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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 $2.3\times10^5\pm2.6\times10^4$ and $1.5x10^6 \pm 8.4x10^5$ 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 Brasiliense* 4 (1.3%) *Byssochlamys nivea* 3 (1%), *Colletotrichum gloeosporioidides* 2(0.6%). *Geotrichum candidum* 70 (24%). The frequency distribution of yeast isolated from the examined kareish cheese samples were Candida *24.8*%, Rhodotorulla 15%, Saccharomyces 13.5%, *Debromyces hansenii* 14.7%, Trichosporon 5.6%, *Yarrowia lipolytica*16.8%, Cryptococus *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 (Moharram *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 toxigenic, 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 1.0 %, 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 examination:
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).
2.3.6. Determination of total mould and yeast count: ISO (2008).

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 1.8×10^4 to 1.2×10^6 with a mean count of $2.3\times10^5 \pm 2.6\times10^4$ cfu/g. The results in table (2) showed that the most prevalent mould isolates from kariesh 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 kariesh 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 (Sarais *et al.,* 1996) .

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

Samples	+ve samples		Total mould Count (cfu/g)		
	No.	%	Min.	Max.	Mean ±S.E.
Kareish cheese	75	100	1.8×10^4	1.2×10^6	$2.3\times10^5 \pm 2.6\times10^4$

The results were presented as mean ± standard error

Table 2 Identification of mould species isolated from examined samples

Mould	Spp.	Kareish cheese	
		No.	%
Aspergillus spp.	*A. flavus*	46	37
	A. niger	49	39.6
	A. fumigatus	10	8.1
	A.parasiticus	19	15.3
Phoma spp.	*Ph. Sorghina*	15	100
Nigrospora spp.	*N. oryzae*	2	100
Penicillium spp.	*P. caseifulvum*	2	4
	P. oxalicum	5	10.2
	P. decumbens	0	0
	P.citrinum	10	20.4
	P. aurantigroseum	11	22.5
	P. coryphilum	14	28.6
	P.crustosum	6	12.3
	P. concentricum	1	2
Cladosporium spp.	*Cl. Cladosporidies*	16	100
Chaetomium spp.	*Ch. Brasiliense*	4	100
Byssochlamys spp.	*By. Nivea*	3	100
Colletotrichum spp.	*Co. gloeosporioidides*	2	100
Geotrichum spp.	*G.candidum*	70	100

*% 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 $2\times10^5$to 6.4×10^7 with a mean count of $1.5\times10^6 \pm 8.4\times10^5$ 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 $5.65\times10^6 \pm 0.69\times10^6$ cfu/g. While it was higher than El-

Diasty and Salem (2007); El- Asuoty (2011); EL-Bagory et al. (2014); El-Komy (2014) and El-Leboudy et al. (2015), who reported that mean values of yeast counts were $1.5\times10^4 \pm 1.3\times10^4$ cfu/g, $2.57\times10^4 \pm 2.11\times10^3$, $45.2\times10^3 \pm 7.8\times10^3$, $4.83\times10^3 \pm 0.09\times10^3$ and $1.27\times10^5 \pm 1.24\times10^4$ cfu/g, respectively. However, these results were lower than that obtained by Khair Allah (2000) and Abd El-Hady (2002), who reported that mean values of mold $3.04\times10^7 \pm 9.39\times10^6$ cfu/g and 2.8×10^8 cfu/g, respectively.

One hundred percent of the examined kareish cheese samples were exceeding the permissible limit with a yeast count not exceeding 400 cfu/g (EOSQ, 2005).

It was evident from tables (4 and 5) that the frequency of distribution of yeast isolated from the examined kareish cheese samples was Candida with 24.8 % (*C. famta* 45.6 %, *C. krusei* 27.2 %, *C. parapsilosis* 27.2 %), Rhodotorulla 16.8 % (*R. mucilaginosa* 46.6 % and *R. glutinis* 53.4 %), Saccharomyces 13.5 %, *Debromyces hansenii* 14.7 %, Trichosporon 5.6 %, *Yarrowia lipolytica* 16.8 %, Cryptococus sp. 2.2 % and Torulopsis 5.6 %.

Table 3 Incidence of total yeast count of examined kareish cheese samples (n=75)

Samples	+ve samples		Count of cfu/g.		
	No.	%	Min.	Max.	Mean ±S.E.
Kariesh cheese	75	100	2×10^5	6.4×10^7	$1.5\times10^6 \pm 8.4\times10^5$

The results were the mean ± standard error.

