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Incidence of fungi in soft skimmed milk cheese from pasteurized milk and trials to control them

Tamer M. H. Elsharawy¹, Hamdi A. Mohamed², Ramadan M. Salem¹ Hend A. ELbarbary² and Eman M. EL-Diasty¹

¹ Mycology and Mycotoxins Department, Animal Health Research Institute (ARC).

² Milk Hygiene and Control Department, Faculty of Veterinary Medicine, Benha University

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ABSTRACT

This study aimed to evaluate the incidence of fungi in soft skimmed milk cheese and manufacturing of soft skimmed milk cheese from pasteurized milk with trials to control fungal growth by application of some substances (chitosan, natamycin and thyme oil) which known to have antifungal activity . Seventy-five soft skimmed milk cheese samples were collected randomly from supermarkets, groceries, small dairies and street-vendors at Gharbia Governorate for mycological examination. In addition, soft skimmed milk cheese was manufactured from pasteurized milk in lab with addition of natamycin (0.02%, 0.015%), chitosan (1.5%, 1%) and thyme oil (1.5%, 1%) and check the quality of cheeses during storage period. The obtained results revealed that 36% of examined samples were contaminated with mould with a mean count of $4.4 \times 10^3 \pm 6.8 \times 10^2$ cfu/g. The profile of the genera and species of mould isolated from soft skimmed milk cheesesamples were *A. flavus* 8 (32%), *Penicillium spp* 14 (56%) *P. decumbens* 7 (28%), *P.citrinum* 2 (8%), *P. coryphilum* 2(8%) and *P. concentricum* 3(12%) and *Byssoschlamys nivea* 3 (12%). While 68% of examined soft skimmed milk cheese samples were contaminated with yeast with a mean count of $1.8 \times 10^4 \pm 2.6 \times 10^3$ cfu/g. The frequency distribution of yeast isolated from the examined soft skimmed milk cheese samples were *Candida* 22.2% (*C. famta* 100%) *Rhodotorulla* 19.5% (*R. mucilaginosa* 42.8% and *R. glutinis* 57.2%), *Saccharomyces* 13.8%, *Debromyceshansenii* 19.5%, *Yarrowia lipolytica* 2.8% and *Torulopsis* 22.2%. The results of this study showed that manufactured cheese containing natamycin and chitosan had better effect on organoleptic properties and total mould count than cheese containing thyme oil. .

1. INTRODUCTION

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 gas and off flavor in cheese due to their proteolytic activity (Viljoen and Greyling, 1995). Contamination of dairy products may occur from the raw material or during manufacturing, storage and distribution (Kure *et al.*, 2004). Such microorganisms 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).

Mycotoxins are secondary metabolites produced naturally by filamentous fungi, which are considered toxic substances when present in food for humans and feed for animals. Studies have demonstrated their toxigenic, nephrotoxic, hepatotoxic, carcinogenic, immune-suppressive and mutagenic characteristics, and most mycotoxins represent a considerable risk to human and animal health (Da Rocha *et al.*, 2014). aflatoxins (AFs) are the most well-known among

the mycotoxins, which are a group of heterocyclic metabolites produced by the fungi of the genus particularly *Aspergillus flavus* and *Aspergillus parasiticus* that frequently contaminate animal feed and human food, causing illness and death to consumers (Magnussen and Parsi, 2013).

Many naturally occurring compounds are effective potential antimicrobial agents against spoilage and pathogenic microorganisms such as natamycin, plant essential oils and nisin (Juneja *et al.*, 2012). Natamycin, as a natural preservative, is used in dairy products and other foods in over sixty countries (Delves-Broughton *et al.*, 2005). Natamycin is identified by the European Union as a natural preservative (EFSA, 2009) and it has been described as generally recognized as safe (GRAS) product for human by FDA (Koontz and Marcy, 2003).

Chitosan, a natural non-toxic biopolymer, which included to the GRAS (Generally Recognized as safe) category by FDA, is known to possess numerous technological and physiological properties useful in foods. In addition to its lack of toxicity and allergenicity, its biocompatibility and bioactivity make it a very attractive substance for diverse

* Corresponding author: **Tamer M. H. Elsharawy**. Mycology and Mycotoxins Department, Animal Health Research Institute (ARC).

application in food fields (Chien *et al.*, 2007; Kim *et al.*, 2007).

