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**ASSOCIATED MYCOBIOTA OF SOME TYPES OF
CHEESE AND COOKING BUTTER**
(With 5 Tables)

By

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الفطريات والخمائر العالقة ببعض أنواع الجبن وزبد الطهي

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أجريت هذه الدراسة للتعرف على مدى تواجد الفطريات والخمائر في بعض أنواع الجبن وزبد الطهي. لذلك تم جمع ١١٩ عينة عشوائية (٩٨ عينة من الجبن الطري "الدمياطي والقريش - محليا"، الجاف، المطبوخ المستورد والمطلي، ٢١ عينة من زبد الطهي) من بعض أماكن البيع وكذلك أماكن التصنيع المختلفة لهذه المنتجات بمحافظة أسيوط. وقد أسفرت النتائج عن تواجد هذه الفطريات والخمائر بمتوسط عدد كلي يتراوح من 1.0×10^3 إلى 1.3×10^4 مستعمرة/جم في الجبن الدمياطي والجاف المستورد على التوالي، أما في حالة زبد الطهي فقد كان متوسط العدد الكلي 1.4×10^6 مستعمرة/جم. وكذلك تم عزل السلالات الآتية: الأسبرجلس، البنسليوم، الكانديدا، الكلاوسيبورم، الميكوسفيريللا، الريزوبس والجيوتركم من كل العينات المفحوصة. وكانت سلالات الكانديدا باراسيلوزس، البنسليوم روكفورتى، الأسبرجلس نيجر تم بعد ذلك الجيوتركم كانديدم، الكلاوسيبورم سفروسبيرم والميكوسفيريللا تسيانا والريزوبس ستوليفر هي السلالات السائدة المتواجدة في الجبن الطري بنوعيه، أما في الجبن الجاف فقد كانت السلالات السائدة هي الكانديدا باراسيلوزس، البنسليوم روكفورتى، والأسبرجلس نيجر، وقد كانت السلالات السائدة في الجبن المطبوخ هي البنسليوم والأسبرجلس، أما في حالة زبد الطهي فقد تم عزل الجيوتركم كانديدم بنسبة ٧٨% والكانديدا باراسيلوزس بنسبة ١٢,٨% أما باقي السلالات فكانت متواجدة بنسب قليلة. وعموماً يتضح من النتائج أن عينات الجبن المختلفة وزبد الطهي ملوثة بالعديد من الفطريات والخمائر التي بعضها يؤثر على جودة المنتج والبعض على صحة المستهلك، لهذا تم مناقشة الاشتراطات الصحية التي يجب إتباعها لمنع تلوث الألبان ومنتجاتها بهذه الميكروبات.

SUMMARY

119 random samples of different kinds of cheese (98) and locally produced cooking butter (21) were collected from different localities in Assiut Governorate and subjected to mycological examination for associated mycobiota. All samples were found to be contaminated by a wide variety of spoilage molds and yeasts at levels ranging, in case of cheeses, from 4×10^3 (the least recorded in Damietta cheese) to 1.3×10^4 (the highest in imported hard cheese) to 1.4×10^5 propagules/g in case of cooking butter. The high level of contamination of cooking butter is due to the highest incidence and count of *Geotrichum candidum* (78% of the total propagules encountered). *Aspergillus*, *Penicillium*, *Candida*, *Cladosporium*, *Mycosphaerella*, *Rhizopus* and *Geotrichum* were the most dominant genera on all substrates investigated, however, they may differ in their incidences and counts from a kind to another. In case of soft cheeses, the most prevalent species on both imported and locally produced were: *Candida parapsilosis*, *Penicillium roquefortii* and *Aspergillus niger* followed by *Geotrichum candidum*, *Cladosporium sphaerospermum*, *Mycosphaerella tassiana* and *Rhizopus stolonifer*. However, some other species were more common either on imported (*P. chrysogenum*, *P. aurantiogriseum* & *A. flavus*) or on locally produced Kareish (*P. viridicatum* & *P. chrysogenum*). The dominant species on hard cheeses were *C. parapsilosis*, *P. roquefortii* and *A. niger*, however, *P. aurantiogriseum* and *P. viridicatum* were more common on the imported type and *M. tassiana* and *G. candidum* on the local one. On the other hand, the highly frequent species on both local and imported processed cheeses were *P. aurantiogriseum*, *P. chrysogenum*, *P. roquefortii*, *A. niger* and *A. flavus*, however, others were more common either on imported (*C. parapsilosis* & *P. verrucosum*) or local type (*M. tassiana*, *C. sphaerospermum* and *R. stolonifer*). With respect to cooking butter, the highly encountered species were *G. candidum* (78%) and *C. parapsilosis* (12.8%) but *P. aurantiogriseum*, *A. flavus*, *P. roquefortii*, *M. tassiana* and *A. niger* were a significant proportion (7.8%) of the spoilage fungi. Public health implications of these findings are discussed.

