

Effect of nisin on the keeping quality of feta cheese

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تأثير النيسين على مدة المحافظة بجبنة الفيتا

في محاولة لتمديد مدة المحافظة بجبنة الفيتا أضيف النيسين أثناء التصنيع بتركيز 40 وحدة عالمية للميلتر من الحليب. كان لإضافة النيسين، بهذا التركيز تأثير طفيف على تخميص الحليب ولكن مراحل التصنيع استمرت بصفة عادية ظاهريا وحصل على جبنة مطابقة للمواصفات الإغريقية. وأظهرت التحاليل الكيماوية على أن إضافة النيسين ليس له أي تأثير على نسبة الأملاح، الدهنيات والرماد. في حين تراجع نسبة المادة اليابسة بشكل ملموس. أما مدة المحافظة فنزلت إلى أقل من 47 يوما من جراء تكاثر بكتيريا كرام السالب (-Gram) المقاومة للنيسين. وقد يكون هذا التكاثر ناتجا عن تنشيط بكتيريا كرام الجيب (+Gram) وإلى حد ما، البكتيريا المستعملة في البادئات المنافسة للبكتيريا الغير مرغوب فيها. بالمقابل، تعدت مدة المحافظة على الشاهد (جبنة بدون إضافة النيسين) الستين يوما.

الكلمات المفتاحية : جبنة الفيتا - التصنيع - النيسين.

Effet de la nisine sur la conservabilité du fromage Feta

Dans une tentative d'extension de la durée de conservation du fromage "Feta", la nisine lui a été ajoutée durant sa fabrication à une concentration finale de 40 UI/ml de lait. À cette concentration, la production d'acide a été légèrement affectée après coupure du caillé sans être complètement inhibée. La fabrication du fromage a continué apparemment normalement aboutissant à un produit conforme aux normes grecs. L'analyse chimique a révélé que l'addition de nisine n'avait pas d'effet significatif ($P < 0,01$) sur les teneurs en sel, matière grasse et cendres. Cependant, le taux de matière sèche a été significativement réduit. La durée de conservation a été réduite à moins de 47 jours à cause d'une croissance intensive des bactéries à Gram négatif qui ne sont pas inhibées par la nisine. La croissance de ces bactéries a été probablement favorisée par l'inhibition, à un certain degré, des bactéries à Gram positif compétitives comprenant celles du ferment. La durée de conservation du témoin (fromage sans ajout de nisine) a, en revanche, dépassé les 60 jours.

Mots clés: Fromage Feta - Conservabilité - Nisine

Effect of nisin on the keeping quality of feta cheese

In an attempt to extend cheese shelf-life, nisin was added to Feta cheese during cheese making to achieve a final concentration of 40 IU/ml of milk. At this concentration, acid production was slightly retarded after the curd cutting but not completely inhibited. Cheese manufacture proceeded apparently normally yielding a product that met Greek regulatory standards. Chemical analysis showed that nisin addition had no significant effect ($P < 0.01$) on salt, fat or ash contents. However, the total cheese dry matter was significantly reduced. The Shelf-life was shortened to less than 47 days due to intensive growth of Gram negative bacteria not inhibited by nisin. Such organisms were probably stimulated by the inhibition of competitive Gram positive bacteria including, the starter culture, to some extent. The shelf-life of the control cheese exceeded 60 days.

Key words: Feta cheese - Shelf-life - Nisin

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INTRODUCTION

Feta is originally home-made Greek cheese characterized by its smooth, creamy, soft and sliceable body and pleasant acidic, salty and mild rancid flavor when ripened (Etthymiou & Mattick, 1964). For a long time, manufacture of Feta cheese relied on the natural flora of milk during the fermentation; therefore, its composition and flavor were not consistent and varied greatly among localities, climatic conditions and traditions. Furthermore, defects in body and flavor were common. Feta cheese is made from sheep or goat milk or blends of both. However, production of these two kinds of milk is not sufficient to yield significant quantities of cheese. Therefore, cow milk now is used as well. Similar dairy products have been described in Near Eastern countries (Fahmi & Sharara, 1950). Egyptian, Domiati cheese is similar to Feta, the main difference being in the salting procedure. Feta cheese is salted in 12% brine. For Domiati cheese, salt is directly added to the milk before fermentation to a final concentration of 5 to 15% (Fahmi & Sharara, 1950). In 1964, Etthymiou & Mattick (1964) developed a procedure to manufacture Feta cheese from pasteurized cow milk using selected starter cultures. The best results were obtained with a mixed starter culture composed of lactococci and *Lactobacilli*. Yogurt starter (e.g. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*) has also been used successfully (Papageorgiou & Marth, 1989).

