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 1.3×10⁴ ± 1.8×10⁰ cfu/g, 6.7×10⁴ ± 2.6×10⁰ cfu/g and 5.9×10⁵ ± 1.3×10⁵ cfu/m³, 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, Saccharomyces cervisiae, 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 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 (Moubasheret 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
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 an sanitized environment which lead to milk and cheese contamination during their production, transportation, manufacturing and storage. Also fungal contamination may be resulted from uncontaminated air, bad environment in some factories as, uncontrolled sterilization, use of low quality milk, unhygienic utensils, equipment and storage. Seker (2010) said that the most isolated fungi in cheese and milk were Aspergillus spp., Penicillium spp., Geotrichum spp., and Fusarium spp., Phoma spp., (14.3%). These results are nearly similar to the results obtained by Nolvenet 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 EI-Kestel 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                      | 1.3 × 10^3 ± 1.8 × 10^2  |
| Soft white cheese     | 20                            | 6                       | 6.7 × 10^3 ± 2.6 × 10^3  |
| Raw milk              | 20                            | 16                      | 5.9 × 10^3 ± 1.3 × 10^2  |

% 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 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 Nolvenet 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 EI-Kestel 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.
and revealed that the most common isolated yeast spp. were (5.00%) Pichia spp. (5.00%) and Cryptococcus spp. (1.70%).

Candida spp. (70.00%), Rhodotorula spp. (11.70%), Trichosporon spp. (6.70%), Geotrichum spp.

Table 2: Incidence of different mould genera isolated from different samples

| Moulds          | Processed cheese | Soft white cheese | Raw milk |
|-----------------|------------------|-------------------|---------|
| Penicillium spp.| 10               | 2                 | 16      |
| Aspergillus spp.| 36               | 62                | 10      |
| Pichia spp.     | 14               | 38.9              |         |
| Total           | 58               | 100               | 100     |

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

Table 3: Incidence of Aspergillus species isolated from examined samples

| Aspergillus Spp. | Processed cheese | Soft white cheese | Raw milk |
|------------------|------------------|-------------------|---------|
| A. flavus        | 24               | 66.7              | 40      |
| A. furocearius   | 2                | 5.6               | 0       |
| A. niger         | 6                | 16.7              | 0       |
| A. sydowii       | 2                | 5.5               | 0       |
| A. terreus       | 0                | 0                 | 0       |
| A. versicolor    | 2                | 5.5               | 0       |
| Total            | 36               | 100               | 100     |

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

Table 4: Incidence of Penicillium spp. isolated from examined samples

| Penicillium Spp. | Processed cheese | Soft white cheese | Raw milk |
|------------------|------------------|-------------------|---------|
| P. antarcticum   | 2                | 20                | 0       |
| P. aurantiogaeum | 0                | 0                 | 2       |
| P. camemberti    | 2                | 20                | 0       |
| P. citrinum      | 2                | 20                | 0       |
| P. crustosum     | 0                | 0                 | 0       |
| P. digitatum     | 0                | 0                 | 0       |
| P. implicatum    | 2                | 20                | 0       |
| P. ostreatus     | 0                | 0                 | 0       |
| P. simplicissum  | 2                | 20                | 0       |
| Total            | 10               | 100               | 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 | Mean ± SE (cfu/ml or gm) |
|-----------------|------------------------------|-------------------------|
| Processed cheese| 20                           | 7.6 \times 10^2 ± 2.8 \times 10^2 |
| Soft white cheese| 20                         | 5 \times 10^2 ± 1.3 \times 10^2 |
| Raw milk        | 20                           | 7.6 \times 10^2 ± 2.8 \times 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 |
|------------------|------------------|-------------------|---------|
| Candida tropicalis| 0                | 0                 | 2       |
| Candida pseudotropicalis | 2              | 14.3              |         |
| Debaromyces hansenii | 14            | 53.8              | 0       |
| Pichia anomala    | 8                | 30.8              | 0       |
| Pichia membranifaciens | 2             | 7.7               | 0       |
| Rhodotorula      | 0                | 0                 | 6       |
| Saccharomyces cervisia | 2             | 14.3              |         |
| Torulaeopsis     | 2                | 14.3              |         |
| Total            | 26               | 100               | 14      |

% 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.

6. REFERENCES

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