Keywords: *Molds, Yeasts, Cheeses, Soft Cheeses, Hard Cheeses, Processed cheeses & Cooking butter.*

INTRODUCTION

Fresh milk, a liquid of neutral pH, is highly susceptible to bacterial spoilage, and hence fungi are rarely a problem. When, milk is processed to cottage cheese or butter, the growth of lactic acid bacteria will cause the pH to fall, favouring the growth of spoilage molds and yeasts (Pitt and Hocking, 1997). Such molds and yeasts can cause gas and off flavor in cheese and rancidity or other flavor defects in butter due to their lipolytic and proteolytic activities and the production of bitter compounds from lactose (Walker & Ayres, 1970 and Viljoen & Greyling, 1995). Mold contamination not only causes deterioration of foods but also adversely affect the health of humans & animals, since they produce toxic metabolites called mycotoxins (Sweeney and Dobson, 1998), which could be regarded as potential health hazard. Also, they are responsible for many serious diseases of liver, kidney, blood, circulation system and blood forming organs (Cole and Cox, 1981) as well as carcinogenic effect (Mossel, 1982).

Bodini *et al.* (1969) found *Mucor*, *Penicillium* and *Aspergillus* to be the most prevalent genera from soft cheeses. Also, *Penicillium*, *Aspergillus* and *Fusarium* of which some species are capable of mycotoxin production were the most common from refrigerated cheddar cheese (Bullerman & Olivigni, 1974). In Egypt, El-Sayed (1981) isolated 14 fungal genera from processed cheese of which *Aspergillus* and *Penicillium* were the most common species. In Australia and New Zealand cheeses, Hocking (1994) found that *Penicillium commune* and *P. roquefortii* were the most common spoilage species, but other penicillia (*P. chrysogenum*, *P. expansum*, *P. solitum*, *P. viridicatum* and *P. brevicompactum*) were a significant proportion of the spoilage mycoflora. In packaged cheeses in Europe, *P. commune* was also the most common spoilage species (Lund *et al.*, 1995), with *P. verrucosum*, *P. solitum*, *P. roquefortii* and *P. nalgiovense* also significant; while species other than *Penicillium* were rarely encountered.

On the other hand, solid perishable dairy products such as butter and margarine are susceptible to the growth of spoilage fungi. Muys *et al.* (1966) concluded that *G. candidum*, *Moniliella suaveolens* and the yeast *Yarrowia lipolytica* could cause spoilage of margarine by their lipolytic action. However, Hocking (1994) found that the most common spoilage fungi on margarine are *Penicillium* species, particularly *P. glabrum*.

P. expansum, *P. chrysogenum* and *Cladosporium* species, with *C. cladosporioides* by far the most common.

Aman (1985) recorded an average yeast and mold count of $99.0 \times 10^5/g$ cooking butter, while a mean count of $2.5 \times 10^5/g$ cooking butter was reported by Ahmed *et al.* (1987). Mohamed *et al.* (1982 & 1983) detected a mean yeast count of $41.87 \times 10^2/g$ and a mean mold count of $55.2 \times 10^2/g$ cooking butter, respectively. While, Tasnim *et al.* (1993) and Patir *et al.* (1995) recorded mean counts of $3.84 \times 10^2/g$ and $9.0 \times 10^5/g$ table butter for yeasts & molds, respectively. *Candida* spp., *Torulopsis* spp., *Debaromyces* spp., *Saccharomyces* spp. and *Rhodotorula* spp. were isolated from salted and unsalted butter (Mohamed *et al.*, 1982 & 1983; Aman, 1985; Nazem, 1991 and Rajaraman *et al.*, 1994).

Therefore, this study was undertaken to emuncerate and identify spoilage fungal species contaminating different kinds of cheeses either imported or locally manufactured in Egypt, as well as, Egyptian cooking butter.

MATERIALS and METHODS

Collection of samples:

A total of 119 samples of either imported or locally manufactured (Damietta and Kareish) soft (31), hard (22), processed cheeses (45) and cooking butter (21) at the stage of consumption were randomly collected from different localities in Assiut, Egypt. Details about the number of samples in each case are recorded in Table 1. The samples once collected were transferred to the laboratory and kept in a refrigerator at 3 - 5°C till fungal analysis.

Detection and enumeration of spoilage mycobiota:

The dilution plate technique was used for the isolation and enumeration of viable fungal propagules. Dilutions were prepared by shaking samples in diluents containing 0.1% (V/V) tween 80 and 85% (w/v) NaCl (Hartog and Notermans, 1988). Serial ten-fold dilutions were prepared and 1ml aliquots of the appropriate dilution was placed in sterile Petri-dishes. Malt extract agar as an isolation medium (Harrigan and McCance, 1976) was used (5 plates for each sample). The plates were incubated at 25°C for 7 - 10 days and the growing fungal colonies were counted, isolated and identified.

Identification of the isolated strains:

Identification was based on macro and microscopic characteristics using the taxonomic methods of Raper and Fennell (1965); Booth (1971); Ellis (1971 & 1976); Pitt (1979); Sivanesan (1984); Samson and van

Reenen - Hoekstra (1988); Moubasher (1993) and Pitt and Hocking (1997).

