

Study of Storage Conditions Effect (Light-Heat) on Natamycin Content and Stability in Some Dairy Products (Cheese-Yoghurt)

Al-Haj Saeed Nihad* and Maysam Salami

Department of Food Control and Analytical Chemistry, Faculty of Pharmacy, Damascus University, Al-Mazzeah Street, Damascus, Syria

Abstract

The study aims to determine changes of Natamycin content in some dairy products (cheese-yoghurt) from different local companies in refrigeration conditions ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$) or different temperatures ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and during exposure to sunlight at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) using ultraviolet spectrophotometer method. This study was based on the collection of samples at the same day (the same type from the same batch) and used to study the effect of temperature and light on natamycin content in these pervious products. Results of analysis showed that exposure to sunlight and different temperature conditions significantly affected the stability of natamycin. A significant negative correlation exists between natamycin and storage time. Natamycin content was more stable in cheese and yoghurt products when stored under refrigeration conditions ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$) in a dark place. A little significant effect on natamycin content during exposure to room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) without exposure to sunlight. A more significant effect on natamycin content was found during exposure to high temperatures ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}$) compared to effect of sunlight at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$. These results showed that dairy products containing natamycin should be kept away from sunlight and high temperatures.

Keywords: Ultraviolet; Spectrophotometer; Natamycin; Stability; Refrigeration conditions; Sunlight; Temperature; Cheese; Yoghurt

Introduction

Foods are prone to microbiological deterioration throughout storage and distribution. Addition of antimicrobial agents can extend the shelf-life and safety of fresh and packaged foods, by reducing or even preventing the growth of pathogenic and spoilage microorganisms [1]. Consumers demand for more natural ingredients in processed foods is a result of a requirement for more healthy and safe foods. As a consequence, the demand for natural food ingredients has increased and the use of natural antimicrobial compounds from a wide variety of sources has been widely investigated [2]. Natamycin is a natural antimicrobial polyene characterized by the presence of a large macrocyclic lactone ring containing a series of conjugated double bonds and one or more sugar residues [3]. Natamycin has an empirical formula of $\text{C}_{33}\text{H}_{47}\text{NO}_{13}$ and a molecular weight of 665.75 [4]. It is produced by *Streptomyces natalensis* and is commonly employed in dairy-based food products to prevent yeasts and moulds contamination [5]. It has been approved as a food additive in over 40 countries and has been considered as a Generally Recognized as Safe (GRAS) product by Food and Drug Administration (FDA) [6] and also indicated as a natural preservative by the European Union (EU). Natamycin (Pimaricin) (Figure 1), a polyene macrolide antibiotic, is a potent antifungal compound that can effectively prevent the growth of yeasts and inhibit aflatoxine formation in molds [7]. Natamycin is widely used in the food industry as a natural food preservative for the prevention of mold contamination of beverage, cheese, fruits and other non-sterile foods (i.e., cured meats, sausages) [8,9]. The activity of natamycin against yeasts and molds, but not bacteria, makes it convenient for use in foods that undergo a ripening period after processing. Its low solubility in water and most organic solvents makes it suitable for the surface treatment of foods [10].

The stability of natamycin is affected by extreme pH values, light, heat, oxidants, chlorine, and heavy metals [11]. It is greatly dependent upon the stability of the tetraene chromophore of the molecule. Inactivation of natamycin in solution occurring by heat, light, or oxidation always begins with the cleaving of these four conjugated double bonds. Solutions of natamycin are quite stable at pH values

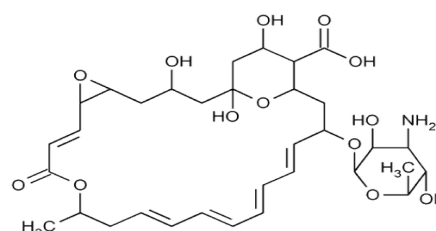


Figure 1: Chemical structure of natamycin [7].

between 5.0 and 9.0 when stored in the dark. Aqueous suspensions of natamycin are more stable than aqueous solutions [12]. Storage of the dry powder at room temperature for several years only resulted in a few percent loss of activity. In its trihydrate form, natamycin is a stable compound when it is protected from light and moisture [13].