2. MATERIAL AND METHODS

2.1. Collection of samples:

A total of 75 soft skimmed milk cheese samples were collected randomly from supermarkets, groceries, small dairies and street-vendors at Gharbia Governorate. The samples were transferred in an icebox at $4 \pm 1^\circ\text{C}$ to the laboratory with a minimum of delay to be examined.

2.2. Mycological examination of 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 soft skimmed milk cheese from pasteurized milk.

2.3.1. Preparation of *A. flavus*:

The fungal strains of *A. flavus* (Gene bank accession number: MF094441) obtained from Mycology Department at Animal Health Research Institute, Giza. Confirmation of *A. flavus* was take place by subculture onto Malt Extract Agar and Czapek Dox agar (Oxoid, 1990). Furthermore, confirmation of *A. flavus* was carried out by Single Spore method, using the identification keys of (Pitt and Hocking, 2009).

2.3.2. Preparation of natamycin:

Natamycin (produced by Danisco Cultor and obtained from Amson International Trading Company , Giza , Egypt) at a level of 15 and 20 mg / kg to milk directly before curdling (Thomas and Broughton, 2001).

2.3.3. Preparation of chitosan:

Chitosan extracted from a shrimp shell was used (Sigma Aldrich. Low molecular weight of (150,000) chitosan is 75-85 percent 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)

2.3.4. Preparation of thyme oil:

Thyme oil (El Captain Company Reg. No 33/2006) was obtained from local market at Tanta, Gharbia, Egypt.

2.3.5. Manufacturing of soft skimmed milk cheese from pasteurized milk:

Fresh raw buffaloes' milk was obtained from a farm at Gharbia Governorate. Buffalos' milk fat was mechanically separated to skim milk for manufacturing

of soft cheese. Commercial fine grade salt (El-Nasr Salines Company, Egypt) and calcium chloride (Sigma Chemical Company, Str. Louis, USA) were used. As

coagulant, rennet powder (CHY- Max powder extra) was purchased from Chr. Hansen's Lab., Denmark. Freeze-dried DVS mixed bacterial starters of CH-1 (containing of *Lb. delbrueckii* sp. *bulgaricus* & *Streptococcus (Str.) thermophilus*). The cultures were incubated at 42°C until curdling of milk. Cultures were prepared 24 h before use. 14 equal portions of skim milk was pasteurized at 72°C for 15 sec and cooled to $39-40^\circ\text{C}$, after that the prepared bacterial starter 3 % (v/v), CaCl_2 solution 40% (0.02%), and rennet (3g/100 kg of milk) were added. All skim milk treatments were stirred well, and held until to coagulate (Hussein and Shalaby, 2014).

Concerning the 1st portion was left without treatment as a control negative. The 2nd portion was prepared from pasteurized milk with addition of 10^6 cfu/kg milk of *Aspergillus flavus* as a control positive. The 3rd and 4th portions were treated with natamycin 0.02% and 0.015%, respectively. 5th and 6th portions were treated with chitosan 1.5% and 1%, respectively. 7th and 8th portions were treated with thyme oil 1.5% and 1%, respectively. All these groups (from the third to eighth) were made with addition of *Aspergillus flavus* 10^6 cfu/kg milk of and kept at refrigerator for periodical mycological examination until the appearance of signs of spoilage .

The groups (from the third to eighth) were made another time without addition of *Aspergillus flavus* and kept at refrigerator for periodical organoleptic examination.

2.3.6. Organoleptic examination:

Organoleptic evaluation was carried out according to the scheme of Bodyfelt and Potter, (2009). Appearance (20), body texture (45) and flavor (35).

2.3.7. Determination of total mould and yeast count:

It was conducted according to ISO (2008).