RESULTS

The obtained results were recorded in Tables 1 - 5.

DISCUSSION

From the results illustrated in Table 1, it could be shown that all samples analyzed (119) either of cheeses (98) or cooking butter (21) were found to be contaminated by molds and yeasts at different levels ranging from 4×10^3 to 1.3×10^4 in case of cheeses to 1.4×10^5 fungal propagules/g in case of butter.

Of the imported cheeses, only hard cheese samples were heavily contaminated (1.3×10^4 propagules/g) approximately twice compared to that locally produced hard cheese (6.9×10^3). Nearly similar results (5.3×10^3) were recorded by Abouzeid et al. (1996). On the other hand, in case of soft cheeses, the least contamination level (4.0×10^3 /g) was detected in locally produced Damietta cheese, while for those imported and Kareish cheese samples were nearly similar (9.6×10^3). Abouzeid et al. (1996) detected slightly higher level of approximately 1.1×10^4 in Kareish cheese. However, in case of processed cheeses, the local samples were slightly more contaminated than imported ones (8.9×10^3 and 7.5×10^3 cfu/g, respectively).

Concerning the Egyptian cooking butter, the contamination level by spoilage fungi was much higher than that of cheeses and this is due to the high incidence and count of *Geotrichum candidum* which accounted approximately 78% of the total counts of spoilage fungi.

I. Spoilage Fungi Recovered from Soft Cheeses:

All samples of soft cheeses either imported or locally manufactured were found to be contaminated by a wide variety of spoilage species of which *Candida* (2 species) and *Penicillium* (10 species) were isolated from the three types. *Candida* was recovered from 50, 60 and 91%, while *Penicillium* from 80, 60 and 73% of the imported, Damietta and Kareish cheese samples, respectively. *Candida* accounted approximately 77.3, 32.5 and 44.9%, while *Penicillium* 15.3, 52.2 and 9.9% of the total fungi in the three substrates. Only *C. parapsilosis* and *P. roquefortii* were the most frequent spoilage species, from the three substrates (Table 2). *P.*

roquefortii was reported from French, Italian, German, English and Danish blue cheeses (Samson *et al.*, 1977). *P. chrysogenum* was isolated moderately from both imported and Kareish, while rarely from Damietta cheese. *P. aurantiogriseum* was moderately isolated from imported soft cheese, while in low frequency from Damietta. *P. viridicatum* was recovered moderately from Kareish, while rarely in Damietta samples. *P. oxalicum* was isolated only and in low frequency from imported cheese. The remainder 5 *Penicillium* species and *C. guilliermondii* were rarely encountered and each only from one substrate (Table 2). Abdel-Sater *et al.* (1995) and Abouzeid *et al.* (1996) found that yeasts were the most commonly encountered from the Egyptian soft cheeses. Also, *Penicillia* were found to be prevalent in Egyptian (Abdel-Rahman and El-Bassiony, 1984; Ibrahim, 1987; Abdel-Sater *et al.*, 1995; Saad and Hemida, 1995; Abouzeid *et al.*, 1996 and Saleh, 1997), Swiss (Bullerman, 1976 & 1980), Australian (King *et al.*, 1981), Greece (Zerfiridis, 1985) and Turkish cheeses (Aran and Eke, 1987). Also, The following *Penicillium* species were reported either from Damietta: *P. chrysogenum*, *P. citrinum* and *P. oxalicum* (Abdel-Sater *et al.*, 1995) or from Kareish: *P. chrysogenum*, *P. citrinum*, *P. duclauxii*, *P. cyclopium*, *P. brevicompactum*, *P. commune*, *P. viridicatum* and *P. urticae* (Abdel-Sater *et al.*, 1995 and Saleh, 1997).

Aspergillus (7 species) was also common and isolated in high frequency from imported and Damietta while moderately from Kareish cheese. It accounted about 2.6, 1.9 and 0.3% of the total propagules in the three substrates, respectively. In agreement with the findings of Abdel-Sater *et al.* (1995) and Saleh (1997). Only *A. niger* being the most common species, was found to be parallel in its frequency and counts to that of the genus. *A. flavus* the aflatoxigenic species, was isolated in low frequency from imported cheese and rarely from Kareish. This species was encountered from Damietta and Kareish cheeses, but in different incidences (Abdel-Sater *et al.*, 1995 and Abouzeid *et al.*, 1996). Galvano *et al.* (1996) stated that the occurrence of aflatoxin M₁ in milk and milk products is widespread, although contamination levels do not seem to be a serious health hazard. The remaining 5 *Aspergilli* were rarely encountered from either Kareish (*A. alutaceus* & *A. carbonarius*), Damietta (*A. restrictus* & *A. terreus*) or imported and Damietta cheese (*A. sydowii*). In previous studies, *A. ochraceus* (= *A. alutaceus*), *A. parasiticus*, *A. sydowii*, *A. tamarii* and *A. terreus* were encountered from either Damietta or Kareish cheese (Abdel-Sater *et al.*, 1995 and Abouzeid *et al.*, 1996).

