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Diversity of some food borne fungi associated with raw milk and some cheese in Egypt

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ABSTRACT

Contamination of milk and cheese with different types of fungi would constitute a public health hazard. These fungi may cause mycotoxicosis in human and/or spoilage of these products. This study aimed to the isolation and identification of mould and yeast in raw milk and some cheese. Sixty samples were collected from Giza Governorate, Egypt including; 20 raw milk, 20 processed cheese and 20 soft white cheese. The results reported that, overall incidence rate of moulds in processed cheese, soft white cheese and raw milk were; 18/20 (90%), 6/20 (30%) and 16/20 (80 %), respectively, while the mean mould counts were $1.3 \times 10^3 \pm 1.8 \times 10^2$ cfu/g, $6.7 \times 10^3 \pm 2.6 \times 10^3$ cfu/g and $5.9 \times 10^3 \pm 1.3 \times 10^3$ cfu/m, respectively. The isolated mould species from these samples were *Aspergillus spp.*, *Penicillium spp.*, *Eupenicillium spp.*, *Phoma spp.*, *Geotrichum spp.* and *Fusarium spp.* Meanwhile, the yeast contamination was detected in soft white cheese and raw milk samples with incidence rate of 10/20 (50%) and 16/20 (80%), respectively, while not detected in processed cheese samples. The most predominant yeast species isolated from soft white cheese and raw milk samples were *Debaryomyces hansenii*, *Pichia anomala*, *Sacharomyces cerevisiae*, *Candida tropicalis*, *Candida pseudotropicalis*, *Torulopsis* and *Rhodotorula*. In conclusion, the presence of high incidence of fungi in raw milk and cheese samples would lead to a public health hazards and/or economic losses due to spoilage of these products, therefore, strict hygienic conditions are required.

1. INTRODUCTION

Raw milk and cheese are generally considered an ideal growth medium for many fungal species, because such milk provides all important nutrients and conditions for fungal growth (Gulbe and Valdovska, 2014). The contamination of raw milk by fungi is influenced by the animal's physiological state breeding condition and the weather that may occur during milking, storage and other pre-processing activities (Callonet et al., 2007).

The spoilage caused by fungal contamination of raw milk and cheese is manifested by the presence of many metabolic by-products, causing off-flavors, odors and visible changes in color and texture. Many yeasts growing in cheese resulting in yeasty tastes by producing alcohol and CO₂, and some yeasts producing sulfides which causes egg odour. The presence of oxygen and low pH are selective media for moulds growth especially in cheese but some moulds can grow under low oxygen tension. *Penicillium spp.* and *Cladosporium spp.* are commonly moulds growing in vacuum-packaged cheeses (Ledenbach and Marshall, 2009). The characterization and identification of fungi in raw milk and cheese were based on phenotypic characteristics, and genotypic features. Also, the fungal count used as an index of sanitary quality of the milk and cheese (Moubasher et al., 2018).

The present study was planned to isolate and identify of fungal contamination in raw milk and some types of cheese samples.

2. MATERIAL AND METHODS

2.1. Samples collection

A total of 60 samples (20 raw milk, 20 processed cheese and 20 soft white cheese) were collected from different farms and supermarkets in Giza Governorate, Egypt. All samples were collected and transferred aseptically in the icebox to the laboratory.

2.2. Preparation of samples (APHA, 2001)

The collected samples were prepared according to the technique recommended by APHA (2001). Twenty-five gm from each sample were carefully and aseptically homogenized in a blender with 225 ml of sterile peptone water 0.1% to form a dilution of 1:10, from which tenth fold serial dilutions were accomplished up to 10⁻⁶.

2.3. Mycological examination

2.3.1. Isolation and counting of fungal spp.

Dichloran Rose Bengal Chloramphenicol (DRBC) agar with chloramphenicol 0.05 mg/L was prepared (ISO, 2008), then the medium was cooled in a water bath and maintained at a

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temperature of 45°C, then the media was dispensed in 15 ml amounts in sterile Petri dishes and allowed to be solidified. Twenty-five grams from each sample were carefully and aseptically homogenized in a blender with 225 ml of sterile peptone water 0.1% to form a dilution of 1:10, from which tenth fold serial dilutions were accomplished up to 10⁻⁶. Then, by sterile pipette, 0.1 ml from each previously prepared dilutions were transferred to a single DRBC agar plate and distributed over the surface of the agar plate by sterile spreader. Then the inoculated plates were incubated in an inverted position at 25°C for 5-7 days.

The isolated colonies were picked up from DRBC (ISO, 2008), into slope of Sabouraud dextrose agar, SDA (Oxoid CM0041) agar and incubated at 25°C for 5-7 days and then transferred on malt extract (MEA) (Oxoid CM0059) and Czapek yeast agar (CYA) plates (Pitt and Hocking, 2009) for identification. The inoculated plates were incubated at 25°C for one week.

2.3.2. Identification of mould spp.

The characterization of mould colonies was applied by microscopical examination and careful observation of the mould colonies according to Pitt and Hocking (2009)

2.3.3. Identification of yeasts spp.

Different yeast colonies were identified by careful observation and microscopical examination according to Lodder and Kreger-van Rij (1970).