3. RESULTS AND DISCUSSION

Yeasts and moulds can grow in milk and its products particularly at suitable conditions of temperature and moisture (Barrios *et al.*, 1997). Yeast and mould usually present in raw milk, do not survive pasteurization; their presence in pasteurized milk and other milk products is caused by recontamination during manufacturing (Jodral *et al.*, 1993). Generally, despite the high mould and yeast counts in some analyzed dairy products samples, they didn't show any visible signs of contamination and they were apparently fit for human consumption.

Data in table (1) showed that 36% of examined soft skimmed milk cheese samples were contaminated with moulds. The total mould ranged from 4.5×10^2 to 1.4×10^4 cfu/g with a mean count of $4.4 \times 10^3 \pm 6.8 \times 10^2$ cfu/g.

Table 1 Incidence of total mould count of soft skimmed milk cheese: (n=75):

Samples of cheese	Positive samples		Total mould Count (cfu/g).		
	No.	%	Min.	Max.	Mean \pm S.E.
Soft skimmed milk Cheese	27	36	4.5×10^2	1.4×10^4	$4.4 \times 10^3 \pm 6.8 \times 10^2$

SE: standard error.

The result achieved in Table (2) showed the profile of the genera and species of mould isolated from soft skimmed milk cheese samples were *A. flavus* 8 (32%), *Penicillium* spp

14 (56%) *P. decumbens* 7 (28%) *P.citrinum* 2 (8%), *P. coryphilum* 2(8%), and *P. concentricum* 3(12%) and *Byssoschlamys nivea* 3 (12%).

The results showed that the most prevalent mouldisolates from soft skimmed milk cheese samples were *Penicillium* spp (56%) followed by *Aspergillus* Spp. (32%) and *Byssoschlamys* Spp. (12%).

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) .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) .

It established from table (3) that 68% of examined soft skimmed milk cheese samples were contaminated with yeast and the count ranged from 4.5×10^2 to 1.1×10^5 cfu/g with a mean count of $1.8 \times 10^4 \pm 2.6 \times 10^3$ cfu/g.

Table 2 Identified mould species isolated from soft skimmed milk cheese:

Sample mould species	Identified mould species	Soft skimmed milk cheese	
		No.	%*
Aspergillus spp.	A. flavus	8	32
Penicillium spp.	P.decumbens	7	28
	P.citrinum	2	8
	P.coryphilum	2	8
	P.concentricum	3	12
Byssoschlamys spp.	By.nivea	3	12
Total		25	100

*% calculated according to total number of positive samples(25).

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

Samples of cheese	Positivesamples		Count of cfu/g.		
	No.	%	Min.	Max.	Mean \pm S.E.
Soft skimmed milk Cheese	51	68	4.5×10^2	1.1×10^5	$1.8 \times 10^4 \pm 2.6 \times 10^3$

S.E ; standarderror.

Also, data presented in Table (4) showed that the frequencydistribution of yeast isolated from the examined soft skimmed milk cheese samples were *Candida* 22.2% (all identified as *C. famta*) *Rhodotorulla* 19.5% (*R. mucilaginos* 8.4% and *R. glutinis* 11.1%), *Saccharomyces* 13.8%, *Debromyces* *hansenii* 19.5%, *Yarrowia lipolytica* 2.8% and *Torulopsis* 22.2%.

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). As an alternative to synthetic preservatives, antimicrobial compounds from plants are becoming a positive selling point, thus creating a modern trend towards so-called 'natural' additives, preservatives and accepted by consumers (Burt, 2004).

Protective food additives should not cause any undesirable sensorial changes to the product. In the present, the manufactured soft skimmed milk 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 recorded in figure (1) determine the sensory evaluation carried out on soft skimmed milk cheese treated with chitosan, natamycin and thyme oil and stored at 4 °C during 0, 7, 15, 21 and 30 days of storage. It is evident that soft skimmed milk cheese containing chitosan, natamycin and thyme oil were significantly different from control one and were more acceptable. Regarding to the control group of cheese, the changes in appearance, texture and flavor were observed on the 15th day. The degree of these changes increased slowly until the 30th day of storage.