Geotrichum (*G. candidum*) was isolated either in high (from all Kareish samples), moderate (30% of Damietta) or rare frequency (10% of imported). Its count was also much higher in case of Kareish (44.4% of the total fungi) compared to that of Damietta (5.18%) or imported cheese (1.6 %). In agreement with Abouzeid *et al.* (1996). This species was also reported from Kareish cheese in high incidence and counts.

Cladosporium (*C. cladosporioides* and *C. sphaerospermum*) was isolated in moderate frequency from both imported (40%) and Damietta (30%) while rarely from Kareish cheese samples (9%). Abouzeid *et al.* (1996) recorded *Cladosporium* spp. from Kareish cheese to be less common, while Abdel-Sater *et al.* (1995) isolated both species rarely but only from Damietta cheese. On the other hand, *Mycosphaerella tassiana* (anamorph: *Cladosporium herbarum*) was isolated in high frequency only from Damietta cheese samples (60%), while in low frequency from both Kareish (18%) and imported cheese (20%). This species accounted approximately 0.8, 0.1 and 0.7% of the total propagules, respectively.

The remaining fungi were isolated either in low or rare frequencies from the three substrates (*Rhizopus stolonifer*), both imported and Damietta (*Emericella nidulans* and *Moniliella suaveolens*), imported and Kareish (*Rhodotorula mucilaginosa*), Kareish and Damietta (*Mucor racemosus*) or only from imported (*Cochliobolus spicifer*, *Gibberella fujikuroi* and *Paecilomyces variotii*), Damietta (*Acremonium strictum*, *Nectria haematococca* and *Neurospora crassa*), and Kareish cheese (*Fusarium oxysporum* and *Mucor hiemalis*). Most of the above species were encountered from either Damietta, Kareish or both (Abdel-Sater *et al.*, 1995; Abouzeid *et al.*, 1996 and Saleh, 1997), or from other milk products (Bullerman and Olivigni, 1974; Sutic *et al.*, 1979; Ismail, 1993 and Ismail and Saad, 1997).

II. Spoilage Fungi Recovered from Hard Cheeses:

A total of 11 and 8 spoilage fungal species were isolated from imported and locally manufactured hard cheeses, respectively. However, the local cheese was found much less contaminated than the imported one (Tables 1&3). The genera *Candida* and *Penicillium* followed by *Aspergillus* were the most dominant on the imported, while *Candida*, *Geotrichum*, *Mycosphaerella* and *Penicillium* on the Egyptian type (Table 3). In previous studies, *Aspergillus*, *Penicillium*, *Scopulariopsis*, *Rhizopus*, *Mucor*, *Cladosporium*, *Geotrichum*, *Candida* and other yeasts were isolated from the Egyptian hard cheese (Abouzeid *et al.*, 1996 and Saleh, 1997).

Only *Penicillium* (6 species) and *Candida* (*C. parapsilosis*) were highly encountered from both substrates. They accounted approximately in 14.9 & 0.8% and 84.6 & 89.0% of the total propagules encountered from imported and Egyptian hard cheese, respectively. *P. roquefortii* was dominant on both substrates (40% and 67% of the samples, accounting 12.9% and 0.7% of the total propagules, respectively), however, *P. aurantiogriseum* and *P. viridicatum* were also common and isolated moderately but only from imported type. The other 3 *Penicillia* were less frequent either on imported (*P. duclauxii* & *P. oxalicum*) or on Egyptian cheese (*P. citrinum*). Saleh (1997) isolated the following *Penicillium* species from the Egyptian hard cheese with the most common being *P. urticae*, *P. viridicatum* & *P. commune* and the least being *P. brevicompactum*, *P. chrysogenum* and *P. cyclopium*.

Mycosphaerella tassiana was isolated in high frequency from the local type, while in low frequency from the imported, however, accounted nearly the same percentage from both (0.25% of the total propagules).

Aspergillus (3 species) was recovered moderately (40% of the samples) from the imported cheese, while in low frequency (17%) from the local. The count and frequency of *A. niger* were parallel to those of the genus. *A. flavus* and *A. terreus* were isolated in low frequency, however the former only from the Egyptian and the latter from the imported. Both of *A. flavus* and *A. niger* have been reported to be commonly encountered from the Egyptian hard cheese (Saleh, 1997). Also, *A. ochraceus*, *A. niger*, *A. parasiticus* and *A. wentii*, followed by *A. flavus* and *A. glaucus* were detected from the Egyptian hard cheese by Abouzeid et al. (1996).

Two species were isolated only from one substrate not from the other: *Geotrichum candidum* (in high incidence) and *Rhodotorula mucilaginosa* (in low incidence) from only the Egyptian cheese, while *Cladosporium sphaerospermum* and *Rhizopus stolonifer* (low) from the imported type (Table 3). *Geotrichum*, *Rhizopus* and *Cladosporium* were also encountered previously from hard cheeses (El-Bassiony et al., 1980; Northolt et al., 1980; Hocking and Faedo, 1992; Kivance, 1992 and Abouzeid et al., 1996).

