



The role of natamycin fortification to extend shelf life of plain yoghurt

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ABSTRACT

The present study was carried out to evaluate the shelf life and sensory evaluation of yoghurt supplemented with natamycin during refrigerator storage. Yoghurt samples were prepared and divided into two groups; G1, control group contains *Lactobacillus delbrueckii subspp bulgaricus* (*Lb. bulgaricus*) and *Streptococcus thermophilus* (yoghurt starter cultures) and G2, natamycin group contains yoghurt starter cultures with natamycin 10 ppm. The overall sensory score of the examined yoghurt samples G2 were higher than G1. The mean counts of *Lactobacillus bulgaricus* in G1 and G2 increased gradually till 7th day of refrigeration storage reached 10.51 ± 0.10 and 10.55 ± 0.01 then decreased gradually till end of shelf life reached 9.43 ± 0.68 and 9.37 ± 0.66 . The mean counts of *Streptococcus thermophilus* in both G1 and G2 were 9.55 ± 0.68 and 9.50 ± 0.62 at zero day then decreased gradually till the end of shelf life reach 9.33 ± 0.67 and 7.94 ± 0.05 . Total yeast and mold counts were detected in G1 at 7th, 14th and 21st day of refrigeration storage with mean values of 1.20 ± 0.10 , 3.20 ± 1.09 and 3.61 ± 0.46 log₁₀ cfu/g; respectively. Molds and yeasts failed to be isolated from the G2 during 31 days of refrigeration storage period. The mean values of yeast and mold counts in G2 were 1.00 ± 0.00 , 1.10 ± 0.10 and 3.36 ± 0.66 after 34, 37 and 40 days; respectively. The current study proved that natamycin fortification for plain yoghurt increases its shelf-life up to 40 days with keeping its sensorial characters.

Keywords: Yoghurt, Natamycin, Sensory score, Shelf-life

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1. INTRODUCTION

Naturally occurring antimicrobial compounds could be applied as food preservatives to protect food quality and extend the shelf-life of foods. These compounds are naturally produced and isolated from various sources, including plants, animals and microorganisms, in which they constitute part of host defense systems. Many naturally occurring compounds, such as nisin, plant essential oils, and natamycin, have been widely studied and are reported to be effective in their potential role as antimicrobial agents against spoilage and pathogenic microorganisms (Juneja *et al.*, 2012). Natamycin (also known as pimaricin) is a natural antifungal agent with a wide range of antimicrobial spectrum against yeasts and molds produced during fermentation by the bacterium *Streptomyces natelensis* and

is widely used in the food industry for the prevention of mold contamination in meats, cheese and fruits (Reps *et al.*, 2002; Jay *et al.*, 2005; Welscher *et al.*, 2008). The use of natamycin as a natural preservative in dairy products and other foods has been approved in over sixty countries (Delves-Broughton *et al.*, 2005). More specifically, natamycin is commonly used in dairy products such as cottage cheese, sour cream and yoghurt (Chen *et al.*, 2008). Its superiority over other natural antifungals has been attributed to its wide spectrum of antifungal activity at low concentrations and its effectiveness without changing organoleptic characteristics of the food products such as cheese, meats and juices (Food Standards Australia New Zealand, 2004; Dzigbordi *et al.*, 2013). Natamycin has a broad spectrum of activity against spoilage fungi and is