Table 4 Incidence of yeast species isolated from the examined soft skimmed milk cheese Samples:

Yeast	Species	Soft skimmed milk cheese positive samples	
		No.	%
<i>Candida famta</i> .		8	22.2
<i>Rhodotoroulla</i> sp.	<i>R. mucilaginos</i>	3	8.4
	<i>R. glutinis</i>	4	11.1
<i>Debromyces</i> .	<i>D. hansenii</i>	7	19.5
<i>Yarrowia lipolytica</i>		1	2.8
<i>Torulopsis</i>		8	22.2
<i>Saccharomyces</i>		5	13.8
Total		36	100

*% calculated according to total number of positive samples (36).

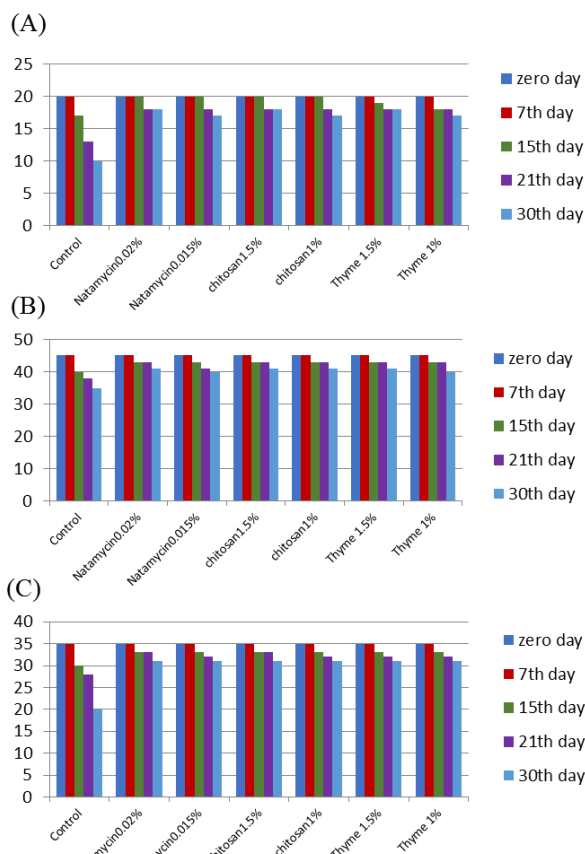


Figure 1 Organoleptic evaluation of manufactured soft skimmed milk cheese, (A) Appearance, (B) Body texture and (C) Flavor

On the other hand, the natamycin –treated cheese (0.02% and 0.015%) showed an improvement of shelf-life extended up to the 30th day of storage. These results agreed with

results reported by Hameed (2016) who reported that the application of natamycin on the feta cheese inhibits mould and yeast growth and extends the shelf- life. While the chitosan –treated cheese (1% 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 on the kareish cheese inhibits mould and yeast growth and extends the shelf- life. While thyme oil –treated cheese (1% and 1.5%) showed an improvement of shelf-life extended up to the 15th and 30th day of storage.

The results recorded in table (5) determine the mean total mould count of experimentally preserved soft skimmed milk cheese from pasteurized milk . The mean total mould counts detected in the control (non-treated) cheese were $1.7 \times 10^4 \pm 0.5 \times 10^4$ and $3.4 \times 10^4 \pm 5.3 \times 10^3$ cfu/g of cheese at 0 and 9th day of examination, respectively. After that the soft skimmed milk cheese (control negative) spoiled.

In natamycin treated cheese (0.02% and 0.015%) the mean total mould count was $1.7 \times 10^4 \pm 0.5 \times 10^4$ at 0 day for both concentrations and $0.4 \times 10^4 \pm 0.1 \times 10^4$ at 3rd day for concentration 0.02% , after 3rd day no mould growth till 30th day, while the other natamycin treated cheese the mean total mould count was $0.4 \times 10^4 \pm 0.1 \times 10^4$ at 6th day. after that no mould growth till 30th day.

