The effect of natamycin on keeping quality and organoleptic characters of yoghurt

(Received: 10.07. 2008; Accepted: 19. 07 .2008)

Eman M. El-Diasty ; R. M. El-Kaseh and R.M. Salem
Faculty of Veterinary Medicine ,Omar El-Mokhtar University, Libya.

ABSTRACT

This study was planned to evaluate the shelf life and the changes occurring in the organoleptic characters of yoghurt supplemented with natamycin during the storage period. Yoghurt samples were prepared and divided into two groups; natamycin was added to the first group at the levels of 10 and 20 mg / kg,. while the second group of yoghurt was prepared without natamycin and kept as a control .Yoghurt cups were stored all over the experimental period (35 days) at 4 ± 1 °C. Organoleptic examinations, estimation of pH and titertable acidity as well as enumeration of total moulds and yeasts were done on both treated and control yoghurts. The yoghurts were examined at the 3rd and 7th days, then weekly till the end of storage period. The treated group of yoghurt showed acceptable degree of organoleptic examination, while yeasts and moulds were not detected till the end of the storage time. On the other hand, control samples of yoghurt showed unacceptable degrees of examination and contamination with moulds and yeasts. Natamycin was proved to be a suitable and effective antifungal agent which increases the shelf life of yoghurt without changing in the normal characters of the products.

Keywords: *Natamycin, organoleptic characters, yoghurt.*

INTRODUCTION

Yoghurt is a fermented milk product known and consumed by a large segment of our population either as a part of diet or as a refreshing beverage .It is a nutritiously balanced food containing almost all the nutrients present in milk but in a more assimilable form. It is believed that yoghurt has valuable therapeutic properties and helps curing gastrointestinal disorders (Athar, 1986).

Yoghurt is produced by adding a starter of active yoghurt containing a mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. These industrial bacteria produce lactic acid during fermentation of lactose .The lactic acid lowers the pH , makes it tart, causing milk protein to thicken and acts as a preservative since pathogenic bacteria cannot grow in acid conditions.

In case of milk processed to cream, cottage cheese, butter and yoghurt, the growth of lactic acid bacteria will cause pH to fall, favouring the growth of spoilage yeasts. Yeasts are very common in yoghurts and can sometimes cause spoilage. The characteristics which enable yeasts to cause spoilage in yoghurt are growth at low temperatures (<10 °C), production of proteolytic and lipolytic enzymes to hydrolyze milk protein and fats, ability to ferment or utilize lactose and sucrose (the main sugars of plain and flavoured yoghurt) and ability to assimilate lactic and citric acids which are the main organic acids in yoghurt (Fleet and Mian ,1987).

Yoghurts containing fruits can also be spoiled by fungi introduced with the fruit preparation. Fermentative spoilage of fruit flavoured yoghurt caused by *Mucor circinelloides* which grows strongly at refrigeration temperatures and can grow under extremely low oxygen tensions (Foschino *et al.*, 1993).

Natamycin (pimaricin) is a naturally antimicrobial agent produced by the bacterium *Streptomyces natalensis* and related species. Natamycin acts by disrupting cell membranes of yeasts and moulds, causing leakage and eventual lysis. Natamycin is an effective antimicrobial preservative against yeasts and moulds, exhibiting a wide spectrum of activity and effectiveness at very low concentration. Natamycin has strong cidal activity towards susceptible microorganisms and is particularly effective against fungi, which may produce mycotoxins (Food Standards, 2004).

The objectives of this study were to determine the effects of natamycin on the shelf life of yoghurt and to measure its optimum concentration needed to prevent the growth of moulds and yeasts during the storage time of yoghurt till consumption.

MATERIALS AND METHODS

Preparation of yoghurt

Raw milk was used for preparing yoghurt according to the method described by Darwish (2005). Fresh cow milk was obtained from dairy shop. The milk was boiled for concentration by evaporation and also to kill bacteria present, left to cool gradually, where a layer of fat is formed on the surface. The container are filled with warm milk, thus another fat layer was formed on the surface of the milk in the container, after which the whole milk at 40 C^{o} was mixed with the starter. Starter used is an old Zabady prepared a day before mixing with the boiled milk.