considered to be a very stable powder with efficacy against *Aspergillus flavus* and aflatoxin production (Ruas-Madiedo *et al.*, 1988; Juneja *et al.*, 2012), *Aspergillus carbonarius* and ochratoxin A production (Medina *et al.*, 2007), *Aspergillus niger*, *Aspergillus versicolor*, *Penicillium chrysogenum*, *Penicillium glabrum*, *Penicillium commune*, *Penicillium verrucosum*, *Byssochlamys nivea* and others (Stark, 2003). Recently, the European Food Safety Authority (EFSA) has published a favorable scientific opinion on the use of natamycin as a food additive (EFSA, 2009). Yoghurt is a very popular dairy product in Egypt. Its production and consumption is growing continuously due to its health benefits beside its high nutritive value. Being nutritionally rich in protein, calcium, riboflavin, vitamin B6 and B12 (Karagul *et al.*, 2004; Ashraf and Shah, 2011). Yoghurt is produced through the fermentation of milk lactose by *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp bulgaricus* (Tamime and Robinson, 2007). Despite yoghurt is generally considered as microbiologically stable, they may be subjected to contamination with acid tolerant fungi. Fungi are responsible for the spoilage of various dairy products. As, most molds can grow at wide pH range of 3 to 8 and can withstand low water activity levels 0.7 to 0.8. Fungal sources of contamination into milk are air, equipment, dust, and soil which cause problems, both economic and sensory (Krue *et al.*, 2004). *Candida parapsilosis*, *Candida diffluens*, *Kluyveromyces marxianus*, *Rhodotorula mucilaginosa*, *Yarrowia lipolytica*, *Zygosaccharomyces bailii* or *Penicillium brevicompactum* are among the most frequently encountered fungal contaminants in yoghurt (Mayoral *et al.*, 2005; Delavenne *et al.*, 2013). Besides, mold contamination may lead to the production of mycotoxin such as aflatoxins, which cause disease of man (Nwagu and Amadi, 2010). The present study aims to determine the effect of natamycin fortification on plain yoghurt shelf life and

sensory properties during refrigeration storage.

2.2. MATERIAL AND METHODS

2.1. Determination of antifungal activity

Indicator pathogenic yeast strain (*Saccharomyces cerevisiae*) and mold strain (*Aspergillus fumigatus*) were obtained from Cairo MIRCEN (Microbiological Resource Center) Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The strains were used for antifungal assay of natamycin. They were cultivated and maintained in Sabouroud dextrose agar slants. The agar slants were preserved in a refrigerator at 4°C until use within 48 h. The organisms were activated for three successive times till obtaining the concentration of 10³ cfu/ml (Laref and Guessas, 2013). Natamycin was obtained from Lanzhou Weiri Bio-Engineering Co., Ltd. Lanzhou, China. According to manufacturer's instructions, natamycin stock solution was prepared by dissolving in low concentration of HCl (0.02 N HCl solution) and kept refrigerated. The concentration of natamycin for yoghurt preservation has been suggested to be in the range of 5-10 ppm (Thomas and Delves-Broughton, 2001). The antifungal activity assay was conducted under complete aseptic condition as following; 4.5 ml of yeast extract peptone dextrose broth, 0.5 ml of fungal culture suspension (to have a final concentration of 10³ cfu/ml) and natamycin (appropriate amount of natamycin stock solution to have a final concentration of 10 ppm) were mixed into a sterile test tube and incubated at 25°C for 24 h. A control was made only with yeast extract peptone dextrose broth and the fungal culture suspension. 100 µl from the already prepared serial dilutions was spread onto a petri dish dry surface of sterile Sabouroud dextrose agar with chloramphenicol (0.01%) and incubated at 25°C for 48-72 h. After this period, colonies were counted (Moita *et al.*, 2005). The experiment was repeated 3 times.

2.2. Yoghurt manufacturing

2.2.1. Activation of starter cultures

Lactobacillus delbrueckii subsp *bulgaricus* and *Streptococcus thermophilus* (yoghurt starter cultures) were obtained from Cairo MIRCEN Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Both strains were activated on MRS broth and M17 broth, incubated at 37°C/24 h, three culture transfers were performed to activate each culture. The activated strains were transferred into sterile 11% reconstituted skimmed milk powder then incubated at 40°C/24 h. Serial dilutions were prepared till obtain concentration of 10⁷-10⁹ cfu/ml. The active starter cultures were kept in refrigerator until use within 24 h (Badawi and El- Sonbaty, 2004).

