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The inhibitory Effect of Natamycin and Potassium Sorbate on Mold Growth in Egyptian Fresh Soft Cheese (Tallaga Cheese)

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Abstract

One of the most common problems in fresh soft cheese manufacture is fungal growth during storage period. In this respect, this study aimed to control fungal growth in Tallaga cheese by natural preservatives as natamycin (pimaricin) and other allowed substances as potassium sorbate. In this study, we evaluated the effect of different concentrations of natamycin (5, 10 and 20 ppm) and potassium sorbate (0.5 and 1%) and combination of natamycin (10 ppm) with potassium sorbate (1%) on molds growth in Tallaga cheese stored at 4 ± 1 ºC for up to 30 days. The results showed that natamycin and potassium sorbate have inhibitory effect on survival of mold in Tallaga cheese kept at refrigeration temperature and the inhibition percentage is concentration dependent. Natamycin showed strong antifungal activity at different concentrations especially when used at 20 ppm than potassium sorbate. The combination of natamycin with potassium sorbate showed synergistic antifungal activity.

1. INTRODUCTION

Tallaga cheese (Cold Stored Cheese) is one of the most popular local type of soft cheeses in Egypt, it favored by all socioeconomic classes due to its nutritional value, good taste, clean pleasant creamy low salty taste with a spreadable smooth soft body and mainly ready for consumption within one month of storage at refrigerator temperature (Mehanna and Rashed, 1990 and El-Kholy, 2016)

Cheese is the greatest part of the dairy market in Egypt. One of the most challenges that threaten cheese manufacturers is mold growth and spoilage of cheese during storage, that is commonly attributed to the increased surface area of cheese, extra handling during cutting of cheese, packaging facilities and from other various sources as starter cultures and processing equipments (Kure et al., 2004).

Fungal contamination could make cheese unsuitable for consumption as a result of production of undesirable flavors, aromas and other metabolic products (Banjara et al., 2015). Moreover, some molds are able to produce mycotoxins that adversely affect human and animal health causing gastroenteritis and may cause some health problems and some are carcinogenic (Garcia et al., 2009 and Dalie et al., 2010).

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).

Natamycin and sorbic acid have antimicrobial preservative effect and commonly used as antifungal

agents in cheese. Natamycin (pimaricin) is produced naturally by *Streptomyces natalensis* bacterium, which act through binding to fungal cell membrane and change its permeability (Deacon, 1997).

Natamycin is very effective in preventing mold growth and has been observed to have an inhibitory effect on toxin production by toxigenic molds at very low concentrations (Ray and Bullerman 1982).

In the same context, sorbic acid and its more water-soluble salts, potassium sorbate, are widely used throughout the world as preservatives for various foods (Sofos, 1989). Potassium sorbate is a naturally occurring organic acid that has been used extensively as a fungistatic agent for foods (Rajapaksha et al., 2013). It may be added directly to the food, or incorporated into the packaging method. According to the Code of Federal Regulations it is regard as generally recognized as safe (GRAS) when used in accordance with good manufacturing or feeding practice.

This study aimed to control fungal growth in experimentally manufactured Tallaga cheese by addition of natural preservatives as natamycin (pimaricin) and other allowed substances as potassium sorbate.

2. MATERIALS AND METHODS

2.1 Preparation of natamycin and Potassium sorbate:

Solutions of natamycin (Natamax®, Danisco) and potassium sorbate (Monsato Co., St. Louis) were prepared and sterilized by filtration. Concentrations of potassium sorbate used were in the range of commercial usage levels. Levels of natamycin used were around the minimum inhibitory concentration to fungi in foods, which is 1 to 20 ppm (Stark, 2003).

Six different suspensions of natamycin (5 ppm), natamycin (10 ppm), natamycin (20 ppm), potassium sorbate (0.5%), potassium sorbate (1%) and combination of natamycin (10 ppm) with potassium sorbate (1%) were prepared in distilled water by stirring. These suspensions were separately used during the manufacture of Tallaga cheese at laboratory to study the effect of such preservatives on mold growth.

2.2. Isolates and culture preparation:

Four isolates of Aspergillus spp. (*A. flavus, A. fumigatus, A. niger* and *A. terrus*) previously isolated and identified from dairy products were used. The isolates are obtained from laboratory of Food Hygiene and control, Faculty of Veterinary Medicine, Sadat City University. The isolates were propagated in Sabouraud broth then incubated for 48 h at 25°C. One mL of the culture was serially diluted in 1% peptone water to attain the desired inoculum levels.

2.3. Preparation of Tallaga cheese samples:

Tallaga cheese manufacturing was carried out according to El-Kholy et al. (2016), with some modification. Briefly, fresh buffalo's milk was heated at 80 °C for 15 sec, then immediately cooled to 37 °C. Enough broth culture of mold added to the warmed (37 °C) heat treated milk to provide approximately 10^3 cfu/ml. Further, Calcium chloride, sodium chloride and rennet were added at the ratios of 0.02, 3 and 0.05% (w/v), respectively.

