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Original Article

Fungal diversity in different types of cheese and the effect of natamycin on their survival during Feta cheese manufacture and storage

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ABSTRACT

Objective: This study was planned to assess the distribution of yeasts and moulds in different types of cheese, and to study the effect of natamycin on their survival during manufacture of Feta cheese and its storage.

Materials and methods: A total of 150 samples of local manufactured cheese were collected randomly from markets of Qena city in Egypt which were subjected for mycological examination by traditional microbiological examinations. The effects of different concentrations of natamycin on survival of yeasts and moulds in Feta cheese at room temperature, refrigeration condition, and pH values were evaluated.

Results: Highest yeasts and moulds contaminations were recorded in Kareish cheese with a mean value of $2.40x10^6 \pm 9.72x10^5$ and $4.64x10^2 \pm 1.18x10^2$, respectively. The isolated moulds were mostly *Cladosporium*, *Penicillium* and *Aspergillus*, while yeast genera were species of *Candida* and *Debaryomyces hansenii*. Yeasts and moulds could not be detected after 24 h, and after curd in Feta cheese samples containing natamycin (at 0.2% or 0.4%) when the cheese samples were stored either at room or refrigeration temperature. Gradual decrease of pH value was also recorded in the cheese.

Conclusion: Natamycin has strong antifungal activity and can extend cheese shelf-life during storage period.

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INTRODUCTION

Cheese is universally recognizes as first class food, due to richness of high proteins, fat, calcium, phosphate, multiple vitamins and minerals. Production and handling of white soft cheese, is still under-way especially those produced by cheese maker in most villages distributed all over the country leading to contamination of the cheese yeasts and moulds that gaining access to the cheese from various sources, as starter cultures and processing equipements (Kure et al., 2004).

The fungal diversity which presented in cheese depends on the microbial quality of milk, heat treatment of milk, manufacturing temperature, humidity during ripening, amount of salting, and microbial contamination during and after manufacture (Torkar and Teger, 2006).

Contaminating cheese with fungi lead to spoilage or produce undesirable flavors, aromas, or other metabolic products which rendering them unsuitable for consumption (Banjara et al., 2015). Some moulds are able to produce mycotoxins that adversely affect human and animal health causing as gastroenteritis and cancer (Garcia et al., 2009; Dalie et al., 2010)

The most common problem for the cheese manufacture is fungal growth on cheese during ripening and for the retailer and consumer during refrigeration storage. So, there is a need for natural preservatives which possess antimicrobial activity but cause no problems to the handlers and consumers. In this respect, natamycin (pimaricin) is a natural polyene macrolid produced by *Streptomyces natalensis* bacterium. Such bacteria inhibit microbial activity through binding to and change permeability of fungal cell membrane (Deacon, 1997). In over sixty countries, Natamycin has been used in dairy products and other foods as a natural preservative (Delves-Broughton et al., 2005).

Natamycin has been described as General Recognized Safe product for human by FDA (Koontz et al., 2003). It is identified by the European Union as a natural preservative (EFSA, 2009).

Many researchers recommended the use of natamycine as a natural antimycotic polyene in dairy based food products to prevent contamination with yeasts and moulds (Dzigbordi et al., 2013; Kallinteri et al., 2013; Dervisoglu et al., 2014).

Therefore, objectives of the study were to know fungal distribution in different types of cheese generally consumed in Egypt and to evaluate the effect of different natamycin concentrations of on survival of yeast and mould during manufacture of Feta cheese which stored at room and refrigerator temperature.

MATERIALS AND METHODS

Samples collection: Total of 150 samples of local manufactured cheese was collected randomly from markets of Qena city of Egypt including Damietta, Kareish and processed cheese (50 samples each). kariesh cheese were collected from farmer's houses, groceries and markets. Damietta and processed cheese samples were purchased from different dairy shops and supermarkets in their packages ready for sale. Samples were collected in dry, clean and sterile glass containers. The samples were transported to laboratory in ice box (4±2°C) within 1–2 h of collection and immediately analyzed after arrival.