2.2.2. Preparation of yoghurt

Yoghurt was prepared as described by Nighswonger *et al.* (1996). A total of 4 L of fresh raw mixed milk of cows and buffalos (1:1) were obtained from the herd of Faculty of Veterinary Medicine, Benha University. Milk fat was standardized to 3% then milk was heated to 85°C for 30 min. and immediately cooled to 45°C. The bulk volume of milk was divided into 2 groups (2 L, each) and inoculated by the activated starter cultures (*Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus*) as follow:

G1: 2% yoghurt starter cultures 1:1.

G2: 2% yoghurt starter cultures 1:1 + Natamycin 10 ppm.

Then samples of each group were mixed and put into cups (100 ml) and incubated at 42°C until curd formation (pH~ 4.6) then transferred to refrigerator at 4°C. The yoghurt samples were examined for sensorial and microbiological evaluation at appropriate intervals till the appearance of spoilage. The yoghurt preparation and examinations were repeated for three times.

2.3. Sensory evaluation

The sensory evaluation of yoghurt samples was carried out according to Mehanna *et al.* (2000). The score given were 60 points for flavor, 30 points for body and texture and

10 points for appearance with an overall score of 100 points.

2.4. Microbiological examination

2.4.1. Preparation of serial dilutions

Yoghurt samples were thoroughly mixed under complete aseptic condition. Serial dilution was prepared; one g of each thoroughly mixed yoghurt sample was added to 9 ml sterile distilled water to make tenth fold serial dilution, from which decimal dilutions were prepared (APHA, 1992).

2.4.2. Determination of lactic acid bacterial count

Lactobacillus delbrueckii subsp *bulgaricus* was enumerated by pouring plate method. One ml from each of the previously prepared serial dilutions was transferred into Petri dishes and thoroughly mixed with MRS agar and incubated at 37°C/48 h while, *Streptococcus thermophilus* was enumerated on M17 agar supplemented with glucose 0.5% at 42°C/48 h (Ryan *et al.*, 1996).

2.4.3. Determination of total mold and yeast counts

From the already prepared serial dilutions, one ml was transferred into duplicate Petri dishes and thoroughly mixed with Sabouraud dextrose agar medium supplemented with chloramphenicol (0.01%) as described by IDF (1990). The plates were incubated at 25°C for 5-7 days. The first examination was done after 3 days to determine the degree of yeast and mold growth.

2.5. Statistical analysis

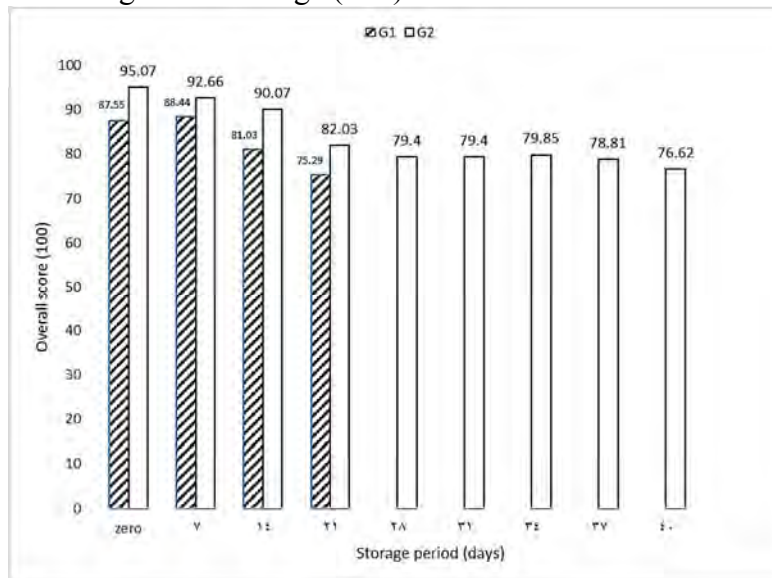
Statistical analysis of the data was done using the analysis of variance in SPSS 16.0. Statistical comparisons were made by using one-way analysis of variance (ANOVA). The results were considered significantly different with $P < 0.05$ as described by Clarke and Kempson (1997).