Addition of natamycin and experimental design

The prepared yoghurt was divided into three sets, The first and second groups was supplied with natamycin (produced by Danisco Cultor and obtained from Amson International Trading Company, Giza, Egypt) at levels of 10 and 20 mg / kg to yoghurt directly before pouring yoghurt into containers (Thomas and Broughton , 2001). The third group was made without natamycin treatment and kept as a control.

Storage of yoghurt

The yoghurt containers of the three groups were kept at warm rooms (38- 40° $C^{)}$ till complete coagulation took place, and then they were kept in a refrigerator at 4 °C ± 1 for 35 days.

Organoleptic evaluation of yoghurt

The yoghurt was evaluated according to the method mentioned by ADSA (2002) using a **20** points system with **10** points for flavour, **5** for body and texture and **5** for appearance .

Measuring of yoghurt pH

The pH of yoghurt was measured during the storage period using electronic digital type (Hana *pH* meter No.H6726) according to AOAC (.1990).

Total Titertable acidity

Titertable acidity of yoghurt was measured according to Ling (1963).

Mycological evaluation of yoghurt

Moulds and yeasts were enumerated as described by Pitt and Hocking (1997). All mould isolates were identified according to the key of Pitt and Hocking (1997), while yeast isolates were identified according to Kriger van Rij (1984).

RESULTS AND DISCUSSION

Results recorded in Table (1) summarize the sensory evaluation of yoghurt made with and without natamycin during the storage period. It is obvious that yoghurt supplemented with natamycin gained high scores of the evaluation. This could be attributed to the effect of natamycin which improves the keeping quality in addition to preventing the growth of yeasts and moulds till the end of storage time. Also, natamycin imparts no adverse flavour to yoghurt. On the other, hand the control yoghurt samples had lower scores as a result of yeasts and moulds growth, which impart off- flavour, bitterness (Rhodotorula), discolouration and changes of textures (Tudor and Board, 1993).

Table (1): Organoleptic evaluation of treated and control yoghurt.

Time of Storage (days)	*Flavour (10 points)*			*Body and texture (5 points)*			*Appearance (5 points)*			*Total points (20 points)*		
	10 mg	**20 mg**		**10 mg**	**20 mg**	**control**	**10 mg**	**20 mg**		**10 mg**	**20 mg**	**control**
3rd	9	9	9	4	4	4	4	4	4	17	17	17
7th	9	9	9	4	4	3	4	4	3	17	17	15
14th	9	9	8	4	4	3	4	4	3	17	17	14
21st	9	9	5	4	4	3	4	4	3	17	17	11
28th	9	9	5	4	4	2	4	4	2	17	17	9
35th	8	9	5	4	4	1	3	4	2	14	17	8

Off flavour of yoghurt may be caused by contaminating microorganisms, mainly yeasts and moulds. The off-flavours may be characterized as yeasty, fruity, musty, cheesy or bitter and occasionally, soapy –rancid. A flavour threshold is generally reached at a count of about 10^4 yeasts + moulds / ml (Walstra *et al.*, 1999).

Results in Table (2) revealed that the mean pH of natamycin treated yoghurt samples (10 and 20 mg /kg) at 3rd ,14th and 35th days were 4.56 ± 0.03 , 4.53± 0.03 and 4.46 ± 0.03, respectively While in case of control samples, the values were 4.56 ± 0.03 , 4.53± 0.03 and 4.14 ± 0.09, respectively . These results are in line with the findings reported by Varnam and Sutherland (1994). There was no significant variation in pH of either treated or control yoghurt samples .The pH lies within the normal pH of ordinary market yoghurt (4.1 – 4.6) which is acceptable by consumers(Walstra *et al.*,1999). Concerning the titratable acidity of treated and control yoghurt, it was ranging from 0.78 ± 0.01 to 0.89 ± 0.02 for treated yoghurt and for control samples the acidity ranged from 0.78 ± 0.01 to 0.92 ± 0.01. These results are in accordance with usual yoghurt of markets as reported by Walstra *et al.* (1999).