The shelf-life of Feta cheese is variable and depends on raw milk quality and cleanness during manufacture. Papageorgiou & Marth (1989) showed that high quality Feta cheese could be stored for more than 90 days. The shelf-life of Feta cheese may be shorter, especially if the curd has been cut before sufficient acid has been produced (Etthymiou & Mattick, 1964). It is well known that at least 25% of the world's food supply spoils before it can be consumed. The cost of this, while difficult to accurately estimate, is nonetheless astronomical, especially in lost income (Cousin, 1982). Therefore, intensive research work has been done to extend the shelf-life of perishable foods. The most common means used are heat treatments, refrigeration, use of food additives, bacteriocins or bacteriocin-producing microorganisms and the use of viable lactic acid bacteria. Nisin has long been used for this purpose in dairy products, meats, canned foods, and beverages (Campbell *et al.*, 1959; Delves-

Broughton, 1990; Eckner 1991). In the present study an attempt was made to extend the shelf-life of Feta cheese made from pasteurized cow milk by addition of nisin.

MATERIALS AND METHODS

• Starter Culture

Commercial lyophilized starter culture (Visbyvac, yogurt 231) consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* (1:1) was obtained from Laboratorium Wiesby (Germany). The starter was regenerated in 10% sterile reconstituted non-fat dry milk (NDM) by overnight incubation (16 to 18 hours) at 37°C immediately before use in fermentation.

• Nisin

Nisin (Nisaplin, 37x 10⁶ IU/g) was purchased from Aplin and Barrett, Ltd., Trowbridge, England. Stock solution (37 x 10³ IU/ml) was prepared by dissolving 0.1 g of nisin in 80 ml of 0.02 N HCl solution and holding at room temperature for 2 hours to complete dissolution. The volume was then made up to 100 ml with 0.02 N HCl and the solution was filter-sterilized by passage through a 0.22 µm Millipore membrane and stored at -20°C.

• Manufacture of Feta Cheese

Feta cheese was manufactured as described by Papageorgiou and Marth (1989) in the pilote dairy plant of "The Federal Dairy Research Institute" of Kiel (Germany). Two batches were made simultaneously in each of three trials. In each batch, whole cow milk (10 l) was pasteurized (75°C for 16 seconds), tempered and placed in stainless steel vats partially submerged in tepid water (35-40°C) to maintain the milk temperature at 35°C. Starter culture was then mixed (1% v/v) to milk along with 0.01% (w/v) of calcium chloride. When the pH was reduced to 6.4, 2.5 ml of 1/10,000 strength calf rennet was added. To one batch, nisin was added to a final concentration of 40 IU/ml with the rennet and thoroughly mixed. We previously demonstrated that nisin concentration of 50 IU/ml and below has no noticeable effect on yogurt starter performances (unpublished data). No nisin was added to the second batch which served as a control. The coagulum was cut with 0.63-cm knives 40 to 50 min after rennet addition.

Curds were then transferred into rectangular perforated stainless steel forms (30 x 22 x 12 cm) and allowed to drain at room temperature (c.a. 22°C) for 6 hours. Forms were turned twice at 2-hour intervals. After draining, the cheese was cut into six pieces (10 x 11 x 12 cm) and placed into 12% salt brine for 24 hours at room temperature. The pieces of cheese were then cut in half (10 x 5.5 x 12 cm) and placed in 6% salt for 4 days ripening at room temperature. After ripening, each piece was placed into a sterile metal can, covered with 6% brine and stored at 4°C.

• Chemical analysis of feta cheese

Measurement of pH during fermentation and storage of cheese was done with a Corning pH meter equipped with a combination electrode. Total dry matter (TDM), fat, salt and ash were determined as described in Standard Methods for the Examination of Dairy Products (Richardson, 1985).