3. RESULTS

Figure (1) showed the overall sensory evaluation of yoghurt samples. There are significance differences in the mean overall sensory scores between G1 and G2 yoghurt samples. The mean overall sensory scores in G1 were 87.55 ± 0.25 , 88.44 ± 0.11 , 81.03 ± 0.22 and 75.29 ± 0.20 at zero, 7th, 14th and 21st day of refrigeration storage; respectively. On the other hand, the overall sensory evaluation of the examined yoghurt samples in G2 gave the highest score at zero time with a mean value of 95.07 ± 0.25 and maintained high scores during refrigeration storage with mean values of 92.66 ± 0.33 , 90.07 ± 0.09 , 82.03 ± 0.72 , 79.40 ± 0.13 , 79.40 ± 0.09 , 79.85 ± 0.30 , 78.81 ± 0.37 and 76.62 ± 0.77 after 7, 14, 21, 28, 31, 34, 37 and 40 days of refrigeration storage; respectively. Figure (2) showed the total viable counts of *Lactobacillus delbrueckii subsp bulgaricus*. The total viable counts in both G1 and G2 were the same at zero time 10.31 ± 0.08 and 10.31 ± 0.10 then increased gradually after 7 days of storage period to 10.51 ± 0.10 and 10.55 ± 0.01 then

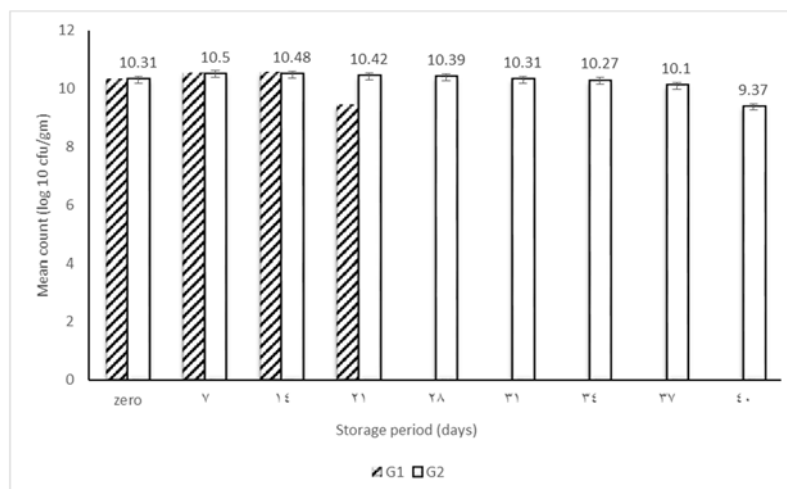
decreased gradually till the end of shelf life in all yoghurt samples reached 9.43 ± 0.68 and 9.37 ± 0.66 . Figure (3) showed the total viable counts of *Streptococcus thermophilus* which were decreased gradually after 7 days till the end of shelf life of all yoghurt samples. The mean counts of *Streptococcus thermophilus* in G1 were 9.55 ± 0.68 , 9.46 ± 0.67 , 9.36 ± 0.66 and 9.33 ± 0.67 at zero, 7th, 14th and 21st day of refrigeration storage; respectively, while in G2 they were 9.50 ± 0.62 , 9.48 ± 0.60 , 9.44 ± 0.63 , 9.31 ± 0.62 , 9.31 ± 0.63 , 9.23 ± 0.61 , 7.98 ± 0.07 , 7.95 ± 0.06 and 7.94 ± 0.05 at zero, 7th, 14th, 21st, 28th, 31st, 34th, 37th and 40th day of refrigeration storage; respectively. Table (1) illustrated the changes in yeast and mold counts of yoghurt samples during the refrigeration storage period. These results revealed that the highest yeast and mold counts were recorded for G1 when compared with G2. Molds and yeasts failed to be isolated from the G2 during 31 days of refrigeration storage period.

