1998); Barakat and Abdel-Sater (1999) and El-Shrief (2000). The remaining species were detected as a single representative of the genera in one type of the examined products and are absent or in a rare occurrence in the other products (Table 1).

Furthermore, the data presented in Table 1 show that the total counts of yeasts isolated from the four different types of milk products examined fluctuated between 6-131 colonies/g, representing 10-43.3% of the samples tested. Also, the results indicated that the samples of ice cream and rice with milk were highly contaminated with yeast, but mehallabia and condensed milk were less polluted. These observations have tended towards those recorded by Aboul-Khier *et al.* (1985) who proved that all samples of dried milk products examined were contaminated by yeasts. Also, Abdel-Sater *et al.* (1995) noticed that the counts of yeasts in different cheeses examined fluctuated between 3900-11700 colonies/g, while Barakat and Abdel-Sater (1999) showed that yeast in raw butter was fluctuating between 84-91 colonies/g.

B- Osmophilic molds and yeast:

It is clearly evident from the results presented in Table 2, that 34 species appertaining 15 genera were detected in the examined samples of milk products on 15% sucrose-Czapek's agar at 25°C. The results obtained from the four types of milk products on malt extract agar are basically similar to those obtained on 15% sucrose-Czapek's agar, with few exceptions. The total counts in all samples were higher on malt extract than on 15% sucrose-Czapek's. On the other hand, sucrose-Czapek's agar allows the isolation of highest number of species and genera of mold. As previously mentioned *Aspergillus*, *Penicillium*, *Cladosporium* and *Rhizopus* which are the most prevalent genera encountered in the examined samples. El-Prince and Ismail (1998) observed that the average counts of molds isolated from the mozzarella cheese were higher on dicloran rose bengal than on malt extract agar and similar results were achieved by King *et al.* (1979), Saad and Hemaida (1995) and Weidenborner *et al.* (1999). While different results recorded by Abdel-Halcm (1995) proved that higher count of total molds were obtained on malt extract agar than that recovered on 15% sucrose Czapek's agar containing dicloran rose bengal.

It is obvious from the achieved data in Tables 1&2 that there are some species of molds were recovered on one medium and not on the other. *Alternaria alternaria*, *Aspergillus ochraceus*, *Penicillium aurantiogriseum*; *P. conescens*; *P. duclauxii*; *P. funiculosum*; *P. nigricans* and *Ulocladium botrytis* could be isolated from the examined products on malt extract agar medium, whereas, *Alternaria chlamydospora*, *Aspergillus flavus*; *A. terricolor*; *Emericella nidulans*; *Geotrichum candidum*; *Humicola grisea*; *Nigrospora sphaerica*; *Penicillium*

camembertii; *P.fellutanum*; *P.lilacenum*; *P.roseopurpureum* and *P.verrucosum* recovered on 15% sucrose-Czapek's agar medium. The incidence and species of such molds recovered from the examined products were previously isolated from different food stuffs examined by several investigators with some trends (Natarajan & Nambudripad, 1980; Aboul-Khier *et al.*, 1985 and Abdel-Haleem, 1995). It is worthwhile from the recorded results that most of isolated molds are toxigenic types and have the ability to produce mycotoxins whenever, the conditions are right and become of public health hazard. Some members of isolated fungi were incriminated in cases of pulmonary infection, urinary tract infection, arthritis, osteomyelitis, dermatitis endocarditis, meningitis and eye infection (Mossel, 1982).

Aspergillus species may induce pulmonary aspergillosis, allergy, skin infection, sinusitis, as well as, nail and ear infection (external otitis). Some *Aspergillus* species produce aflatoxin while others can produce sterigmatocystin, potulin and ochratoxin. Aflatoxins have a carcinogenic effect in human in addition to chronic damage of bone (Deger, 1976).

Some species of genus *Penicillium* may induce also pulmonary infection, external ostomycosis, mycotic keratitis and endocarditis. Some pathogenic species of *Penicillium* cause penicillosis in human, involving the skin, nails, ear, upper respiratory tracts and lungs (Pseudotuberculosis). Penicillic acid sterigmatocystin are mycotoxins produced by *Penicillium* species which had carcinogenic effect (Mossel, 1982).