III. Spoilage Fungi Recovered from Processed Cheeses:

The level of contamination by fungi of the local processed cheese is slightly higher (8.9×10^3) than that of the imported (7.5×10^3 propagules/g), (Tables 1 & 4).

A total of 18 genera and 37 species were recovered from both Egyptian (17 and 33) and imported cheese (10 and 23) of which *Aspergillus* and *Penicillium* were the most predominant genera (Table 4).

Penicillium (11 species) was the commonest fungus with respect to its count (56.9% and 59.3% of the total propagules on imported and Egyptian cheese, respectively), and frequency (77% and 81%). A lower percentage count (9.5%) and frequency (16.7%) for *Penicillium* was obtained from the Egyptian processed cheese by Abdel-Sater et al. (1995). *P. aurantiogriseum* (24.1% and 11.6% of the total propagules), *P. chrysogenum* (15.2% and 6.2%) and *P. roquefortii* (15.2% and 38.8%) were the most prevalent species on both types of cheeses. The rest of *Penicillia* were less commonly encountered either from imported (*P. camembertii* & *P. citrinum*), Egyptian (*P. duclauxii*, *P. glabrum* & *P. purpurogenum*) or both cheeses (*P. oxalicum*, *P. verrucosum* and *P. viridicatum*) (Table 4). Only two *Penicillium* species have been encountered and rarely (*P. chrysogenum* and *P. citrinum*) from the Egyptian type by Abdel-Sater et al. (1995).

Aspergillus (9 species) came behind *Penicillium* in its frequency and isolated from 69% and 78% of the imported and Egyptian samples, respectively. It accounted to 5.7% and 2.0% of the total propagules. Contrariwise to our finding, a much higher count of *Aspergillus* (31%) was obtained from the Egyptian processed cheese by Abdel-Sater et al. (1995). The most prevalent *Aspergilli* were *A. niger* and *A. flavus*. The other 7 species were less commonly encountered either from imported (*A. versicolor*), Egyptian (*A. parasiticus* and *A. tamarii*) or both cheeses (*A. fumigatus*, *A. penicilloides*, *A. sydowii* and *A. terreus*). *A. niger* was encountered frequently, while *A. flavus*, *A. sydowii*, *A. tamarii* and *A. terreus* were less common on the Egyptian processed cheese (Abdel-Sater et al., 1995). It is worth to mention that sterigmatocystin produced by *A. versicolor* (which is rarely encountered herein from imported processed cheese) was detected in the surface layer of hard cheeses in the Netherlands (Northolt et al., 1980).

Mycosphaerella tassiana was highly encountered from the Egyptian processed cheese (53% of the samples), while rarely from the imported type (8%). It accounted to 1.1% and 0.2% of the total propagules, respectively.

Candida parapsilosis, *Cladosporium sphaerospermum* and *Rhizopus stolonifer* were encountered moderately from one type of cheese, while in low frequency from the other (Table 4). They accounted to 36.3%

and 35.5%; 0.1% and 0.5%; and 0.2% and 0.2% of the total propagules, respectively. Yeasts were highly encountered, representing 52.7% of the total count, while *Rhizopus stolonifer* was rarely recovered and accounted to 1.4% from the Egyptian processed cheese (Abdel-Sater et al., 1995).

The remainder of species were isolated in low or rare frequencies either from imported (*Geotrichum candidum*), local (*Acremonium strictum*, *Alternaria chlamydospora*, *Alternaria tenuissima*, *Cochliobolus lunatus*, *Epicoccum nigrum*, *Gibberella fujikuroi*, *Scytalidium lignicola*, *Sporidesmium densum* and *Ulocladium chartarum*) or both cheeses (*Emericella nidulans*, *Nectria haematococca* and *Paecilomyces variotii*) (Table 4).

The level of mold & yeast contamination in the Egyptian Damietta, Egyptian hard and processed cheese is generally low. In processed cheese, this could be attributed to the fact that processed cheese is prepared in small packages for retail markets and each piece of cheese is wrapped in aluminum foil which lower the possibility of contamination unless the foil is injured. These results disagree with those recorded by Taniwaki and Dender (1992), as they could not isolate molds from processed cheese. In addition the low count in Egyptian Damietta & hard cheeses may be due to high salt content as stated by Shehata et al. (1978).

IV. Spoilage Fungi Recovered from the Egyptian Cooking Butter:

Much higher level of contamination was detected in butter as compared to all kinds of cheeses investigated (Tables 1 & 5). Twelve genera represented by 20 species of which *Aspergillus*, *Geotrichum*, *Mycosphaerella* and *Penicillium* were the most predominant on butter (Table 5). However, Saleh (1997) found only *Aspergilli*, *Penicillia*, *Rhizopus* and *Scopulariopsis* to be the contaminants of butter.