Figure (1) The mean values* of overall sensory scores (100) for the examined yoghurt groups during their refrigeration storage (4°C).



G1: 2% yoghurt starter cultures 1:1 (*Lactobacillus delbrueckii subsp bulgaricus*: *Streptococcus thermophilus*). **G2:** 2% yoghurt starter cultures 1:1 + 10 ppm natamycin.*Results shown were means of triplicates of each treatment. Zero day examination was done after yoghurt preparation.

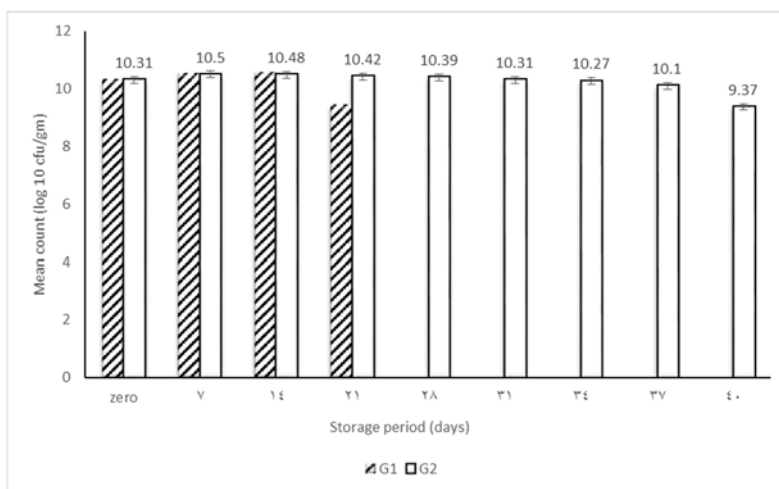
Figure (2) The mean counts* of *Lactobacillus delbrueckii subspp bulgaricus* (log₁₀ cfu/g) for the examined yoghurt groups during their refrigeration storage (4°C).



G1: 2% yoghurt starter cultures 1:1 (*Lactobacillus delbrueckii subspp bulgaricus*: *Streptococcus thermophilus*). G2: 2% yoghurt starter cultures 1:1 + 10 ppm natamycin.

*Results shown were means of triplicates of each treatment.

Figure (3) The mean counts* of *Streptococcus thermophilus* (log₁₀ cfu/g) for the examined yoghurt groups during their refrigeration storage (4°C).



G1: 2% yoghurt starter cultures 1:1 (*Lactobacillus delbrueckii subspp bulgaricus*: *Streptococcus thermophilus*). G2: 2% yoghurt starter cultures 1:1 + 10 ppm natamycin.

*Results shown were means of triplicates of each treatment.

Table (1) The mean total yeast and mold counts* (\log_{10} cfu/g) for the examined yoghurt groups during their refrigeration storage (4°C).

Storage (days)	G1 (mean± S.E*)	G2
Zero	<10 ²	<10 ²
7	1.20±0.10	<10 ²
14	3.20±1.09	<10 ²
21	3.61±0.46	<10 ²
28	S	<10 ²
31	S	<10 ²
34	S	1.00±0.00
37	S	1.10±0.10
40	S	3.36±0.66

G1: 2% yoghurt starter cultures 1:1 (*Lactobacillus delbrueckii subspp bulgaricus*: *Streptococcus thermophilus*). G2: 2% yoghurt starter cultures 1:1 + 10 ppm natamycin. ND: Not detected. S: The spoilage samples. *S.E.: Standard Error. Results shown were means of triplicates of each treatment.

