Incidence of fungi in kareish cheese from raw milk and trials to control them

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ABSTRACT

This study aimed to evaluate the incidence of fungi in kareish cheese and trial to control them. Seventy-five Kareish cheese samples were collected randomly from supermarkets, groceries, small dairies and street-vendors at Gharbia Governorate for mycological examination. In addition, kareish cheese was manufactured in laboratory with addition of natamycin (0.02%, 0.015%), chitosan (1.5%, 1%) and thyme oil (1.5%, 1%). The quality of these cheeses was checked during storage period. The obtained results revealed that all examined samples were contaminated with moulds and yeast with a mean count of $2.3 \times 10^4 \pm 2.6 \times 10^2$ and $1.5 \times 10^4 \pm 8.4 \times 10^1$ cfu/g, respectively. The profile of the genera of mould isolated were Aspergillus spp 124 (42.8%), Penicillium spp 49 (16.8%), Phoma sorghina 15 (5%), Mucor 7 (2.4%), Nigrospora oryzae 2 (0.6%), Cladosporium cladosporidies 16 (5.5%), Chaetomium Brasiilense 4 (1.3%), Byssochlamys nivea 3 (1%), Coletotrichum gloeosporioides 20 (0.6%), Geotrichum candidum 70 (24%). The frequency distribution of yeast isolated from the examined kareish cheese samples were Candida 24.8%, Rhodotorula 15%, Saccharomyces 13.5%, Debryomyces Hansenii 14.7%, Trichosporon 5.6%, Yarrowia lipolytica 16.8%, Cryptococcus spp 2.2% and Torulopsis spp 5.6%. The results of this study showed that manufactured cheese containing natamycin and chitosan had better properties than cheese containing thyme oil.

1. INTRODUCTION

Kareish cheese is one of the most popular cheese varieties consumed in Egypt especially in countryside owing to its high protein, low fat and reasonable price (Metwalli, 2011). Yeast and mould counts are used as an index for the proper sanitation and quality control of certain dairy products (Jay, 1986). Such yeast and moulds can produce undesirable gas and off flavor in cheese due to their proteolytic activity (Viljoen and Greyling, 1995).

Contamination of kareish cheese with yeast or moulds may occur from the raw material or during manufacturing, storage and distribution (Kure et al., 2004), which influence the biochemical characters and flavor of such products as well as their appearance rendering them commercially undesirable and often resulting in decreasing the grading of the dairy product (Demarigny et al., 1997; Muir and Banks, 2000).

Most of kareish cheese is made by dairy farmers, who often don’t follow the correct hygienic measures (Moharam et al., 2018). Mycotoxins are secondary metabolites produced naturally by filamentous fungi, which are considered toxic substances when present in food for humans and animal feed.

Studies have demonstrated their toxicogenic, nephrotoxic, hepatotoxic, carcinogenic, immunosuppressive and mutagenic characteristics, and most mycotoxins represent a considerable risk to human and animal health (Da Rocha et al., 2014).

The food industry is now under pressure to reduce the use of synthetic antimicrobial chemical compounds, which appear to be experiencing a trend for green consumerism and clean labeling of food products (Tajkarimi et al., 2010). As an alternative to synthetic preservatives, natural antimicrobial compounds from plants are becoming a positive selling point, thus creating a modern trend towards so-called natural preservatives which are accepted by consumers (Burt, 2004). Thyme herb has various functions and medical uses. It has an antimicrobial, antifungal and antioxidant effects (Gramza-Michalowska et al., 2008). Chitosan is widely recognized for its potent antimicrobial activity with, broad spectrum, and high killing rate but low toxicity toward mammalian cells. Chitosan acts as water binding agent and inhibits various enzymes (Kulkarni 2017). Natamycin used as a natural antimycotic polyene in dairy based food products to prevent contamination with yeasts and moulds (Dzigbordi et al., 2013).

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2. MATERIAL AND METHODS

2.1. Collection of samples:
A total of 75 Kareish cheese samples were collected randomly from supermarkets, groceries, small dairies and street-vendors at Gharbia Governorate. The samples were transferred as soon as possible in an ice box at 4 ± 1 °C to the laboratory with a minimum of delay to be examined.

2.2. Mycological examination of kareish cheese samples:
2.2.1. Preparation of ten folds serial dilutions according to ISO (2017).
2.2.2. Determination of total mould and yeast count according to ISO (2008)
2.2.3. Identification of mould isolates according to Pitt and Hocking, (2009)
2.2.4. Identification of yeast isolates according to Lodder and Krieger (1970)

2.3. Application of some antimycotic agents in kareish cheese.

2.3.1. Preparation of natamycin:
Natamycin at a level of 15 and 20 mg / kg to milk directly before curdling (Thomas and Broughton, 2001).