Geotrichum candidum was the most dominant spoilage species, contaminating all butter samples and accounting to 78.1% of the total propagules.

Aspergillus (6 species), *Penicillium* (4) and *Mycosphaerella* (1) were found in high incidences being isolated from 71%, 67% and 52% of the samples, accounting approximately 2.8%, 3.9% and 1.4% of the total propagules, respectively. Of these genera, *A. flavus*, *A. niger*, *P. aurantiogriseum* and *M. tassiana* were the most dominant spoilage species. The remaining *Aspergilli* (*A. carbonarius*, *A. fumigatus*, *A. parasiticus* and *A. versicolor*) and *Penicillia* (*P. chrysogenum*, *P. funiculosum* and *P. roquefortii*) were less common. Saleh (1997) recorded *A. niger*, *P. cyclopium*, *P. brevicompactum*, *P. commune* and

P. viridicatum from the Egyptian butter samples, but in different incidences.

Candida (*C. parapsilosis*) came after *Geotrichum* in its count (12.8%) but came behind *Aspergillus*, *Penicillium* and *Mycosphaerella* in its frequency (29%).

Alternaria alternata, *Cladosporium sphaerospermum*, *Mucor hiemalis* and *Rhizopus stolonifer* were isolated in low frequencies (14-24% of the samples), accounting collectively a minor proportion of the total propagules (0.66%). Of the above, only *R. stolonifer* was recorded in 20% of butter samples examined by Saleh (1997). On the other hand, *Emericella nidulans*, *Moniliella suaveolens* and *Paecilomyces variotii* were rarely encountered (Table 5).

Most of the above fungi were reported as contaminants of different milk products all over the world (Bullerman, 1980; King *et al.*, 1981; Zerfiridis, 1985; Aran and Eke, 1987; Ismail, 1993; Hocking, 1994; Abdel-Sater *et al.*, 1995; Saad and Hemida, 1995; Abouzeid *et al.*, 1996; Ismail and Saad, 1997 and Saleh, 1997).

From the a fore-mentioned results it could be concluded that the occurrence of fungi reported in this study indicates to what extent cheeses & cooking butter are exposed to contamination. The contamination by mold could be resulted from different sources including the milk used, air, water and equipments, as well as, during manufacturing process or handling of the product. The invading organisms may find the opportunity to grow and multiply in the product, producing undesirable changes, and render the product unmarketable.

Yeasts and molds that spoil dairy products could be isolated from the processing plant, packing equipment, the air, salt brine, manufacturing equipment, and from the general environment (floors, walls, ventilation, ducts., etc.). Control efforts must be taken to limit the exposure of pasteurized products to these sources (Doyle and Marth, 1975). If the initial contamination level is limited, strategies to inhibit growth are more likely to succeed. These strategies include packaging to reduce oxygen, cold storage and the use of antimycotic chemicals such as sorbate, propionate and natamycin (Hocking and Faedo, 1992).

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Table 1. Showed the number of samples analyzed (NSA), number of contaminated samples (NCS), number of isolated genera (NG) and species (NS) and counts of fungi of the investigated cheeses and butter samples.

Substrate	NSA	NCS	NG	NS	Counts of Fungi/g		
					Min.	Max.	Average
Soft cheese:							
Imported	10	10	13	21	2.6×10^2	5.0×10^4	9.6×10^3
Egyptian Danicetta	10	10	13	21	1.8×10^2	1.8×10^4	4.0×10^3
Egyptian Kareish	11	11	10	18	4.0×10^3	1.9×10^4	9.6×10^3
Hard cheese:							
Imported	10	10	6	11	5.0×10^2	4.2×10^4	1.3×10^4
Egyptian	12	12	6	8	6.0×10^1	3.0×10^4	6.9×10^3
Processed cheese:							
Imported	13	13	10	23	1.6×10^2	3.1×10^4	7.5×10^3
Egyptian	32	32	17	33	1.0×10^2	6.2×10^4	8.9×10^3
Egyptian cooking butter	21	21	12	20	1.2×10^3	2.9×10^5	1.4×10^5

Table 2. Mycobiota recovered from soft cheese.