3. RESULTS AND DISCUSSION

Fungal contamination of raw milk and cheese occur as a result of wide distribution of fungal spores in un sanitized environment which lead to milk and cheese contamination during their production, transportation, manufacturing and storage. Also fungal contamination may be resulted from contaminated air, bad environment in some factories as, uncontrolled sterilization, use of low quality milk, unhygienic utensils, equipment, workers' clothes or re-contamination after pasteurization, transportation or storage (Younis *et al.*, 2016). Raw milk and cheese are generally considered an ideal growth medium for many fungal species, because of providing all important nutrients and conditions for fungal growth (Gulbe and Valdovska 2014).

In the current study a total of 60 raw milk and cheese samples (20 processed cheese, 20 soft white cheese and 20 raw milk samples) collected from Giza Governorate, Egypt, were examined mycologically. The results showed in Table 1 revealed that, overall incidence rate of the mould in processed cheese, soft white cheese and raw milk samples were; 18/20 (90%), 6/20 (30%) and 16/20 (80%) respectively with mean mould counts $1.3 \times 10^3 \pm 1.8 \times 10^2$ cfu/g, $6.7 \times 10^3 \pm 2.6 \times 10^3$ cfu/g and $5.9 \times 10^3 \pm 1.3 \times 10^3$ cfu/ml, respectively. The obtained results were nearly similar to El-Kestet *et al.*, (2015) who examined 30 samples from raw milk and 20 samples from processed cheese for mould contamination. They found that 90% of examined processed cheese samples and 76.6% of raw milk samples were positive for presence of moulds. Also Amer (2002) reported that the incidence of moulds in samples of soft white cheese was 95% of total examined samples. While EL-Bagory *et al.* (2014) obtained higher results.

Delavenne *et al.* (2011) stated that fungal contaminated samples of cow milk contained high fungal diversity up to 15 species in a single sample, also a maximum of 4 or 6 different species were isolated from goat and sheep milks, respectively.

In the present study as shown in table 1 the predominant isolated genera in processed cheese were *Aspergillus spp.* (62%), followed by *Penicillium spp.* (17.2%) While, *Eupenicillium spp.* (10.3%), *Fusarium spp.* (7%) and *Phoma spp.* (3.5%). The only two genera isolated in soft white cheese were *Aspergillus spp.* (83.3%), *Penicillium spp.* (16.7%). The most common isolated mould species from raw milk samples were *Penicillium spp.* (44.5%), *Aspergillus spp.* (38.9%) and *Geotrichum spp.* (16.6%). These results are nearly similar to the results obtained by Nolwennet *et al.* (2014) and Silva *et al.* (2015), who reported that the most isolated fungi in cheese and milk were *Aspergillus spp.*, *Penicillium spp.*, *Geotrichum spp.* and *Fusarium spp.*. While El-Kestet *et al.* (2015) revealed that the most predominant mould spp. in raw milk samples were *Aspergillus spp.* 10 (33.3%), *Penicillium spp.* 7 (23.3%), *Rhizopus spp.* 10 (33.3%) and other spp. in the same incidence 1 (3.3%) were *Fusarium spp.* and *Mucor spp.* while most predominant spp. in the processed cheese were *Aspergillus spp.*

Table 1 Incidence and statistical analytical results of mould isolated from the examined samples

Type of samples	Total No. of examined samples	No. of positive samples	%	Mean ± SE (cfu/ml or gm)
Processed cheese	20	18	90	$1.3 \times 10^3 \pm 1.8 \times 10^2$
Soft white cheese	20	6	30	$6.7 \times 10^3 \pm 2.6 \times 10^3$
Raw milk	20	16	80	$5.9 \times 10^3 \pm 1.3 \times 10^3$

% According to total No. of examined samples for each type of samples.

In the present study the yeast contamination was not detected in processed cheese. This result was in coordinating with Egyptian Organization Standards (2005) who said that processed cheese should be free from any visible fungal contamination, but yeast contamination detected in soft white cheese and raw milk samples with incidence rate of 10/20 (50%) and 16/20 (80%); respectively (Table 5). The most predominant yeast species isolated from soft white cheese were *Debaryomyces hansenii* 14 (53.8%), *Pichia anomala* 8 (30.8%), *Pichia membranifaciens* 2 (7.7%) and *Sacharomyces cerevisiae* 2 (7.7%). While, *Candida tropicalis*, *Candida pseudotropicalis*, *Sacharomyces cerevisiae* and *Torulopsis* in the equal incidence of 2 (14.3%) were detected in raw milk. Also, *Rhodotorula* 6 (42.8%) detected in raw milk. These results agreed with El-Shazly, 2007 and Sayed *et al.*, 2011 who examined mycologically 50 soft white cheese samples and showed that the incidence of yeasts in these samples was 60% and the more identification of those spp. may resulted from contaminated air, different season, bad environment in some factories as, uncontrolled sterilization, use of low quality milk, unhygienic utensils, equipment and storage. Seker (2010) said that the most common non-starter yeasts species which have been isolated from milk and cheeses were *Candida intermedia*, *Candida lusitanae*, *Candida parapsilosis*, *Candida rugosa*, *Cryptococcus curvatus*, *Issatchenkia orientalis*, *Pichia fermentans*, *Saccharomyces cerevisiae*, *Torulasporea delbrueckii* and *Yarrowia lipolytica*. While, Spanenberg *et al.* (2014) examined 588 raw milk samples

and revealed that the most common isolated yeast spp. were *Candida spp.* (70.00%), *Rhodotorula spp.* (11.70%), *Trichosporon spp.* (6.70%), *Geotrichum spp.* (5.00%) *Pichia spp.* (5.00%) and *Cryptococcus spp.* (1.70%).