• Microbiological analysis of cheese

Samples of milk, curd and cheese were serially diluted in a sterile 2% sodium citrate solution and plate counts were performed on appropriate agar media. For curd and cheese, a 1:10 dilution, was prepared by weighing 10 grams into a sterile stomacher bag containing 90 ml of sterile 2% sodium citrate solution, then blending in a Stomacher for 2 to 4 min. Microorganisms enumerated in this study were total mesophilic aerobes, coliforms, psychrotrophs and lactic acid bacteria (LAB). Media and incubation conditions are summarized in Table 1.

Table 1. Media and incubation conditions used in the enumeration of different groups of micro organisms in Feta cheese

Group of Microorganisms	Medium	Incubation
Total count	Plate Count Agar (Difco)	37°C for 72 hours
Coliforms	VRBA (Difco) ¹ +1.5% agar (Difco)	32°C for 24 hours
Psychrotrophs	Plate Count Agar (Difco)	7°C for 10 days
LAB ²	MRS ³ (Difco)+1.5% agar (Difco)	30°C for 24 hours

¹ VRBA = Violet Red Bile Agar

² LAB = Lactic Acid Bacteria

³ MRS = De Man Rogosa and Sharpe

• Statistical analysis

Statistical analysis (analysis of variance and student *t* test) of data were done by computations using "warm stat" software.

RESULTS

• Effect of nisin on the manufacture of Feta cheese

Table 2 shows variations in the pH occurring during manufacture and storage of Feta cheese with and without added nisin. The pH varied similarly both in control (nisin free) and test (40 IU/ml of nisin) samples during the first hour but it decreased slower in the test thereafter. After the sixth day, an increase of the pH was observed for both trials to reach 4.73 and 5.01, respectively, at 47 days. The pH in samples with added nisin remained higher than in the control since curd cutting.

Table 2. Variation of pH during manufacture and storage of Feta cheese with and without nisin added (6 determinations)

Time	Control (without nisin)		Test (+40 IU/ml of nisin)	
	Average	SD ¹	Average	SD
0	6.65	0.15	6.65	0.15
0.5	6.56	0.11	6.57	0.11
0.7	6.45	0.04	6.49	0.06
1 ²	6.41	0.02	6.41	0.01
1.7 ³	5.46	0.13	6.11	0.53
5.5	5.19	0.08	5.79	0.54
7.5	5.24	0.24	5.63	0.39
24	4.74	0.21	4.89	0.15
5 (days)	4.65	0.16	4.79	0.03
6 (days)	4.55	0.05	4.70	0.06
11 (days)	4.68	0.17	4.82	0.07
47 (days)	4.73	0.10	5.05	0.05

¹ Standard deviation (±)

² Rennet addition

³ Curd cutting

• Effect of nisin on the chemical composition of Feta cheese

Table 3 summarizes results of determinations of fat in the dry matter (FDM), salt, ash and total dry matter (TDM) content in cheese after brining in 6% NaCl (e.g. the first day of storage). These data show

that addition of 40 IU/ml of nisin during Feta cheese manufacture had no significant effect ($P < 0.01$) on the fat, NaCl, ash contents or TDM.

Table 3. Chemical analysis of Feta cheese with and without nisin added (6 determinations)

Analysis	Control (nisin free)		Test (+40IU/ml of nisin)	
	Average	SD ¹	Average	SD
TDM (%) ²	44.95	0.83	43.42	0.28
FDM (%) ³	47.07	0.60	46.63	3.48
Salt (%)	3.1	0.89	3.01	0.74
Ash (%)	4.31	0.47	4.41	0.40

¹ Standard deviation (\pm)

² Total Dry Matter

³ Fat in the Dry Matter

• Effect of Nisin on the shelf-life of Feta cheese

During manufacture and storage of Feta cheese, enumerations of total count, coliforms, psychrotrophs and LAB (Figure 1: A, B, C and D) were performed. Cheese appearance was assessed

on a regular basis during storage at 4°C up to 60 days. Figure 1 shows that all groups of microorganisms enumerated in this study grew well in Feta cheese, regardless of whether or not it contained nisin. This figure also shows that coliforms and psychrotrophs were not found in the pasteurized milk but were detected in the cheese in relatively high numbers, higher in the test than in the control (Figure 1: B & C). The average coliform counts at 47 days were 9.8×10^6 CFU/ml in the test and 1.8×10^5 CFU/ml in the control. The average psychrotroph counts in the test and in the control were 1.8×10^6 CFU/ml and 6.8×10^4 CFU/ml, respectively. Total aerobic and LAB counts varied in similar ways. They reached a maximum within 5 days and remained relatively constant during storage (Figure 1: A and D).