The mean total mould counts ranged from the beginning $1.7 \times 10^4 \pm 0.5 \times 10^4$ to $0.3 \times 10^4 \pm 0.1 \times 10^4$ cfu /g at the 9th day of storage period in cheese samples treated with chitosan 1.5%, while in samples treated with chitosan 1% the count ranged from $1.7 \times 10^4 \pm 0.5 \times 10^4$ to $0.3 \times 10^4 \pm 0.1 \times 10^4$ cfu /g at the 12th day of storage period.

The mean total mould counts ranged from the beginning $1.7 \times 10^4 \pm 0.5 \times 10^4$ to $0.4 \times 10^4 \pm 0.1 \times 10^4$ cfu /g at the end of storage period in cheese samples treated with thyme oil 1.5%, while in samples treated with thyme oil 1% the count ranged from $1.7 \times 10^4 \pm 0.5 \times 10^4$ to $1.1 \times 10^4 \pm 0.7 \times 10^4$ cfu /g at the end of storage period.

Regarding to the results recorded in Table (6) the mould and yeast counts detected in the control (non-treated) cheese was $1.3 \times 10^5 \pm 0.8 \times 10^5$ and $1.5 \times 10^5 \pm 0.5 \times 10^5$ cfu/g of cheese at 0 and third day of examination, respectively. After that the soft skimmed milk cheese (control negative) spoiled.

In natamycin treated cheese (0.02% and 0.015%) *A.flavus* count was $9.3 \times 10^4 \pm 1.1 \times 10^4$ and $3 \times 10^2 \pm 1.3 \times 10^2$, respectively. After third day there was no mould growth till 30th day.

A. flavus counts ranged from the beginning $1.1 \times 10^3 \pm 5.1 \times 10^2$ to $3.9 \times 10^2 \pm 2.1 \times 10^2$ cfu /g at the end of storage period in cheese samples treated with chitosan 1.5%, while in samples treated with chitosan 1% the count ranged from $8.2 \times 10^3 \pm 3.6 \times 10^3$ to $5.9 \times 10^2 \pm 0.3 \times 10^2$ cfu /g at the end of storage period. 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. From the achieved results, it is clear that the addition of chitosan at concentration of 1.5% is relatively more effective than 1% in suppressing the moulds and yeasts growth in the manufactured soft skimmed milk cheese. Sagoo *et al.*, (2002) reported a similar sensitivity to chitosan for yeasts and moulds.

Table 5 Mean total mould count of experimentally preserved soft skimmed milk cheese from pasteurized milk.

Treatment	Mean total mould count cfu/g									
	0 day	3rd	6th	9th	12 th	15 th	18th	21th	30 th	
Control –ve	$1.7 \times 10^4 \pm 0.5 \times 10^4$	$1.7 \times 10^4 \pm 3.3 \times 10^4$	$1 \times 10^4 \pm 3.3 \times 10^3$	$3.4 \times 10^4 \pm 5.3 \times 10^3$	Spoiled					
NAT 0.02%	$1.7 \times 10^4 \pm 0.5 \times 10^4$	$0.4 \times 10^4 \pm 0.1 \times 10^4$	Not detected							
NAT. 0.015%	$1.7 \times 10^4 \pm 0.5 \times 10^4$	$1 \times 10^4 \pm 1.3 \times 10^3$	$0.4 \times 10^4 \pm 0.1 \times 10^4$	Not detected						
Chitosan 1.5%	$1.7 \times 10^4 \pm 0.5 \times 10^4$	$0.9 \times 10^4 \pm 0.1 \times 10^4$	$0.5 \times 10^4 \pm 0.2 \times 10^4$	$0.3 \times 10^4 \pm 0.1 \times 10^4$	Not detected					
Chitosan 1%	$1.7 \times 10^4 \pm 0.5 \times 10^4$	$1.1 \times 10^4 \pm 0.1 \times 10^4$	$0.7 \times 10^4 \pm 0.3 \times 10^4$	$0.6 \times 10^4 \pm 0.2 \times 10^4$	$0.3 \times 10^4 \pm 0.1 \times 10^4$	Not detected				
Th. O1.5%	$1.7 \times 10^4 \pm 0.5 \times 10^4$	$2.1 \times 10^4 \pm 1.2 \times 10^4$	$1.2 \times 10^4 \pm 0.9 \times 10^4$	$1.2 \times 10^4 \pm 0.9 \times 10^4$	$1.2 \times 10^4 \pm 0.9 \times 10^4$	$0.9 \times 10^4 \pm 0.1 \times 10^4$	$0.4 \times 10^4 \pm 0.1 \times 10^4$	$0.4 \times 10^4 \pm 0.1 \times 10^4$	$0.4 \times 10^4 \pm 0.1 \times 10^4$	$0.4 \times 10^4 \pm 0.1 \times 10^4$
Th. O 1%	$1.7 \times 10^4 \pm 0.5 \times 10^4$	$2.9 \times 10^4 \pm 1.3 \times 10^4$	$0.9 \times 10^4 \pm 0.3 \times 10^4$	$0.5 \times 10^4 \pm 0.1 \times 10^4$	$0.5 \times 10^4 \pm 0.1 \times 10^4$	$1 \times 10^4 \pm 0.6 \times 10^4$	$1 \times 10^4 \pm 0.4 \times 10^4$	$1.1 \times 10^4 \pm 0.3 \times 10^4$	$1.1 \times 10^4 \pm 0.7 \times 10^4$	$1.1 \times 10^4 \pm 0.7 \times 10^4$