Samples preparation: Cheese samples were released aseptically from their plastic package and thoroughly mixed in a sterile mortar. 10 gm of each sample were mashed in stomacher at 45°C for 2 min. Thereafter to obtain a dilution of 1:10, 90 mL of 2% sterile sodium citrate solution was added. The samples were diluted serially and plated on YGC agar media (0.5% yeast extract, 2% glucose, 1% agar and 0.1% chloramphenicol) and aerobically incubated at 25°C for 5 days. The individual colonies were selected according to their color and morphology. The selected colonies were restreaked on YGC agar, then incubated for 4 days at 25°C, to ensure pure culture and kept at 4°C till identification.

Identification of yeasts and moulds: Fungal cultures were kindly identified by stuff members of the Assiut University Mycological Centre (AUMC), Assiut, Egypt, using the following references: Kwon-Chung and Bennett (1992), Odds and Bernaerts (1994) and Hoog et al. (2000). The isolated yeasts were identified according to Kurtzman et al., (2003) and by API20 C AUX system (bioMérieux, France).

Culture preparation: Yeast and mould used in this study were previously isolated from Damietta cheese, and were propagated in YGC enrichment broth then incubated for 4 days at 25°C. One mL of the culture was serially diluted in 1% peptone water to attain the desired inoculum levels.

Manufacture and treatment of Feta cheese: Feta cheese was prepared in the laboratory of Assiut Dairy Plant from pasteurized milk at 78°C for 15 Sec. Enough broth culture of yeast and mould were added to the warmed (40°C) pasteurized milk to provide approximately $2x10^8$ c.f.u./mL. The inoculated pasteurized milk was