The appearance of cheese containing nisin was altered after 40 days of storage. only surface of the cheese became slimy and soft, an alteration typically caused by *Pseudomonas* bacteria (Cousin, 1982). No such visible alteration was seen in control samples even after 60 days.

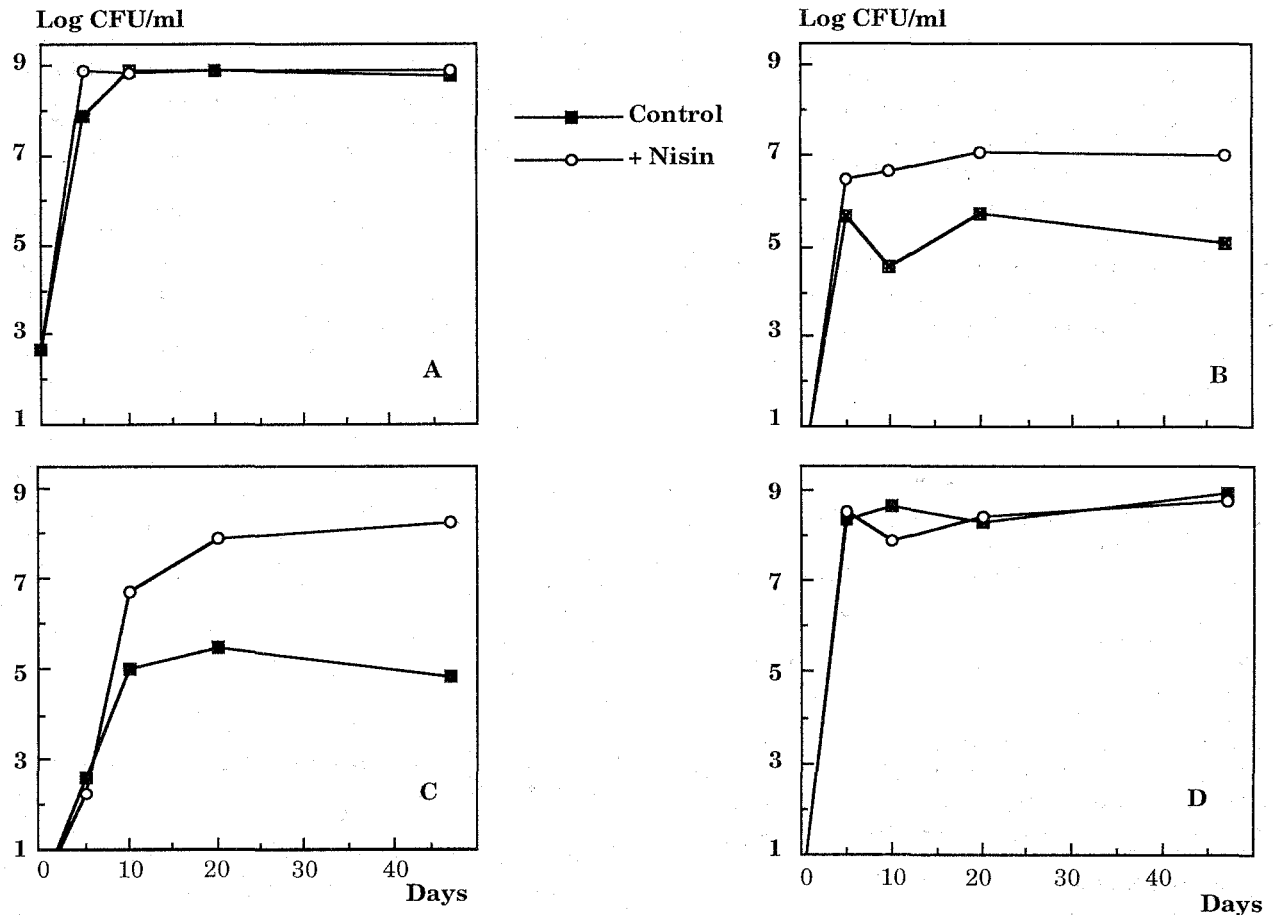


Figure 1. Effect of nisin on the shelf-life of Feta cheese

A : Aerobic plate count ; B : Coliforms ; C : Psychrotrophs ; D : Lactic acid bacteria

DISCUSSION

Results of this study showed that addition of 40 IU/ml of nisin to Feta cheese reduced its shelf-life. Acid production was retarded in samples with added nisin as compared to the control since curd cutting.