Table 4 Incidence of yeast species isolated from the examined samples:

yeast species	Kareish cheese	
	No.	%
Candida	22	24.8
Rhodotorula.	15	16.8
Saccharomyces	12	13.5
Debromyces	13	14.7
Trichosporon	5	5.6
Yarrowia lipolytica	15	16.8
Cryptococus sp.	2	2.2
Torulopsis	5	5.6
Total	89	100

* % calculated according to total number of isolated yeast species in each sample.

Table 5 Identification of yeast species isolated from examined samples

Yeast	Species	Kareish cheese	
		No.	%*
Candida sp.	*C. famta*	10	45.6
	C. krusei	6	27.2
	C. parapsilosis	6	27.2
Rhodotroulla sp.	*R. mucilaginosa*	7	46.6
	R. glutinis	8	53.4
Debromyces sp.	*D. hansenii*	13	100

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

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).

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 21th 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 extends 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 21th 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 $1.3\times10^4 \pm 1.8\times10^2$ and $1.4\times10^4 \pm 1.2\times10^2$ 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 $4.4\times10^3 \pm 6.5\times10$ and $7.5\times10^4 \pm 1.4\times10^2$, 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 mean total mould counts ranged from the beginning $6.5\times10^3 \pm 1.6\times10^2$ to $8.9\times10^2 \pm 1.3\times10$ cfu /g at the end of storage period (30th day) in cheese samples treated with chitosan 1.5% while in samples treated with chitosan 1.0 %, the count ranged from $7.7\times10^3 \pm 2.6\times10^2$ to $1.4\times10^3 \pm 2.2\times10^2$ cfu/g at the end of storage period. From the achieved results, it was clear that the addition of chitosan at concentration of 1.5% was relatively more effective than 1.0 % in suppressing the moulds and yeasts growth in kareish cheese. Sagoo *et al.* (2002) reported a similar sensitivity to chitosan for yeasts and moulds in chilled pork products. In the same context, the mean total mould counts ranged from the $2.1\times10^5 \pm 2.8\times10^3$ at the third day to $1.3\times10^4 \pm 2.6\times10^2$ cfu/g at the end of storage period in cheese samples treated with thyme oil 1.5 %, while in samples treated with 1.0 % thyme oil, the count ranged from $2.1\times10^5 \pm 1\times10^4$ to $1.6\times10^4 \pm 2.4\times10^2$ cfu/g at the end of storage period. From the achieved results, it was clear that the addition of thyme oil at concentration of 1.5 % is relatively more effective than 1.0 % in suppressing the moulds and yeasts growth in kareish cheese.

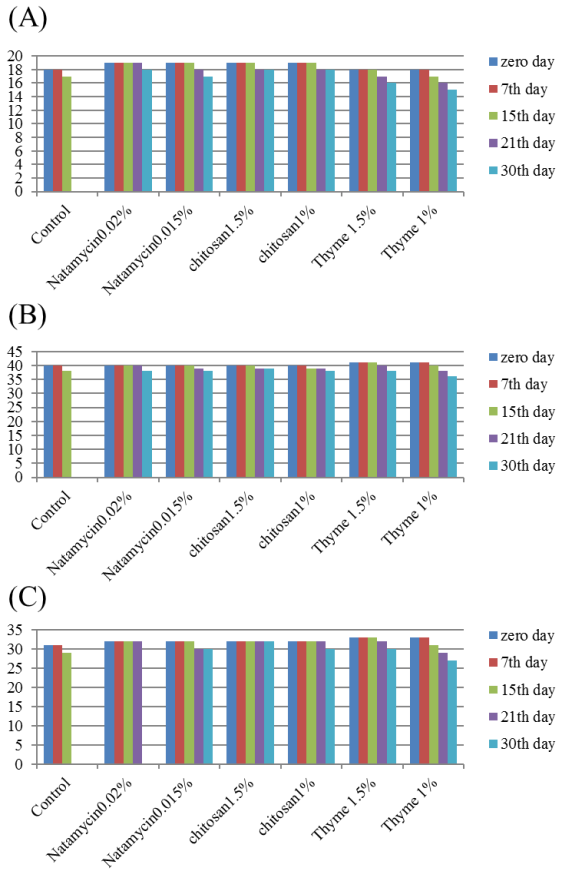

Fig 1 Organoleptic evaluation of manufactured kareish cheese, (A) Appearance, (B) Body texture and (C) Flavor.

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.

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