4. DISCUSSION

The results of the overall sensory scores agreed with those obtained by El-Diasty *et al.* (2009); Misirlilar *et al.* (2012). This could be attributed to the effect of natamycin which improves the keeping quality in addition to preventing the growth of yeasts and molds till the end of storage time. Also, natamycin has no adverse flavor to yoghurt. El-Diasty *et al.* (2009) found that overall sensory evaluation (total score 20) were 17, 17, 17, 17, 17 and 14 at 3rd, 7th, 14th, 21st, 28th and 35th day of storage period; respectively, while control group showed 17, 15, 14, 11, 9 and 8 at 3rd, 7th, 14th, 21st, 28th and 35th day of storage period; respectively. Misirlilar *et al.* (2012) also found that the sensory properties of strained yoghurt with natamycin gain high score than control group. Yoghurt samples with natamycin (total score 5) showed 4.9±0.01, 4.9±0.02, 4.7±0.05, 4.5±0.06 and 4.2±0.09 at 1st, 7th, 14th, 21st and 28th of storage period; respectively. While control samples showed 4.9±0.01, 4.9±0.00, 4.6±0.05, 3.1±0.04 and 3.0±0.03 at 1st, 7th, 14th, 21st and 28th of storage period; respectively. El-Diasty *et al.* (2009) reported that natamycin proved a suitable and effective antifungal agent, which increases the shelf life of yoghurt without

changing its organoleptic characteristics. The gradual decrease of sensory scores of yoghurt samples may be due to the proteolytic activity and the development of acidity by the used LAB (Aly *et al.*, 2004).

The results of *Lactobacillus delbrueckii subspp bulgaricus* counts agreed with those obtained by Dave and Shah (1997) who found that the viable counts of LAB gradually increased up to the 5th day, but their survival decrease gradually during 35 days of refrigerated storage. The mean counts of *Lactobacillus delbrueckii subspp bulgaricus* were 6×10^8 cfu/g at zero day then increased to 6.6×10^8 cfu/g at 5th day then decreased gradually to be 2.20×10^8 cfu/g at 35th day. During storage time, the viability of *Streptococcus thermophilus* was decreased gradually after 7 days till the end of shelf-life of all yoghurt samples. This result nearly similar to Mohammed (2011), while El-Sayed *et al.* (2013) found that the viable counts of *Streptococcus thermophilus* were 8.98±0.04, 9.04±0.04, 8.78±0.04 and 8.30±0.04 log cfu/ml at zero time, 3rd, 9th and 15th of storage period; respectively. The low counts of *Streptococcus thermophilus* may be due to the increase in acidity of yoghurt samples, which affects *Streptococcus* spp while *lactobacilli* spp could tolerate such conditions to some extent (El-Nagar and

Shenana, 1998; El-Nagar and Bernnan, 2001). Food industries that rely upon fermentation by bacteria have found that natamycin is very useful because it does not interfere with fermentation or ripening processes (Davidson and Branen, 1993). 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 lysis. As bacterial membrane does not contain sterol, natamycin is not effective against bacteria (Adams and Moss, 2008). This illustrated the viability of LAB count during refrigerated storage period in G2. No molds and yeasts were isolated from the G2 during 31 days of refrigerated storage period. These results nearly similar to El-Diasty *et al.* (2009); Var *et al.* (2004) who mentioned that no growth of molds was detected in yoghurt samples in the presence of natamycin after 30 days of storage. The mean values of yeast and mold in G2 were 1.00 ± 0.00 , 1.10 ± 0.10 and 3.36 ± 0.66 at 34th, 37th and 40th day; respectively. Yoghurt samples with natamycin showed physical alteration after 40 days as unacceptable appearance, dried texture and high acidity but no visual appearance of mold growth on cups compared to control yoghurt samples. According to EOS (2005), yeast and mold counts in yoghurt must not exceed 10 cfu/g, the presented results for natamycin yoghurt samples were satisfactory with permissible limits until 37 days of refrigerated storage. The yeast and mold counts in G1 were in agreement with those obtained by Küçüköner and Tarakci (2003). According to EOS (2005), the presented results for control yoghurt samples (G1) were satisfactory with permissible limits of fungal counts till 7 days of refrigerated storage.