Table 2 Incidence of different mould genera isolated from different samples

Moulds	processed cheese		Soft white cheese		Raw milk	
	No of positive isolates	%	No of positive isolates	%	No of positive isolates	%
<i>Asperigellus spp.</i>	36	62	10	83.3	14	38.9
<i>Penicillium spp.</i>	10	17.2	2	16.7	16	44.5
<i>Eupenicillium spp.</i>	6	10.3	0	0	0	0
<i>Phoma spp.</i>	2	3.5	0	0	0	0
<i>Fusarium spp.</i>	4	7	0	0	0	0
<i>Geotrichum spp.</i>	0	0	0	0	6	16.6
Total	58	100	12	100	36	100

% calculated related to total number of isolates for each type of samples

Table 3 Incidence of *Aspergillus* species isolated from examined samples

Asperigellus Spp.	processed cheese		Soft white cheese		Raw milk	
	No. of positive isolates	%	No. of positive isolates	%	No. of positive isolates	%
<i>A.flavus</i>	24	66.7	4	40	6	42.8
<i>A.flocculosus</i>	2	5.6	0	0	0	0
<i>A.fumigatus</i>	0	0	2	20	0	0
<i>A.niger</i>	6	16.7	0	0	4	28.6
<i>A.sydowii</i>	2	5.5	0	0	0	0
<i>A.terreus</i>	0	0	0	0	4	28.6
<i>A.ustus</i>	0	0	4	40	0	0
<i>A.versicolor</i>	2	5.5	0	0	0	0
Total	36	100	10	100	14	100

% calculated related to total number of isolates for each type of samples

Table 4 Incidence of *Penicillium* spp. isolated from examined sample.

<i>Penicillium</i> Spp.	processed cheese		Soft white cheese		Raw milk	
	No. of positive isolates	%	No. of positive isolates	%	No. of positive isolates	%
<i>P.aethiopicum</i>	2	20	0	0	0	0
<i>P.aurantiogriseum</i>	0	0	2	100	6	37.5
<i>P.cammberti</i>	2	20	0	0	0	0
<i>P.citreonigrum</i>	2	20	0	0	0	0
<i>P.crustosum</i>	0	0	0	0	6	37.5
<i>P.digitatum</i>	0	0	0	0	2	12.5
<i>P.implicatum</i>	2	20	0	0	0	0
<i>P.oxalicum</i>	0	0	0	0	2	12.5
<i>p.simplicissimum</i>	2	20	0	0	0	0
Total	10	100	2	100	16	100

%calculated related to total number of isolates for each type of samples

Table 5 Incidence and statistical analytical results of yeasts isolated from examined samples

Type of samples	Total No. of examined samples	No. of positive samples	%	Mean ± SE (cfu/ml or gm)
Processed cheese	20	0	0	0
Soft white cheese	20	10	50	5 × 10 ± 1.3 × 10
Raw milk	20	16	80	7.6 × 10 ² ± 2.4 × 10 ²

% According to total No. of examined samples for each type of samples

Table 6 Incidence of yeast species isolated from examined samples

Yeast Spp.	processed cheese		Soft white cheese		Raw milk	
	No. of positive isolates	%	No. of positive isolates	%	No. of positive isolates	%
<i>Candida tropicalis</i>	0	0	0	0	2	14.3
<i>Candida pseudotropicalis</i>	0	0	0	0	2	14.3
<i>Debaryomyceshanseni</i>	0	0	14	53.8	0	0
<i>Pichia anomala</i>	0	0	8	30.8	0	0
<i>Pichia membranifaciens</i>	0	0	2	7.7	0	0
<i>Rhodotorula</i>	0	0	0	0	6	42.8
<i>Sacharomyces cervisioe</i>	0	0	2	7.7	2	14.3
<i>Torulopsis</i>	0	0	0	0	2	14.3
Total	0	0	26	100	14	100

%calculated related to total number of isolates for each type of samples

5. CONCLUSION

In conclusion, fungal contamination of raw milk and cheese samples collected from markets in Giza Governorate, Egypt, was clearly observed in this study indicating unsatisfactory Hygienic measures during milk production, handling and cheese manufacture. The most prevalent mould species were *Aspergillus* and *Penicillium spp.* The growing fungi may represent potential health hazards for consumers. Thus, strict hygienic measures should be adopted during milk production handling, cheese processing and storage and the end product should be complied with the Egyptian standards.

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