NAT: Natamycin. Th. O: Thyme oil. Similar letters indicate no significant variation. Different letters indicate significant variation

Table 6 Mean total *Aspergillus flavus* count of experimentally preserved soft skimmed milk cheese:

Treatment	Mean count cfu/g									
	0 day	3rd	6th	9th	12 th	15th	18th	21th	30 th	
Control –ve	$1.3 \times 10^5 \pm 0.8 \times 10^5$	$1.5 \times 10^5 \pm 0.5 \times 10^5$	Spoiled							
Control +ve	$1.3 \times 10^5 \pm 1.2 \times 10^5$	$1.2 \times 10^5 \pm 8.5 \times 10^4$	4.6×10^4	Spoiled						
Nat. 0.02%	$1.3 \times 10^5 \pm 1.5 \times 10^5$	$9.3 \times 10^4 \pm 1.1 \times 10^4$	Not detected							
Nat. 0.015%	$1.3 \times 10^5 \pm 2.2 \times 10^5$	$3 \times 10^2 \pm 1.3 \times 10^2$	Not detected							
Chitosan 1.5%	$1.3 \times 10^5 \pm 3.2 \times 10^5$	$1.1 \times 10^5 \pm 5.1 \times 10^4$	$9.6 \times 10^4 \pm 0.6 \times 10^4$	$6.5 \times 10^4 \pm 0.2 \times 10^4$	$2.7 \times 10^4 \pm 2.6 \times 10^4$	$2.9 \times 10^4 \pm 0.2 \times 10^4$	$3.8 \times 10^4 \pm 1.5 \times 10^4$	$3.9 \times 10^4 \pm 1.9 \times 10^4$	$3.9 \times 10^4 \pm 2.1 \times 10^4$	$3.9 \times 10^4 \pm 2.1 \times 10^4$
Chitosan 1%	$1.3 \times 10^5 \pm 1.2 \times 10^5$	$8.2 \times 10^4 \pm 3.6 \times 10^4$	$5.9 \times 10^4 \pm 4.6 \times 10^4$	$1.2 \times 10^5 \pm 6.9 \times 10^4$	$5.9 \times 10^4 \pm 5.2 \times 10^4$	$5.1 \times 10^4 \pm 3.3 \times 10^4$	$5.4 \times 10^4 \pm 2.4 \times 10^4$	$5.5 \times 10^4 \pm 2.7 \times 10^4$	$5.9 \times 10^4 \pm 0.3 \times 10^4$	$5.9 \times 10^4 \pm 0.3 \times 10^4$
Th. O1.5%	$1.3 \times 10^5 \pm 1.3 \times 10^5$	$2.1 \times 10^5 \pm 5.2 \times 10^4$	$2.6 \times 10^5 \pm 6.9 \times 10^4$	$7.8 \times 10^4 \pm 4.7 \times 10^4$	$8 \times 10^4 \pm 4.7 \times 10^4$	$7.7 \times 10^4 \pm 2.1 \times 10^4$	$7.8 \times 10^4 \pm 2.1 \times 10^4$	$7.8 \times 10^4 \pm 2.1 \times 10^4$	$7.8 \times 10^4 \pm 1.7 \times 10^4$	$7.8 \times 10^4 \pm 1.7 \times 10^4$
Th. O 1%	$1.3 \times 10^5 \pm 1.8 \times 10^5$	$2.9 \times 10^5 \pm 8.4 \times 10^4$	$3.8 \times 10^5 \pm 1.4 \times 10^5$	$1.7 \times 10^5 \pm 1.7 \times 10^5$	$1.7 \times 10^5 \pm 2 \times 10^5$	$2 \times 10^5 \pm 9.1 \times 10^4$	$2.3 \times 10^5 \pm 9.4 \times 10^4$	$2.4 \times 10^5 \pm 1 \times 10^5$	$2.4 \times 10^5 \pm 1.4 \times 10^5$	$2.4 \times 10^5 \pm 1.4 \times 10^5$