Table (2) :The pH values and titratable acidity of treated and control yoghurt samples.

Time of storage (days)	Treated samples				Control samples	
	10 mg / kg		20 mg /kg			
	pH	*TA**	*pH*	*TA**	*pH*	*TA**
3rd	4.56 ± 0.03	0.78 ± 0.01	4.56 ± 0.03	0.89± 0.02	4.56 ± 0.03	0.78 ± 0.01
7th	4.53 ± 0.01	0.78 ± 0.01	4.53 ± 0.01	0.83 ± 0.01	4.53 ± 0.01	0.78 ± 0.01
14th	4.53 ± 0.03	0.78 ± 0.01	4.53 ± 0.03	0.82 ± 0.04	4.53 ± 0.03	0.82 ± 0.01
21st	4.46 ± 0.03	0.82 ± 0.04	4.46 ± 0.03	0.78 ± 0.01	4.46 ± 0.03	0.88 ± 0.01
28th	4.50 ± 0.03	0.83 ± 0.01	4.50 ± 0.05	0.78 ± 0.01	4.14 ± 0.09	0.89 ± 0.02
35th day	4.46 ± 0.03	0.89 ± 0.02	4.46 ± 0.03	0.78 ± 0.01	4.14 ± 0.09	0.92± 0.01

TA* = Titratable acidity

Data presented in Table (3) summarize the results of examination of yoghurt of both treated and control for total yeast counts. It was found that the mean yeast count in control samples at 3rd ,7th ,14th , 21st, 28th and 35th days were < 10^2 , 3.7× 10^2 , 3.6×10^3 ,4.3× 10^3, 4.5 ×10^3 and 5.5× 10^3 cfu ml^{-1}, respectively . Meanwhile, after 35 days of storage, no growth of yeast was detected in yoghurts in the presence of natamycin (10 and 20 mg / kg.) .This result was achieved by Food Standards (2004), Thomas and Broughton (2001) Var et *al.* (2004) who reported that no yeast growth was detected in yoghurt samples in the presence of natamycin after 30 days of storage.

Table (3): Densities of yeasts of yoghurt (cfu ml^{-1}) .

Time of storage (days)	Natamycin treated samples						Control samples		
	10 mg/ kg			20 mg / kg					
	Min.	*Max.*	*Mean*	*Min*	*Max.*	*Mean*	*Min.*	*Max.*	*Mean*
3rd	ND	ND	ND	ND	ND	ND	<10^2	1.8×10^2	<10^2
7th	ND	ND	ND	ND	ND	ND	3.0×10^2	4.3×10^2	3.7×10^2
14th	ND	ND	ND	ND	ND	ND	3.3×10^2	6.0×10^3	3.6×10^3
21st	ND	ND	ND	ND	ND	ND	5.0×10^2	8.0×10^3	4.3×10^3
28th	ND	ND	ND	ND	ND	ND	5.1×10^2	8.5×10^3	4.5×10^3
35th	ND	ND	ND	ND	ND	ND	3.0×10^3	9.0×10^3	5.5×10^3

ND = Not detected.

It could be seen from Table (4) that the mean mould counts in control samples at 3rd, 7th, 14th, 21st, 28th and 35th days were < 10^2, 1.3× 10^2, 3.6 ×10^2, 5 × 10^2, 2.8 × 10^3 and 3.8 × 10^3 cfu ml^{-1} respectively. On the other hand, no moulds were isolated from the treated yoghurt till the end of the storage period. These results are in harmony with those reported by Food Standards (2004), Thomas and Broughton (2001), Var *et al.*(2004) who mentioned that no growth of moulds was detected in yoghurt in the presence of natamycin after 30 days of storage.