Accordingly, 7 groups were prepared as follows. The 1st group was untreated control, while the other 6 groups were treated, directly before addition of rennet, by natamycin (5, 10 and 20 ppm), potassium sorbate (0.5% and 1 %) and mixed natamycin (10 ppm) and potassium sorbate (1 %). All groups, either control or treated, were subjected to mycological assessment at day zero (within 2 hours after treatment) then stored at 4 ±1 °C, and periodically checked every week (1st, 2nd, 3rd and 4th weeks).

2.4. Determination of mold count:

Mold counts were determined by pour plate technique using Sabouraud Dextrose agar media as described elsewhere (ICMSF, 1996)**.** The inoculated plates were incubated at 25 °C for 5-7 days. During the incubation period, the inoculated plates were examined daily for mold colonies and the mold count/ g was calculated and recorded.

3. RESULTS

The present study evaluated the effect of different concentrations of natamycin (5, 10 and 20 ppm) and potassium sorbate (0.5 and 1%) and combination of natamycin (10 ppm) with potassium sorbate (1%) on the molds growth pattern in Tallaga cheese stored at 4± 1 °C for up to 30 days.

Data in Fig. 1 showed the inhibitory effect of different concentrations of natamycin and potassium sorbate on survival of mold, in addition Table 1 showed the inhibition percentage in mold growth in treated samples of Tallaga cheese kept at refrigeration temperature.

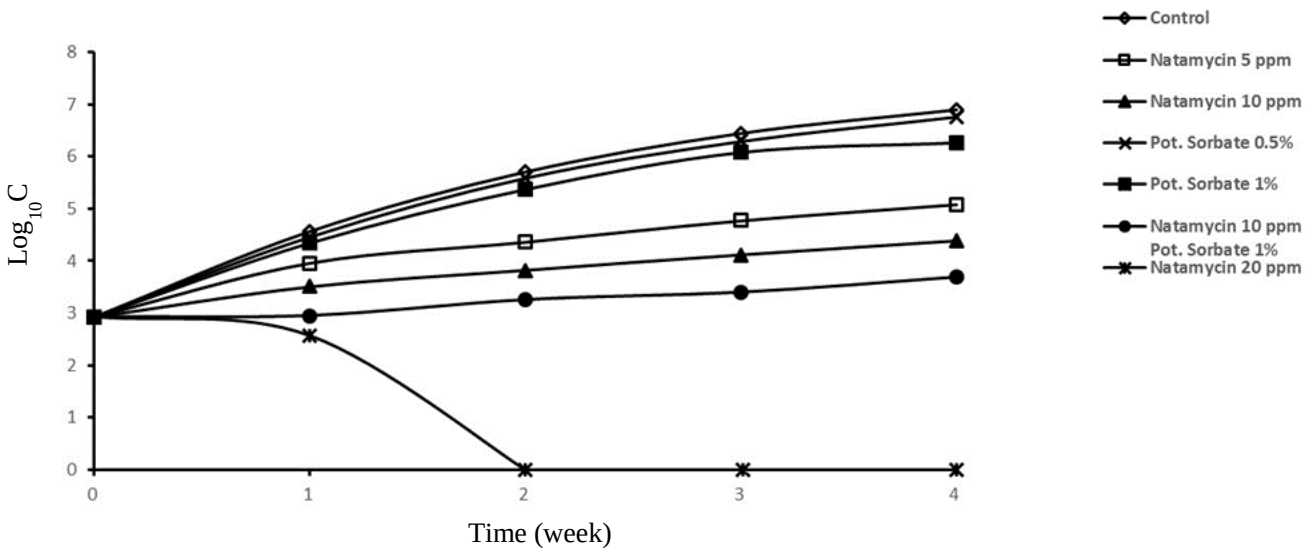

Fig 1. Survival and growth patterns of mold (log CFU/gm) in control untreated cheese samples *(absence of natamycin and potassium sorbate)* and treated samples with different concentrations of natamycin and/or potassium sorbate in Tallaga cheese during refrigerated storage at 4±1 °C.