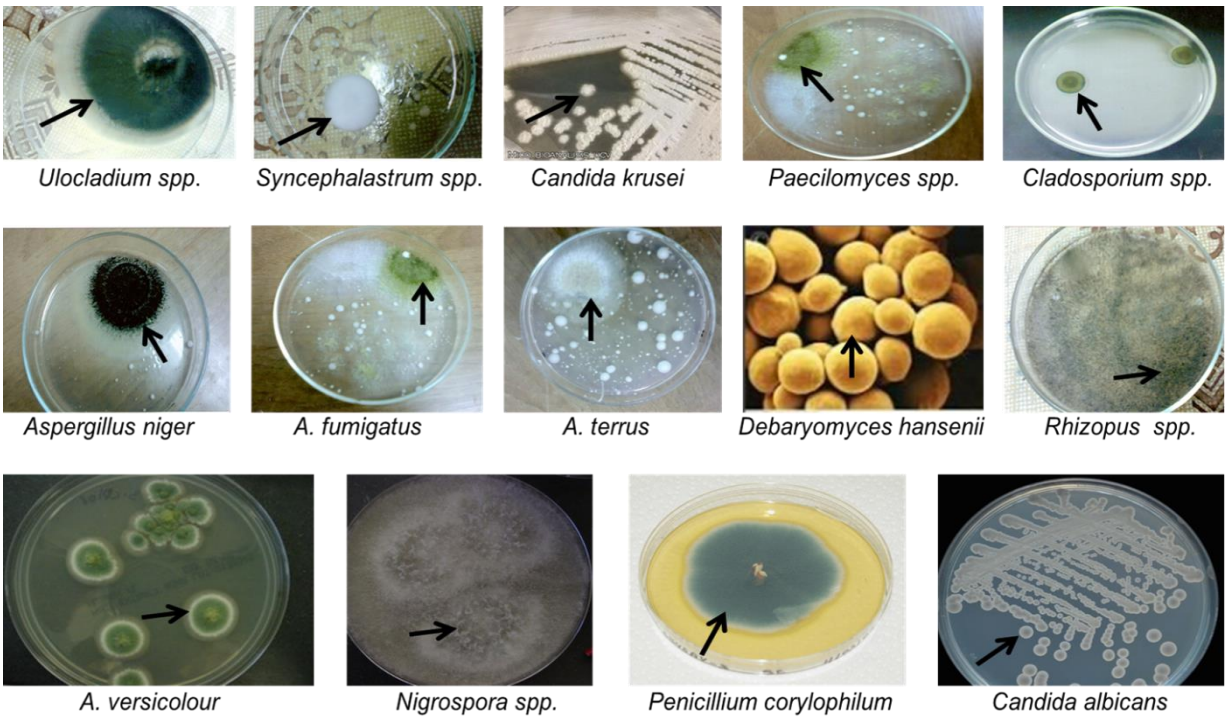

Figure 1. Different yeast and mould genera isolated in this study

salted to provide a concentration of 4% of rennet and calcium chloride (0.02%) and Glucorodelta lactone (GDL) 2.5% were added and mixed continuously.

Addition of natamycin (Natamax) to pre cheese after mixing, natamycin provided by Danisco (Als. Dk-7200 Grindsted, Denmark) was added in concentration of 0.2 and 0.4% (WHO, 1976). Cheese was manufactured and divided into three sections to be tested in this part of the study. Two concentrations of natamycin (Piramycin) in aqueous suspension (0.2 and 0.4%) were prepared in which cheese immersed before ripening. The third cheese section was left without natamycin suspension and kept as control.

Each section was divided into two portions, the first was kept at room temperature (25±2°C) and the other was stored at refrigerator temperature (5±2°C). The effect of natamycin aqueous suspension on the growth of yeast and mould was determined for inoculated milk, curd and after filtration, as well as, at zero time day of cheese manufacture and every 24 h.

Control samples were tested for pH values according to standard methods of APHA (1978) with a pH meter (Orion Model 201) equipped with standard combination electrodes which was inserted into the sample for at least 45 sec. then the pH value was recorded directly.

Statistical analysis: yeast and mould incidence was calculated by dividing number of positive samples by the total number of examined samples. The obtained data were entered into Microsoft Excel Spreadsheet.

RESULTS AND DISCUSSION

Dairy products manufactured from raw milk might carry a wide variety of pathogenic agents as yeasts and moulds (Melville et al., 2011; Lavoie et al., 2012).

Table 1 showed contaminated samples from Damietta, Kareish and processed cheeses with yeast at percentages of 66,100 and 36% respectively with mean values of 2.63 x 10^5 ± 1.21 x 10^5, 2.40 x 10^6 ± 9.72 x 10^5 and 8 x 10 ± 9.22 x 10, respectively.

The obtained result of the examined Kareish cheese samples run parallel to that obtained by Soliman and Aly et al. (2012). Lower counts obtained by El-Diasty and Salem (2007) and by ELbagory et al. (2014). In case of Domiati cheese samples the results were similar to that obtained by Saad (2010). Lower counts obtained by Aly et al. (2007). For processed cheese samples higher counts were obtained by ELbagory et al. (2014).

Results presented in **Table 2** reveals that the examined samples of Damietta, Kareish and processed cheeses

were contaminated with moulds at percentages of 46, 52 and 20% respectively with mean values of $4.45x10^2±1.61x10^2$, $4.64x10^2±1.18x10^2$ and 1.7x10±2.55, respectively.

In case of the examined Domiati cheese samples, higher counts were obtained by Aly et al. (2007). Concerning Kareish cheese samples higher findings were reported by ELbagory et al., (2014). Lower findings were observed by El Sayed et al. (2011), Hussein et al. (2011) and Aly et al. (2012). Regarding results of processed cheese lower incidence were observed by Hussein et al. (2011). While relatively, higher counts were recorded by EL-Shibiny el al. (2013) and ELbagory et al. (2014).

Counts of yeasts and moulds are used as an index of the proper sanitation quality in cheese. In addition, some species constitutes public health hazard due to its production of mycotoxins (Garcia et al., 2009; Dalie et al., 2010). The main defects caused by yeasts in cheese are gas production, off flavors, texture and discoloration changes.