Results in Table (5) showed that moulds and yeasts were isolated only from the control yoghurt samples. Isolated moulds included Mucor spp., *Aspergillus niger* , *Aspergillus flavus Penicillium* and *Alternaria spp.* Yeasts included *Candida spp* , *Debaromyces hansenii* and *Rhodotorula spp.* ,while no moulds or yeast were detected in natamycin treated yoghurt. These results agree with Pitt and Hocking (1997). The presence of moulds and

yeasts in yoghurt is undesirable and may create health hazards to the consumer as well as these lead to economic losses as food contamination with moulds and yeasts are unfit for human consumption.

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 processes (Davidson and Branen ,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, benefiting both consumers and manufactures by reducing product losses, extending shelf- life and protecting public health and safety (Food Standards, 2004).

Table (4): Counts of moulds of yoghurt (cfu ml^{-1}).

Time of storage (days)	Natamycin treated samples						Control samples		
	10 mg/ kg			20 mg / kg					
	Min.	*Max.*	*Mean*	*Min*	*Max.*	*Mean*	*Min.*	*Max.*	*Mean*
3rd	ND	ND	ND	ND	ND	ND	$<10^2$	$<10^2$	$<10^2$
7th	ND	ND	ND	ND	ND	ND	$<10^2$	2×10^2	1.3×10^2
14th	ND	ND	ND	ND	ND	ND	3.0×10^2	4.2×10^3	3.6×10^3
21st	ND	ND	ND	ND	ND	ND	4.0×10^2	6.0×10^3	5.0×10^3
28th	ND	ND	ND	ND	ND	ND	4.3×10^2	7.5×10^3	2.8×10^3
35th	ND	ND	ND	ND	ND	ND	6.0×10^3	9.2×10^3	3.8×10^3

ND = Not detected .

Table (5): Isolated moulds and yeasts from yoghurt (natamycin treated and control).

Isolates	Treated samples		Control samples
	10 mg /kg	20 mg /kg	
- Moulds :			
Mucor spp.	-ve	-ve	+ve
Aspergillus niger	-ve.	-ve	+ve
Apergillus flavus	-ve	-ve	+ve
Penicillium spp.	-ve	-ve	+ve
Alternaria spp.	-ve	-ve	+ve
- Yeasts :			
Candida spp.	- ve	-ve	+ve
Debaromyces hansenii.	- ve	-ve	+ve .
Rhodotorula spp.	-ve	-ve	+ve.

CONCLUSION

Natamycin is an effective antimicrobial preservative against yeasts and moulds, exhibiting a wide spectrum of activity and effectiveness at very low concentrations. Natamycin has strongcidal activity towards susceptible microorganisms and is particularly effective against fungi, which may produce mycotoxins and create public health hazard. Yoghurt treated with natamycin (either 10 or 20 mg /kg) showed good characteristics of organoleptic examination during storage period as well as the inhibition of moulds and yeast growth. This effect leads to increasing keeping quality of yoghurt which is desired by manufacturers and human consumers.

REFERENCES

ADSA American Dairy Science Association (2002). Committee on Dairy Products Evaluation. Score card and guide for strawberry ,Swiss style yoghurt.

Official Methods of Analysis of Association of Official Analytical Chemists AOAC. (1990)., 15th Ed. Virginia 22201, Arlington .

Athar, I, H. (1986). Preparation of cheese and yoghurt (Dahi) at household level . Pak. Agri. Res. Council, Islamabad.

Darwish , S. (2005). Fermented milks. Notes on hygiene of dairy products, edible fats and oils and eggs. Faculty of Veterinary Medicine , Cairo, Egypt .

Davidson , P.M. and Branen ,A.L.(1993). Antimicrobial in Foods. 2nd Ed. USA

Fleet, G., H. and Mian , M. A. (1987). The occurrence and growth of yeasts in dairy products. Int. J. Food Microbiol., 4 : 145- 155 .**Food Standards Australia Neo Zealand (2004) .**Natamycin extension use as a food additive. Initial Assessment Report. Application, A 542.