Mucor and *Rhizopus* species are frequent contaminants of food these members may involved the rhinofacial cranial area, the lungs, gastrointestinal tract, skin and possibly other organ systems as well as the mucosa. Also, some species of *Mucor* fungi are responsible for mucor-mycosis in man causing lung lesions similar to tuberculosis in their clinical manifestations, keratitis and pseudotuberculosis in the liver (Pyatki, 1967). *Rhizopus* species can induce intraocular infection and deep wound infection (Pyatki, 1967). While, *Alternaria* species can cause skin and nail infection, and some species produce aflatoxin while others can produce sterigmatocystin potulin and ochratoxin. in addition aflatoxins have a carcinogenic effect in human and induce a chronic damage of bone (Deger, 1976).

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Table 1. Total counts (TC/g) and frequency percentages (F%) of fungal genera and species recovered from the examined samples of milk products on malt extract agar medium at 25°C.

Genera & Species	Ice cream samples			Rice with milk samples			Mehallabeia samples			Condensed milk samples		
	TC	F		TC	F		TC	F		TC	F	
		N	%		N	%		N	%		N	%
<i>Acremonium strictum</i>	-	-	-	2	2	8	-	-	-	-	-	-
<i>Alternaria</i>	1	1	3.3	9	9	36	-	-	-	-	-	-
<i>A. alternata</i>	1	1	3.3	-	-	-	-	-	-	-	-	-
<i>A. tenuissima</i>	-	-	-	9	9	36	-	-	-	-	-	-
<i>Aspergillus</i>	36	11	36.6	54	17	68	16	7	46.6	12	6	60
<i>A. flavus</i>	28	9	30	7	7	28	1	1	6.6	11	5	50
<i>A. fumigatus</i>	-	-	-	2	2	8	1	1	6.6	-	-	-
<i>A. niger</i>	6	5	16.6	38	12	48	13	6	40	1	1	10
<i>A. ochraceus</i>	-	-	-	4	3	12	-	-	-	-	-	-
<i>A. sydowii</i>	2	1	3.3	1	1	4	1	1	6.6	-	-	-
<i>A. terreus</i>	-	-	-	1	1	4	-	-	-	-	-	-
<i>A. versicolor</i>	-	-	-	1	1	4	-	-	-	-	-	-
<i>Cladosporium</i>	19	7	23.3	113	22	88	27	10	66.6	8	8	80
<i>C. cladosporioides</i>	10	5	16.6	56	14	56	11	6	40	6	6	60
<i>C. herbarum</i>	5	4	13.3	49	14	56	15	8	53.3	2	2	20
<i>C. sphaerospermum</i>	4	2	6.6	8	4	16	1	1	6.6	-	-	-
<i>Cochliobolus spicifer</i>	-	-	-	1	1	4	-	-	-	-	-	-
<i>Epicoccum purpurascens</i>	-	-	-	8	4	16	-	-	-	-	-	-
<i>Fusarium oxysporum</i>	1	1	3.3	-	-	-	-	-	-	-	-	-
<i>Mucor hiemalis</i>	1	1	3.3	1	1	4	-	-	-	-	-	-

Table 2. Total counts (TC/g) and frequency percentages (F%) of fungal genera and species recovered from the examined samples of milk products on 15% sucrose Czpek's agar medium at 25°C.