As reported in **Table 3** and **Figure 1**, *Candida*, *Cladosporium*, *Penicillium* and *Aspergillus* were the most common genera recovered from the examined cheese samples. Similar results were recorded by Saad (2010) and ELbagory et al. (2014).

Candida albicans was clearly the dominant fungus in all examined cheese types. *Candida* species secrets toxic metabolites, which cause many symptoms (Colombo et al., 2006; Silva, 2010). The pathogenicity of *Candida* species in gastrointestinal tract is related to adhesion factors that mediate yeast binding to cell surface and production of lipase, protease, and phospholipase, which help in their propagation and invasion (Schulze and Sonnenborn, 2009).

D. hansenii present in 12.12, 16 and 27.7% of Damietta, Kareish and Processed cheese respectively. *D. hansenii* prevalence in cheese is due to its ability to grow at high salt content, low pH, low temperature, low water activity as well as its ability to use lactate as a source of carbon. Moreover, it produces lipolytic and proteolytic enzymes

Table 1. Statistical analytical results of yeast count in the examined cheese samples.

Types of cheese samples	Examined samples (n)	Positive		Counts/gm		
		No.	%	Min.	Max.	Mean±SE
Damietta cheese	50	33	66	1.x10	$2.25x10^5$	$2.63x10^5±1.21x10^5$
Kareish cheese	50	50	100	$1.6x10^2$	$2.27x10^6$	$2.40x10^6±9.72x10^5$
Processed cheese	50	18	36	1 x10	$2.23x10^2$	8x10±9.22x10

Table 2. Statistical analytical results of mould count in the examined cheese samples.

Types of cheese samples	Examined samples (n)	Positive		Counts/gm		
		No.	%	Min.	Max.	Mean±SE
Damietta cheese	50	23	46	1x10	$4.82x10^3$	$4.45x10^2±1.61x10^2$
Kareish cheese	50	26	52	1x10	$3.14x10^2$	$4.64x10^2±1.18x10^2$
Processed cheese	50	10	20	1x10	3.6x10	1.7x10±2.55

Table 3. Incidences of fungi isolated from the examined cheese samples.

Isolated fungi	Damietta cheese		Kareish cheese		Processed cheese	
	No.	%	No.	%	No.	%
Yeast						
Candida albicans	8	24.24	15	30	12	66.66
Candida krusei	3	9.09	2	4	0	0.00
Debaryomyces hansenii	4	12.12	8	16	5	27.77
Mould						
Rhizopus spp.	1	4.34	0	0.00	0	0.00
Syncephalastrum spp.	2	8.69	0	0.00	0	0.00
Paecilomyces spp.	1	4.34	0	0.00	1	10
Aspergillus versicolour	1	4.34	0	0.00	0	0.00
Aspergillus fumigatus	1	4.34	3	11.53	1	10
Aspergillus niger	2	8.69	5	19.23	1	10
Aspergillus terrus	1	4.34	0	0.00	1	10
Penicillium corylophilum	2	8.69	10	38.46	6	60
Cladosporium cladosporidis	5	21.73	7	26.92	5	50
Nigrospora spp.	1	4.34	0	0.00	0	0.00
Ulocladium spp.	0	0.00	0	0.00	1	10

Table 4. Effect of natamycin on the growth of yeast and mould during manufacture and storage of Feta cheese at room temperature (25±2°C).

Storage period	Control (0%)	Natamycin Concentrations		pH of control cheese
		0.2%	0.4%	
Inoculated milk	20×10^7	20×10^7	20×10^7	6.4
After curd	20×10^7	20×10^6	20×10^3	6.4
After filtration	30×10^6	14×10^3	4×10	6.0
Cheese at 0 h	14×10^7	12×10	ND	5.8
Cheese at 24 h	50×10^5	ND	ND	5.5
Cheese at 72 h	22×10^5	ND	ND	5.2

**Initial count ≃ 2×10^8 c.f.u. / mL or gm.*
ND: Not Detected

Table 5. Effect of natamycin on the growth of of yeast and mould during manufacture and storage of Feta cheese at refrigerator temperature (5±2°C).