Foschino, R.; Garzaroli , C. and Ottogalli , G. (1993). Microbial contaminants cause swelling and inward collapse of yoghurt packs . Lait., 73: 395- 400.

Kriger van Rij ,N.J.W.(1984). The Yeast. A taxonomic Study. 3rd Ed. Amsterdam, Elsevier, pp 345.

Ling, E.R. (1963). A Textbook of Dairy Chemistry. 3rd Ed. Chapman and Hall Ltd., London .

Pitt ,J.I. and A.D. Hocking (1997) . Fungi and Food Spoilage. 2nd Ed. Published by Blackie Academic and Professional. Academic Press New York ,London .

Thomas, L.V. and Broughton, J.D. (2001). Applications of the natural food preservative natamycin. Research Advances in Food Science, 2: 1- 10 .

Tudor, D.A. and Board ,R.G.(1993) . Food Spoilage Yeasts I. The yeast. Vol.5 Yeasts technology . 2nd Ed.

Var ,I. ; Sahan , N.; Kabak ,B. and Golge , O. (2004) . The effect of natamycin on the shelf life of yoghurt. Archiv Fur Lebensmittelhygiene, 55 (1): 7-9.

Varnam , A.H. and Sutherland J.P.(1994) . Milk and Milk Products : technology , chemistry and microbiology .Chapman and Hall , London . pp 351- 364.

Walstra, P.; Geurts, T.J.; Noomen, A.; Jellema, A. and Van Boekel , M.A. (1999). Dairy Technology .Principles of milk properties and processes. USA .

الملخص العربي

تأثير الناتاميسين على فترة الصلاحية والخصائص الحسية لليوغرت

إيمان محمود الدياسطي , رافع مصطفي الكاسح , رمضان مصطفي تاج الدين
كلية الطب البيطري ، جامعة عمر المختار- مدينة البيضاء- ليبيا

تم في هذه الدراسة تقييم فترة الحفظ بالإضافة إلى دراسة التغيراتِ التي تحدث في الخصائص الحسية لليوغرت (الزبادي) المضاف إليه الناتاميسين أثناء فترةِ تخزينَ أو حفظ اليوغرت للبيع بالمحلات . تم تجهيز اللبن الطازج وقُسّمَ إلى مجموعتين , حيث تم إضافة الناتاميسين إلى المجموعةِ الأولى بمعدل ° 10 و20 ملليجرام ناتاميسين / كيلوجرام من اللبن بينما المجموعة الثانية للبن تم تجهيزها بنفس طريقة المجموعة الأولى ولكن بدون إضافة الناتاميسين . تم حفظ جميع الأكواب المحتوية على الزبادي أثناء فترة الدراسة (35 يوما) عند درجة حرارة 4 ± 1 درجة مئوية. أثناء فترة التخزين للزبادي أجريت الفحوص الحسية المختلفة ، وقدر الأس الهيدروجيني والحموضة العيارية بالإضافة إلى حسابِ العدد الكليِّ للفطريات والخمائر لكل من مجموعة اليوغرت المعالج بالناتاميسين ومجموعة اليوغرت الكنترول. وقد تم فحص هذه المجموعات من اليوغرت عند اليوم الثالث والسابع من بداية التجربة ثم فحصت أسبوعيا بعد ذلك حتى نهاية فترة التخزين . أظهرت المجموعة المعالجة من اليوغرت درجة مقبولة من الفحص الحسي بالإضافة إلى أن الخمائرِ والفطريات لم يتم عزلها من تلك المجموعة حتى النهاية مِنْ وقتِ التخزين. من الناحية الأخرى فأن عينات الكنترول من اليوغرت أظهرت درجاتَ غير مقبولة مِنْ الفحص إلى جانب التلوّثِ بالفطريات والخمائر . ويمكن استخلاص أن الناتاميسين أثبتَ أنه مضاد للفطريات حيث أدى إلى زيادة فترة حفظ اليوغرت بدون حدوث أي تغيرات في الخصائص الطبيعية للمنتج .