Table 1. Inhibition percentages in mold counts in treated samples of Tallaga cheese as compared with untreated ones during refrigerator storage at 4±1 °C

Storage time	Treatment						
	Control	**Natamycin (5 ppm)**	**Natamycin (10 ppm)**	**Pot. Sorbate (0.5%)**	**Pot. Sorbate (1%)**	**Natamycin (10 ppm) + Pot. Sorbate (1%)**	**Natamycin (20 ppm)**
	(Mean ± S.E*)	**Reduction %**					
Zero time	$8.5\times10^{2}\pm 0.3\times10^{2}$	-	-	-	-	-	-
1st week	$3.7\times10^{4}\pm 0.6\times10^{4}$	75.4	91.4	21.6	40.5	97.6	99.9
2nd week	$5.1\times10^{5}\pm 0.9\times10^{5}$	95.5	98.7	23.5	54.3	99.6	100
3rd week	$2.8\times10^{6}\pm 0.4\times10^{6}$	97.8	99.5	29.6	57.5	99.9	100
4th week	$7.9\times10^{6}\pm 1.1\times10^{6}$	98.4	99.7	26.6	77.0	99.9	100

N.B. *Inhibition % was calculated according to deviation of treated groups away from control group at the same storage time*

The initial mold count of Tallaga cheese, at zero time, was $8.5x10^2 \pm 0.3x10^2$ cfu/ gm. The count of mold after one week of refrigerated storage started to increase gradually till reach $7.6x10^6 \pm 1.1x10^6$ cfu/ gm by the end of the 4th week, while in the cheese samples treated with natamycin or potassium sorbate or combination of them, the mold count were much lower than the count of control samples stored for the same period.

In cheese samples treated with 5 ppm natamycin, mold growth inhibition percentages, compared with control group at the same storage time, were 75.4, 95.5, 97.8 and 98.4 after the 1st, 2nd, 3rd and 4th week of refrigerated storage, respectively (Table 1).

The group of Tallaga cheese treated with 10 ppm natamycin, the reduction percentages of mold counts were 91.4, 98.7, 99.5 and 99.7 after the 1st, 2nd, 3rd and 4th week of refrigerated storage, respectively (Table 1) the inhibition percentages in samples treated with 10 ppm concentration were higher than those obtained with 5 ppm concentration.

While, the group of Tallaga cheese treated with 0.5% potassium sorbate, mold count was slightly decreased than the count of the control group. The reduction percentages were 21.6, 23.5, 29.6 and 26.6 after the 1st, 2nd, 3rd and 4th week of refrigerated storage, respectively (Fig.1 & table1). When the potassium sorbate concentration increased to 1%, the reduction percentage of mold counts slightly increased (Table 1).

The treated cheese samples contained both natamycin (10 ppm) and potassium sorbate (1%), mold growth reduction percentages were 97.6, 99.6, 99.9 and 99.9 after the 1st, 2nd, 3rd and 4th week of refrigerated storage, respectively (Fig.1 & table1). Mold growth reduction percentages obtained with this combination (natamycin 10 ppm and potassium sorbate 1%) were slightly higher than the concentration of natamycin (10 ppm) when added in separate from potassium sorbate (1%) signifying that a synergistic relationship may exist.

The last group of treated cheese samples contained natamycin (20 ppm), after the 2nd week of storage mold growth couldn't be detected (Fig.1 & table1).

4. DISCUSSION

Recently, natamycin is used to inhibit mold and yeasts growth in some dairy products (Pintado et al., 2010, Dzigbordi, et al., 2013, Kallinteri et al., 2013; Ollé Resa, et al., 2014; Galal & Hameed, 2016).

In the European Union, natamycin is permitted as a food additive for the surface treatment of hard, semi-hard and semi-soft cheese. In the USA, natamycin is an approved food additive. It may be applied on cheese in amounts not to exceed 20 mg/kg in the finished product as determined by International Dairy Federation Standard 140A: 1992 (21 CFR 172.155). In Canada, natamycin is permitted on 47 named cheeses up to 20 ppm and in shredded/grated cheese up to 10 ppm residual level (Stark, 2003). The Code of Federal Regulations (CFR) defines sorbic acid as a substance that is generally recognized as safe (GRAS) when used in accordance with good manufacturing practice (CFR 2012).

The results obtained in this study showed that the addition of natamycin either singly added or in combination with potassium sorbate to Tallaga cheese, kept under refrigerated storage, had an inhibitory effect on survival of molds. Our results showed that natamycin, at concentrations of 20 ppm, can efficiently suppress the growth of molds in Tallaga cheese.

This result come to an agreement with Kallinteri et al. (2013) who pointed out that natamycin, added either singly or in combination with nisin, efficiently suppressed fungal growth in the Galotyri cheese.

In the same context, Galal and Hameed (2016) reported that yeasts and molds could not be detected in Feta cheese samples containing natamycin (at 0.2% or 0.4%) when the cheese samples were stored either at room or refrigeration temperature.

Potassium sorbate showed less inhibitory effect, than natamycin, against the studied mold species. The obtained results agreed with the findings of Finol et al. (1982) who reported that cheeses with potassium sorbate as a preservative are sometimes spoiled by molds, even if the cheeses are refrigerated. This finding may be attributable to that *A. niger* is more resistant to potassium sorbate than other mold species as observed by Ooraikul (1991).

In conclusion, the results of the present study indicate that natamycin is an effective natural antifungal preservative against molds at very low concentrations. The combination of natamycin with potassium sorbate showed synergistic antifungal activity

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