Storage period	Control (0%)	Natamycin concentrations		pH of control cheese
		0.2%	0.4%	
Inoculated milk	20×10^7	20×10^7	20×10^7	6.4
After curd	60×10^6	10×10^6	30×10^4	6.4
After filtration	20×10^5	10×10^2	10×10	6.0
Cheese at 0 h	23×10^5	10×10	ND	5.8
Cheese at 24 h	12×10^5	ND	ND	5.6
Cheese at 72 h	20×10^4	ND	ND	5.3

**Initial count ≃ 2×10^8 c.f.u. / ml or gm.*
ND: Not Detected

that capable of metabolizing milk fat and proteins, respectively (Gori et al., 2012).

Growth of such genera may responsible for bitterness and rancidity of cheese. *Penicillium* species may lead to softness the surface of cheese Minervini et al. (2001) and have been associated with pulmonary, urinary tract infections and death in man. Many of *Asperigallus*, *Cladosporium*, *Penicillium* and *Fusarium* species were responsible for kerato-conjunctivitis in man while *Asperigellus niger* causes otomycosis and allergic condition Nielsen et al. (1998).

The results achieved in **Table 4** showed that the count of yeast and mould during manufactured and storage of Feta cheese kept at room temperature (25±2°C) containing 0.2% natamycin was gradually decreased during the manufacture of cheese to 12×10 c.f.u./gm at zero time until the pathogen failed to be detected and completely disappeared at the end of 24 h of storage (Initial count was ≃2×10^8 c.f.u./mL or gm). While, the count of yeast and mould in manufactured and storage of Feta cheese containing 0.4% natamycin was gradually decreased during the manufacture of Feta cheese to 4×10 c.f.u./gm after filtration, until the pathogen failed to be detected and completely disappeared at zero time of storage. On the other side, in control samples, the count of yeast and mould decreased from the initial count of 20×10^8 c.f.u./mL or gm to 22×10^5 c.f.u./gm in cheese at 72 h. Gradual decrease of pH value of Feta cheese occurred from 6.0 to 5.2 in cheese samples at zero time to the end of cheese samples at 72 h, respectively.

Results in **Table 5** depicted natamycin effect of on survival of yeast and mould in manufactured and storage of Feta cheese at refrigerator temperature (5±2°C). The initial count of yeast and mould was 2×10^8 c.f.u./mL or gm decreased to 100 organisms/gm at zero time in storage in cheese 0.2% natamycin containing samples, then the pathogens failed to be detected at the end of 24 h of storage cheese samples. While, the count of yeast and mould in Feta cheese containing 0.4% natamycin was gradually decreased during manufacture to 100 organisms /gm, until the pathogen failed to be detected and completely disappeared at the end of zero time of storage of cheese.

On the contrary, the pathogens were still survived in the samples free from natamycin (control) until 72 h of the storage Feta cheese kept at refrigerator temperature. Slight decrease of pH was observed from 6.0 to 5.3 at the end of 72 h of the storage.

From the aforementioned results, it was evident that the increased natamycin concentration with storage of Feta cheese in refrigerator temperature had an inhibitory effect on survival of yeasts and moulds. These results agreed with the theory which pointed out that microorganisms can survive for long period at low temperatures below those which permit growth.

Shahani et al. (1985) reported that natamycine at low concentration was more effective against both yeasts and molds than any other known antifungal. As well the results of this study agreed with the results recorded by Reps et al. (2002), they noted the antifungal effects of Delvocid containing (50% natamycin) at concentration of 0.2 and 0.4% in the cheese surface after salting in brine aquous suspension.

CONCLUSION

Natamycin is an effective natural antifungal preservative against yeasts and moulds at very low concentrations. Cheese treated with natamycin could extend the shelf-life of during refrigeration storage period which is desired by manufacturers and consumers.

CONFLICT OF INTEREST

Nothing to declare.

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