Genera & Species	Ice cream samples			Rice with milk samples			Mehallabeia samples			Condensed milk samples		
	TC	F		TC	F		TC	F		TC	F	
		N	%		N	%		N	%		N	%
<i>Acremonium strictum</i>	9	7	23.3	-	-	-	1	1	6.6	-	-	-
<i>Alternaria</i>	1	1	3.3	4	3	12	-	-	-	-	-	-
<i>A. chlamydospora</i>	-	-	-	3	2	8	-	-	-	-	-	-
<i>A. tenuissima</i>	1	1	3.3	1	1	4	-	-	-	-	-	-
<i>Aspergillus</i>	52	16	53.3	8	7	28	4	3	20	17	7	70
<i>A. flavipes</i>	-	-	-	2	2	8	-	-	-	-	-	-
<i>A. flavus</i>	40	12	40	3	3	12	1	1	6.6	7	5	50
<i>A. fumigatus</i>	3	2	6.6	1	1	4	-	-	-	-	-	-
<i>A. niger</i>	-	-	-	-	-	-	2	1	6.6	-	-	-
<i>A. sydowii</i>	6	5	16.7	-	-	-	1	1	6.6	-	-	-
<i>A. terricolor</i>	-	-	-	-	-	-	-	-	-	9	3	30
<i>A. terreus</i>	2	1	3.3	1	1	4	-	-	-	1	1	10
<i>A. versicolor</i>	1	1	3.3	1	1	4	-	-	-	-	-	-
<i>Cladosporium</i>	12	7	23.3	34	13	52	26	8	53.3	2	2	20
<i>C. cladosporioides</i>	12	7	23.3	27	12	48	15	7	46.6	1	1	10
<i>C. herbarum</i>	-	-	-	1	1	4	1	1	6.6	-	-	-
<i>C. sphaerospermum</i>	-	-	-	6	1	4	6	2	13.3	1	1	10
<i>Cochliobolus spicifer</i>	-	-	-	1	1	4	-	-	-	-	-	-
<i>Emericella nidulans</i>	3	2	6.6	-	-	-	-	-	-	-	-	-
<i>Epicoccum purpurascens</i>	-	-	-	-	-	-	1	1	6.6	-	-	-
<i>Fusarium oxysporum</i>	1	1	3.3	-	-	-	-	-	-	-	-	-

Table2. Continued

Genera & Species	Ice cream samples			Rice with milk samples			Mehallabeia samples			Condensed milk samples		
	TC	F		TC	F		TC	F		TC	F	
		N	%		N	%		N	%		N	%
<i>Geotrichum candidum</i>	3	1	3.3	-	-	-	-	-	-	-	-	-
<i>Humicola griseu</i>	-	-	-	-	-	-	1	1	6.6	-	-	-
<i>Mucor hiemalis</i>	2	2	6.6	-	-	-	-	-	-	-	-	-
<i>Nigrospora sphaerica</i>	-	-	-	1	1	4	-	-	-	-	-	-
<i>Penicillium</i>	33	10	33.3	3	3	12	4	3	20	12	8	80
<i>P.camembertii</i>	5	2	6.6	-	-	-	-	-	-	1	1	10
<i>P.chrysogenum</i>	2	1	3.3	-	-	-	2	1	6.6	-	-	-
<i>P.corylophilum</i>	6	2	6.6	-	-	-	1	1	6.6	-	-	-
<i>P.fellutanum</i>	-	-	-	-	-	-	-	-	-	5	4	40
<i>P.frequentiens</i>	2	1	3.3	-	-	-	-	-	-	-	-	-
<i>P.lanosum</i>	12	3	10	-	-	-	-	-	-	2	2	20
<i>P.lilacenum</i>	6	4	13.3	2	2	8	-	-	-	-	-	-
<i>P.oxalicum</i>	-	-	-	1	1	4	-	-	-	-	-	-
<i>P.roseopurpurenum</i>	-	-	-	-	-	-	-	-	-	4	3	30
<i>P.verrucosum</i>	-	-	-	-	-	-	1	1	6.6	-	-	-
<i>Phoma glomerata</i>	7	3	10	-	-	-	-	-	-	-	-	-
<i>Rhizopus stolonifer</i>	4	4	13.3	2	2	8	1	1	6.6	-	-	-
Sterile mycelium	42	9	30	9	4	16	1	1	6.6	-	-	-
Yeast	140	20	66.6	-	-	-	-	-	-	10	4	40
Gross total count	309	90		62	76		39	73.3		41	80	
No. of Genera	15	12			7			8			3	
No. of species	34	21			15			13			9	