Taxon	Imported			Egyptian Damietta			Kareish								
	ATC	%C	NCS	%F	0	ATC	%C	NCS	%F	0	ATC	%C	NCS	%F	0
<i>Acremonium strictum</i>	244	2.55	8	80	H	76	1.9	6	60	H	26	0.25	5	45.5	M
<i>Aspergillus</i>															
<i>A. alutaceus</i>												0.06	1	9.1	R
<i>A. carbonarius</i>												0.04	1	9.1	R
<i>A. flavus</i>	34	0.36	2	20	L							0.02	1	9.1	R
<i>A. niger</i>	208	2.18	7	70	H	68	1.7	5	50	H	14	0.13	3	27.3	M
<i>A. restrictus</i>															
<i>A. sydowii</i>															
<i>A. terreus</i>	2	0.02	1	10	R	2	0.05	1	10	R					
Candida	3300	77.33	5	50	H	1304	32.51	6	60	H	4760	44.86	10	90.9	H
<i>C. parapsilosis</i>	7378	77.21	5	50	H	1304	32.51	6	60	H	4760	44.86	10	90.9	H
<i>C. guilliermondii</i>	12	0.13	1	10	R										
Cladosporium	24	0.25	4	40	M	184	4.59	3	30	M	8	0.08	1	9.1	R
<i>C. cladosporioides</i>															
<i>C. spheerospirum</i>	24	0.25	4	40	M	180	4.49	2	20	L	8	0.08	1	9.1	R
<i>Cochliobolus spicifer</i>	6	0.06	1	10	R										
Dematiaceous hyphomycete															
<i>Emeticella nidulans</i>	2	0.02	1	10	R	6	0.15	1	10	R	12	0.11	2	18.2	L
Fusarium oxysporum															
<i>Georchium candidum</i>	152	1.6	1	10	R	208	5.18	3	30	M	6	0.06	1	9.1	R
<i>Gibberella fujikuroi</i>	40	0.42	1	10	R						4712	44.4	11	100	F
<i>Moniella suavelens</i>	6	0.06	1	10	R	82	2.05	1	10	R					
Mucor															
<i>M. hiemalis</i>						2	0.05	1	10	R	12	0.11	2	18.2	L
<i>M. racemosus</i>						2	0.05	1	10	R	2	0.02	1	9.1	R
Mycosphaerella tassiana	68	0.71	2	20	L	32	0.8	6	60	H	12	0.11	2	18.2	L
Nectria haematococca						4	0.1	1	10	R					
Neurospora crassa						2	0.05	1	10	R					

Taxon	Imported			Egyptian Damietta			Kareish		
	ATC	%C	NCS	ATC	%C	NCS	ATC	%C	NCS
<i>Paschomyces variotii</i>	130	1.36	1	R					
Penicillium	1464	15.32	8	H	2094	52.22	6	H	1050
<i>P. aurantiogriseum</i>	1034	10.82	4	M	152	3.79	2	L	
<i>P. brevicompactum</i>									
<i>P. chrysogenum</i>	68	0.71	4	M	40	1	1	R	34
<i>P. dactylois</i>									2
<i>P. glabrum</i>	60	0.63	1	R					
<i>P. oxalicum</i>	198	2.07	2	L					
<i>P. purpurogenum</i>	2	0.02	1	R					
<i>P. roquefortii</i>	102	1.07	4	M	1242	30.97	6	H	958
<i>P. spinulosum</i>					600	14.96	1	R	
<i>P. viridicatum</i>					60	1.5	1	R	
Rhizopus stolonifer	18	0.19	2	L	10	0.25	2	L	52
Rhodotorula mucilaginosa	12	0.13	2	L					12
Total	9556	100	10	100	4010	100	10	100	10612

ATC, average total count in all samples analyzed (calculated per g. cheese). Values of ATC are multiplied with 10; %C, percentage counts (calculated per total fungal counts); NCS, number of contaminated samples (out of the total); %F, percentage frequency; 0, occurrence; H = High 50-100%, M = Moderate 25-49%, L = Low 12.5-24%, R = Rare less than 12.5%.

Table 3. Mycobiota recovered from hard cheese*

Taxon	Imported			Egyptian		
	ATC	%C	NCS	ATC	%C	NCS
<i>Aspergillus</i>	28	0.22	4	0	0	0
<i>A. flavus</i>				M	0.24	2
<i>A. niger</i>	24	0.19	4	M	0.05	2
<i>A. terreus</i>	4	0.03	2	L	0.19	2
<i>Candida parapsilosis</i>	10800	84.59	6	H		
<i>Cladosporium sphaerospermum</i>	4	0.03	2	L	88.99	9
<i>Geotrichum candidum</i>				L		
<i>Mycosphaerella tassiana</i>	32	0.25	2	L	9.61	6
<i>Penicillium</i>	1900	14.88	8	H	0.24	6
<i>P. aurantiogriseum</i>	116	0.91	4	M	0.77	8
<i>P. citrinum</i>						
<i>P. daclauxi</i>	8	0.06	1	R	0.05	2
<i>P. oxalicum</i>	24	0.19	2	L		
<i>P. roquefortii</i>	1640	12.85	4	M	0.72	8
<i>P. viridicatum</i>	112	0.88	3	M		
<i>Rhizopus stolonifer</i>	4	0.03	2	L		
<i>Rhodotorula mucilaginosa</i>						
Total	12768	100	10	H	100	12
					8284	100
						16.7
						H

* As the same in Table 2.