5. CONCLUSION

Natamycin is an effective natural antifungal preservative against yeasts and molds,

exhibiting a wide spectrum of activity and effectiveness at very low concentrations. Natamycin has strong antifungal activity toward fungi, which may produce mycotoxins and create public health hazard. Yoghurt treated with natamycin 10 ppm could extend the shelf-life up to 40 days with good characteristics of sensory evaluation during refrigeration storage period as well as the inhibition of fungal growth without alteration of LAB growth pattern. This effect leads to increasing keeping quality of yoghurt, which is desired, by manufacturers and consumers.

6. REFERENCES

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دور التقوية بالناتاميسين في إطالة فترة صلاحية الزبادى الطبيعى

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قسم مراقبة الأغذية كلية الطب البيطري-جامعة بنها

الملخص العربي

يعتبر الزبادي من أكثر منتجات الألبان إستهلاكا في جميع انحاء العالم. تعتبر الفطريات والخمائر من أكثر مسببات الفساد في الزبادى وذلك لوجود البيئة الحامضية الملائمة لنموها. وقد تم في هذه الدراسة تقييم فترة الصلاحية بالإضافة الى دراسة التغييرات التي تحدث في الخصائص الحسية والميكروبيولوجية للزبادى المضاف إليه الناتاميسين أثناء فترة التخزين. تم تجهيز اللبن الطازج وقُسم إلى مجموعتين، حيث المجموعة الضابطة التي تحتوي على بادئ الزبادي والمجموعة الثانية تم تجهيزها بنفس طريقة المجموعة الضابطة مع إضافة الناتاميسين بمعدل 10 جزء في المليون. تم حفظ جميع أكواب الزبادى عند درجة حرارة 4 درجة مئوية. وقد تم تحليل العينات عند يوم انتاجها وخلال فترة التخزين فى الثلاجة تقييما حسيا و ميكروبيولوجيا عن طرق عدد نوعي بادئ بكتيريا الزبادي والعدد الكلى للفطريات والخمائر. أظهرت النتائج ان الزبادي المصنع باستخدام الناتاميسين زادت فترة صلاحيته الى 40 يوم مع الحفاظ على خصائصه الحسية والميكروبيولوجية بصورة جيدة. بينما عينات المجموعة الضابطة كانت فترة الصلاحية 21 يوما. كما وجد ارتفاع فى اعداد البكتريا *Lactobacillus delbrueckii subsp bulgaricus* حتى اليوم السابع ثم حدث انخفاض حتى نهاية فترة التخزين فى كلتا المجموعتين بينما حدث انخفاض فى اعداد *Streptococcus thermophilus* من اليوم السابع حتى نهاية فترة التخزين. كما اوضحت النتائج خلو الزبادى المصنع بإضافة الناتاميسين من الفطريات والخمائر حتى اليوم 31 وظهرت بمعدل $1.00 \pm 0.00 \log_{10} \text{cfu/g}$ عند اليوم 34 ثم بدأ العدد بالتزايد حتى نهاية فترة الصلاحية بمعدل $3.36 \pm 0.66, 1.10 \pm 0.10 \log_{10} \text{cfu/g}$ عند اليوم 37 و 40 على التوالي. بينما ظهرت الفطريات و الخمائر فى المجموعة الضابطة عند اليوم السابع بمعدل $1.20 \pm 0.10 \log_{10} \text{cfu/g}$ و قد تزايد العدد حتى نهاية فترة الصلاحية الى $3.20 \pm 1.09, 3.61 \pm 0.46 \log_{10} \text{cfu/g}$ عند اليوم 14 و 21 على التوالي. ومن الدراسة يمكن استخلاص أن الناتاميسين تأثيره المضاد للفطريات والخمائر أدى إلى زيادة فترة صلاحية الزبادى مع المحافظة على خصائصه الحسية جيدة.

(مجلة بنها للعلوم الطبية البيطرية: عدد 27(2): 140-149 , ديسمبر 2014)