NAT: Natamycin. Th. O: Thyme oil. Similar letters indicate no significant variation. Different letters indicate significant variation

The mean total mould counts ranged from the beginning $2.1 \times 10^4 \pm 5.2 \times 10^3$ to $7.8 \times 10^2 \pm 1.7 \times 10^2$ cfu /g at the end of storage period in cheese samples treated with thyme oil 1.5%, while in samples treated with thyme oil 1% the count ranged from $2.9 \times 10^4 \pm 8.4 \times 10^3$ to $2.4 \times 10^3 \pm 1.4 \times 10^2$ cfu /g at

the end of storage period. From the achieved results, it is clear that the addition of thyme oil at concentration of 1.5% is relatively more effective than 1% in suppressing the moulds and yeasts growth in soft skimmed milk cheese.

Natamycin binds irreversibly to the cell membrane of fungi because of its high affinity for ergosterol. This causes membrane hyperpermeability leading to rapid leakage of essential ions and peptides and ultimately cell do not contain sterol, natamycin is not effective against bacteria. This makes natamycin a suitable antifungal during bacterial ripening and fermentation processes for cheese (Adams and Moss, 2008).

Natamycin is active against nearly all moulds and yeasts but it has no effect on bacteria and viruses. Food industries that rely upon fermentation by bacteria have found that natamycin is very useful because it does not interfere with fermentation or ripening (Davidson and Branan, 1993). Also, natamycin was re-evaluated by the joint FAO / WHO expert committee on Food Additives (JECFA) in 2001 and confirmed as safe for its intended use. Natamycin in foods may provide improved protection against microbial spoilage, exhibiting a wide spectrum of activity and effectiveness at very low concentrations. Natamycin has strongicidal activity towards susceptible microorganisms and is particularly effective against fungi, which may produce mycotoxins and create public health hazard, benefiting both consumers and manufactures by reducing product losses, extending shelf- life and protecting public health and safety (Food Standards, 2004).

Several mechanisms for antifungal action of chitosan have been proposed. For example , it has been suggested that chitosan may inhibits microbial growth by acting as a chelating agent rendering metals , trace elements or essential nutrients unavailable for the moulds and yeasts at normal rates. The growth rates of fungal hyphae have been shown to be sensitive to all factors which influence intracellular calcium ions, including variations in extracellular calcium concentrations and the presence of calcium transport inhibitors (Jackson and Heath, 1993). Therefore, it is conceivable that chitosan limits the growth of filamentous fungi indirectly by making calcium and other essential minerals and nutrients inaccessible. Several authors have proposed that the antimicrobial action of chitosan against filamentous fungi could be explained by a more direct disturbance of membrane performance (Leuba and Stossel, 1986 ; Muzzarelli,1996).

Thyme oil can be used as an alternative natural preservative in the kareish cheese, enhancing the nutritional value, hygienic quality and acceptable taste (El- Bialy, 2016)

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