2.3.2. Preparation of chitosan:
Chitosan (Sigma Aldrich, USA) extracted from a shrimp shell was used Low molecular weight (150,000) chitosan is 75-85 % deacetylated. Stock solution of chitosan (1.0 % and 1.5 %w/v) was prepared in 1.0 % (v/v) acetic acid (El-Diasty et al., 2012)

2.3.3. Preparation of thyme oil:
Thyme oil (El Captain Company Reg. No 33/2006) was obtained from local market (Tanta, Gharbia, Egypt).

2.3.4. Manufacturing of Kareish cheese:
Fresh raw buffaloes’ milk was obtained from a private farm at Gharbia Governorate. Buffalos’ milk fat was mechanically separated for manufacturing of kareish cheese from skim milk.
The skimmed milk (7 kg) was divided into seven groups, each group kept in earthenware pots which were kept undisturbed 24-36 hours during the summer and skimmed milk sours and clots (Todaro et al., 2013). Concerning the first treatment, the sample was made without treatment as a control. The second and third were treated with natamycin 0.02 % and 0.015 %, respectively. Fourth and fifth were treated with chitosan 1.5 % and 1 %, respectively. The sixth and seventh were treated with thyme oil 1.5 % and 1 %, respectively. These groups were examined organoleptically and microbiologically at zero time and every 3 days until the signs of spoilage were appeared, while they were kept at refrigerator.

2.3.5. Organoleptic evaluation:
Organoleptic evaluation was carried out according to the scheme of Bodyfelt and Potter, (2009) at Mycology Department of Animal Health Research Institute, where the total overall score is 100 Appearance (20), body texture (45) and flavour (35).


3. RESULTS AND DISCUSSION

Kareish cheese is a soft cheese commonly made and consumed in Egypt. Environmental conditions prevailing during storage, combined with the composition of the cheese often create possibilities for extensive development of mould on cheese surface, which reduces considerably its quality (Reps et al., 2002). Warm climate and inadequate refrigeration are the principal causes of high level of contamination with fungi lead to defects such as off colour, loss of firmness and loss of aroma following the spoilage of milk products by fungi (Pal, 2014).

Data summarized in table (1) showed that the total mould in kareish cheese ranged from 1.8×10^3 to 1.2×10^6 with a mean count of 2.3×10^5±2.6×10^4 cfu/g. The results in table (2) showed that the most prevalent mould isolates from kareish cheese samples were Aspergillus spp (42.9%) followed by Geotrichum spp (24.2%) and Penicillium spp (16.8%). These results agreed with those reported by El-Diasty and Salem (2007) and El-Asuoty (2011), who found that Aspergillus spp, Geotrichum and Penicillium spp were the most predominant mould species isolated from kareish cheese samples. According to EOSQ (2005) kareish cheese should not contain mould counts more than 10 cfu/g. All examined kareish cheese samples were exceeded this limit.

Yeast spoilage constitutes a major economic loss in the cheese industry through developing undesirable changes, such as slimness, red color and yeasty flavour (Saras, et al., 1996).

Table 1 Incidence of total mould count of examined kareish cheese samples (n=75)

<table>
<thead>
<tr>
<th>Samples</th>
<th>%ve samples</th>
<th>Total mould Count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kareish cheese</td>
<td>75</td>
<td>100</td>
</tr>
</tbody>
</table>

The results were presented as mean ± standard error.

Table 2 Identification of mould species isolated from examined samples

<table>
<thead>
<tr>
<th>Mould</th>
<th>Spp</th>
<th>Kareish cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Aspergillus spp</td>
<td>A. flavus</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>A. niger</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>A. fumigatus</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>A. parasiticus</td>
<td>19</td>
</tr>
<tr>
<td>P. sorghina</td>
<td>Ph. sorghina</td>
<td>15</td>
</tr>
<tr>
<td>Nigrospora spp</td>
<td>N. oryae</td>
<td>2</td>
</tr>
<tr>
<td>Penicillium spp</td>
<td>P. caseicolum</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>P. citrinum</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>P. decumbens</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>P. citrinum</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>P. aurantigenum</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>P. roqueforti</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>P. chrysogenum</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>P. chrysogenum</td>
<td>1</td>
</tr>
<tr>
<td>Cladosporium spp</td>
<td>C. cladosporioides</td>
<td>16</td>
</tr>
<tr>
<td>Chasmothium spp</td>
<td>C. brasiliense</td>
<td>4</td>
</tr>
<tr>
<td>Byssochlamys spp</td>
<td>B. nivea</td>
<td>3</td>
</tr>
<tr>
<td>Colletotrichum spp</td>
<td>C. gloeosporioides</td>
<td>2</td>
</tr>
<tr>
<td>Geotrichum spp</td>
<td>G. candidum</td>
<td>70</td>
</tr>
</tbody>
</table>

*% calculated according to total number of each species/sample.