Table 4. Mycobacteria recovered from processed cheese*

Taxon	Imported				Egyptian			
	ATC	%C	NCS	%F	ATC	%C	NCS	%F
<i>Acremonium strictum</i>					24	0.09	1	3.1
<i>Alternaria</i>					22	0.08	4	12.5
<i>A. chilensispora</i>					2	0.01	1	3.1
<i>A. tenuissima</i>					20	0.07	3	9.4
<i>Aspergillus</i>	558	5.69	9	69.2	564	1.99	25	78.1
<i>A. flavus</i>	224	2.28	3	23.1	260	0.92	13	40.6
<i>A. fumigatus</i>	162	1.65	2	23.1	12	0.04	1	3.1
<i>A. niger</i>	154	1.57	6	46.2	248	0.87	18	56.3
<i>A. parasitica</i>					22	0.08	2	6.3
<i>A. penicilloides</i>	2	0.02	1	7.7	10	0.04	2	6.3
<i>A. sydowii</i>	8	0.08	2	15.4	2	0.01	1	3.1
<i>A. tamaritii</i>					6	0.02	1	3.1
<i>A. terreus</i>	2	0.02	1	7.7	4	0.01	2	6.3
<i>A. versicolor</i>	6	0.06	1	7.7				
<i>Candida parapsilosis</i>	3560	36.27	4	30.8	10082	35.50	5	15.6
<i>Cladosporium sphaerospermum</i>	14	0.14	2	15.4	144	0.51	9	28.1
<i>Cochliobolus lanatus</i>					10	0.04	1	3.1
<i>Emericella nidulans</i>	2	0.02	1	7.7	20	0.07	1	3.1
<i>Epicoccum nigrum</i>					10	0.04	1	3.1
<i>Geotrichum candidum</i>	22	0.22	1	7.7				
<i>Gibberella fujikuroi</i>					4	0.01	2	6.3
<i>Mycosphaerella tassiana</i>	18	0.18	1	7.7	308	1.08	17	53.1
<i>Nectria haematococca</i>	34	0.35	2	15.4	58	0.20	4	12.5
<i>Puccinia variabilis</i>	6	0.06	1	7.7	58	0.20	5	15.6
<i>Penicillium</i>	5582	56.87	10	76.9	16840	59.29	26	81.3
<i>P. aurantiogriseum</i>	2364	24.08	6	46.2	3302	11.63	13	40.6
<i>P. canebertii</i>	10	0.10	1	7.7				
<i>P. chrysogenum</i>	1496	15.24	6	46.2	1750	6.16	18	56.3
<i>P. citrinum</i>	2	0.02	1	7.7				
<i>P. dactylois</i>					20	0.07	2	6.3
<i>P. glabrum</i>					32	0.11	1	3.1
<i>P. oxalicum</i>	6	0.06	1	7.7	186	0.66	3	9.4

Taxon	Imported			Egyptian		
	ATC	%C	NCS	ATC	%C	NCS
<i>P. parvigenum</i>	1490	15.18	9	326	1.15	4
<i>P. roquefortii</i>		69.2		11018	38.79	14
<i>P. verrucosum</i>	194	1.98	4	62	0.22	3
<i>P. viridicatum</i>	20	0.20	1	144	0.51	2
<i>Rhizopus stolonifer</i>	20	0.20	3	50	0.18	10
<i>Seyalidium ligricola</i>		23.1		6	0.02	1
<i>Sporidesmium densum</i>				148	0.52	4
<i>Ulocladium chartarum</i>				54	0.19	2
Total	9816	100	13	28402	100	32

* As the same in Table 2.

Table 5. Mycobiota recovered from the Egyptian cooking butter*.

Taxon	ATC	%C	NCS	%F	O
<i>Alternaria alternata</i>	26	0.08	5	23.8	L
<i>Aspergillus</i>	862	2.84	15	71.4	H
<i>A. carbonarius</i>	8	0.03	2	9.5	R
<i>A. flavus</i>	480	1.58	9	42.9	M
<i>A. fumigatus</i>	84	0.28	3	14.3	L
<i>A. niger</i>	286	0.94	13	61.9	H
<i>A. parasiticus</i>	2	0.01	1	4.8	R
<i>A. versicolor</i>	2	0.01	1	4.8	R
<i>Candida parapsitosis</i>	3886	12.8	6	28.6	M
<i>Cladosporium sphaerospermum</i>	50	0.17	3	14.3	L
<i>Emeticella nidulans</i>	60	0.2	1	4.8	R
<i>Geotrichum candidum</i>	23692	78.08	21	100	H
<i>Moniliella suaveolens</i>	16	0.05	1	4.8	R
<i>Mucor hiemalis</i>	102	0.34	3	14.3	L
<i>Mycosphaerella tassiana</i>	436	1.44	11	52.4	H
<i>Paecilomyces variotii</i>	4	0.01	2	9.5	R
<i>Penicillium</i>	1188	3.92	14	66.7	H
<i>P. aurantiogriseum</i>	716	2.36	9	42.9	M
<i>P. chrysogenum</i>	16	0.05	1	4.8	R
<i>P. funiculosum</i>	4	0.01	1	4.8	R
<i>P. roquefortii</i>	452	1.49	5	23.8	L
<i>Rhizopus stolonifer</i>	20	0.07	4	19.1	L
Total	30342	100	21	100	H

* As the same in Table 2, except for the values of ATC are multiplied with 10².