An increase of the pH after 6 days (Table 3) was observed both in control and test batches, therefore, it could not be attributed to a nisin effect. Similar findings were reported by Etthymiou & Mattick (1964), who showed that the pH of Feta cheese increased from 4.7 to 5.1 - 5.3 during one month of storage. Papageorgiou & Marth (1989), however, showed that the pH of Feta cheese was about 4.3 after brining and this value was maintained through the whole period of storage (90 days). The increase of the pH in Feta cheese after 6 days may be due to contamination of the cheese by microorganisms able to deaminate amino acids, resulting in release of ammonia.

Such microorganisms are widely distributed in nature (Harrigan & McCance, 1976). In this regard *Pseudomonas* was reported to produce ammonia from arginine (Rogul & Carr, 1972).

Otherwise, the chemical composition of Feta cheese was not affected by nisin addition. The TDM was lower in the test than in the control, however, in both cases FDM met Greek regulatory standards (>43%).

The average value of salt content in the control and test cheeses ($3.1\% \pm 0.89$ and 3.01 ± 0.74 , respectively) were higher than the normal salt content in commercial Feta cheese, which is about 2.5% (Papageorgiou & Marth, 1989).

However, there is no standard for the amount of salt. Greek regulations describe only the salting procedure; therefore great variations in salt content of Feta cheese should be expected and are, in fact, tolerated.

As already mentioned, this study showed that 40 IU/ml of nisin reduced the shelf-life of Feta cheese. Numbers of coliforms and psychrotrophs were higher in cheese containing nisin than in cheese without nisin (Figure 1: B & C).

It is well known that nisin inhibits only Gram-positive bacteria and has no effect on Gram-negative bacteria, such as coliforms and

Pseudomonas (Delves-Broughton, 1990; Eckner, 1991; Hurst, 1981; Stevens *et al.*, 1991). Nisin may encourage growth of these microorganisms by limiting the inhibitory action of the lactic starter bacteria against them. *Lb. bulgaricus* has been shown to produce inhibitory substances against a variety of microorganisms including *Pseudomonas* (Abdel Bar *et al.*, 1987; Blazek *et al.*, 1991), which appear to be responsible for the cheese alteration.

According to Taylor and Somers (1985), lactobacilli preserve bacon more efficiently than nisin. Although nisin extends the shelf-life of many foods, including beverages (Delves-Broughton, 1990; Ogden, 1985; Ogden *et al.*, 1988), dairy products (Benkerroum & Sandine, 1988; Delves-Broughton, 1990; Eapen *et al.*, 1983; Fowler, 1979; Kalra *et al.*, 1973; Somers & Taylor, 1987), canned foods (Taylor, 1986) and meat (Delves-Broughton, 1990; Eckner, 1991; Rayman *et al.*, 1981), in other cases it had limited success (Eckner, 1991; Rayman *et al.*, 1983; Taylor & Somers, 1985) or even an adverse effect, especially in fermented products where the starter cultures are sensitive to nisin.

In this regard we have found earlier (data not shown) that a relatively high concentration of nisin (more than 50 IU/ml) impedes the yogurt fermentation.

Also, for nisin to extend efficiently the shelf-life of food products, it is necessary to maintain the "Good Manufacturing Practices" because this bacteriocin does not inhibit many spoilage bacteria, particularly *Pseudomonas*, which are common causes of dairy product deterioration (Cousin, 1982).

The present study showed that the addition of 40 IU/ml of nisin to Feta cheese during manufacture failed to extend its shelf-life due, probably, to the bacteriocin effect on the starter bacteria. The results suggest that nisin may not be a suitable additive for dairy products fermented with sensitive starter cultures. In such case, nisin could be added to the final product as was demonstrated for cottage cheese (Benkerroum & Sandine, 1987).

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