It established from table (3) that all of examined kareish cheese samples were contaminated with yeast. The total yeast count ranged from 2×10^2 to 6.4×10^5 with a mean count of 1.5×10^5 ± 8.4×10^3 cfu/g. The obtained results were nearly similar to that recorded by Mohamed et al. (2017), who found that the mean values of yeast counts were 3.65×10^5 ± 0.69×10^5 cfu/g. While it was higher than El-
In the present study, the kareish cheese samples were examined organoleptically. The panelists who carried out the sensory evaluation detected no significant differences among the treated cheese with respect to the colour, consistency, flavor and odour. The results presented in figure (1) determined the evaluation carried out on kareish cheese treated with chitosan, natamycin and thyme oil and stored at 4 °C during 0, 7, 15, 21 and 30 days of storage. It was evident that kareish cheese containing chitosan, natamycin and thyme oil were different from the control one and were more acceptable. Regarding to the control group of cheese, the changes in appearance, texture and flavor were observed on 15th day. The degree of these changes increased gradually until 21st day of storage.

Natamycin-treated cheese (0.02 % and 0.015 %) showed an improvement of shelf-life extended up to 30th day of storage. These results agreed with those reported by Hameed (2016), who found that the application of natamycin on the feta cheese inhibited mould and yeast growth and extend the shelf-life, while the chitosan-treated cheese (1.0 % and 1.5 %) showed an improvement of shelf-life extended up to the 30th day of storage. Similar findings have been reported by El-Diasty et al. (2012), who reported that the application of chitosan in kareish cheese inhibited mould and yeast growth and extend the shelf-life. While thyme oil-treated cheese (1.0 % and 1.5 %) showed an improvement of shelf-life extended up to the 15th and 21st day of storage, respectively. Also, the antifungal effects and organoleptic properties of natamycin and chitosan were found to be higher than those of thyme essential oil.

Regarding to the results recorded in table (6), the mould and yeast counts detected in the control (non-treated) cheese was 1.3×10^4 ± 1.8×10^2 and 1.4×10^5 ± 1.2×10^4 cfu/g of cheese at 0 and third day of examination, respectively, and after that the kareish cheese (control negative) spoiled.

In natamycin treated cheese (0.02 % and 0.015 %), the mean of total mould count was 4.4×10^4 ± 6.5×10^2 and 7.5×10^3 ± 1.4×10^2, respectively. After the third day of storage, there was no mould growth till 30th day. The treatment of cheese with chitosan lead to the inhibition and retardation of moulds and yeasts growth and lowered the maximum growth levels in the cheese.

The high yeast counts often indicates neglected hygienic measures during production and handling, contamination of raw material, unsatisfactory sanitation, or unsuitable time and temperature during storage and/or production (Soliman and Aly, 2011). However, the food industry is now under pressure to reduce the use of synthetic antimicrobial chemical compounds, which appear to be experiencing a trend for ‘green’ consumerism and ‘clean labeling’ of food products (Tajkarimi et al., 2010).
Fig 1 Organoleptic evaluation of manufactured kareish cheese. (A) Appearance, (B) Body texture and (C) Flavor.

4. CONCLUSION

From the present study we concluded that kareish cheese had been contaminated by a wide variety of spoilage mould and yeast. Natamycin (0.02 % and 0.015 %), chitosan (1.5 % and 1.0 %) and thyme oil (1.5 % and 1.0 %) showed antifungal effect against both mould and yeast at all concentrations, with the highest reduction rate obtained from using natamycin. In addition, treatment of kareish cheese with natamycin and chitosan inhibited mould and yeast growth and extended the shelf life of treated kareish cheese to 30 days. Therefore, natamycin and chitosan have the potential to be used in food as natural preservative to control food spoilage.

5. REFERENCES

16. ISO (21527-1:2008): Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and mould. Part 1: Colony count technique in products with water activity greater than 0.95.
17. ISO 6887 (all parts) (2017): Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal for microbiology examination.