Natamycin in aqueous solutions at temperatures below 100°C exhibits strong biological stability. However, the exposure to 121°C results in the reduction of biological stability of natamycin solution. Neutral aqueous suspensions of natamycin can tolerate a temperature of 50°C for several days with only a slight decrease in activity [14]. Natamycin efficacy appears to be improved at refrigerated temperatures that allow the slow growth of yeast. The stability of natamycin is also enhanced under refrigeration [15]. Oxidation of natamycin proceeds more readily at higher temperatures. Oxidation was prevented by

*Corresponding author: Al-Haj Saeed Nihad, Department of Food Control and Analytical Chemistry, Faculty of Pharmacy, Damascus University, Al-Mazzeah Street, Damascus, Syria, E-mail: nihadhajsaheed@gmail.com

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ascorbic acid, hydroquinone, and Gallic acid [16]. The antimicrobial activity of natamycin is rapidly destroyed when exposed to chemical oxidants, such as peroxides, organic acid anhydrides, perchlorates, persulfates, permanganates, iodates, bromates, hypochlorite's, and sulphites [17]. Several metal ions, especially Fe (III), Ni(II), and Cr(III), promote oxidative inactivation of natamycin. The addition of complexing agents, such as EDTA or polyphosphates, can prevent this metal promoted oxidation [18]. The polyene macrolide antibiotics are very light sensitive antimicrobial substances [19]. Photolytic oxidation clearly poses a problem in the handling and storage of the polyene antibiotics [20]. The photolysis of natamycin is a very rapid upon light exposure to a xenon lamp with a spectrum similar to that of natural sunlight [21].

The objective of this study was to estimate the stability and changes of natamycin content in cheese and yoghurt products during exposure to different storage conditions (light - heat) by ultraviolet spectrophotometer method which it was previously suggested by International Dairy Federation (IDF) [22] as a simple, sensitive, inexpensive and non-consuming time.

Materials and Methods

Equipment's and instrumentals

UV-Spectrophotometer (OPTIZEN POP) - Lambda-2 with matched 1 cm quartz absorption cells connected to a computer equipped with PECSS software suitable for recording UV-visible spectra between 200 and 800 nm.

- Analytical Balance from Shimadzu A*200
- Magnetic stirrer (Snijders NO 34532)

Reagents

All reagents were analytical reagent grade. Methanol (high pure 99.6%) purchased from Panreac (Germany), Glacial acetic acid (high pure 99.5%) purchased from SCP (England), Deionized water.

Collection of samples

Dairy products used for this research work were obtained from different sources of local companies. Two types of cheese (processed - cream) from M company and two types of yogurt (common-flavored) from HS Company. All the samples were collected at the same day (the same type from the same batch) and used to study the effect of temperature and light on natamycin content in cheese and yoghurt products.

Effect of Temperature

Fourteen closed containers of two types of cheese (processed-cream) and 14 closed containers of two types of yoghurt (common-flavored) were maintained in refrigeration conditions (at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in a dark place) within six months according to expiry date at monthly intervals.

Thirty two closed containers of two types of cheese (processed-cream) and two types of yoghurt (common-flavored) were maintained at varying temperature conditions ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$) without exposure to sunlight within three months at monthly intervals.

Effect of light

Sixteen closed containers of two types of cheese (processed-cream) and two types of yoghurt (common-flavored) were maintained at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) exposed to sunlight within three months at monthly intervals.

Sample treatment

An amount of 10 g homogenized yoghurt was weighed in a conical flask and 75 mL of methanol acidified with 0.1% glacial acetic acid was added. Sample was stirred for 90 min in a magnetic stirrer. 25 ml of deionized water was added and placed in the conical flask in the freezer for about 60 min. Cold extract was filtered through a filter paper; first filtrate was discarded. Filtrate was warmed at room temperature and filled with methanol acidified with 0.1% glacial acetic acid in 100 ml-volumetric flask. 2 ml of filtrate was taken and put in 100 ml-volumetric flask. The same treatment is applied on cheese samples.

Analytical procedure

Natamycin content in the experimental design above were assayed using UV-Spectrophotometer method at maximum absorption wavelength 303 nm. All determinations were done in triplicates.

Results and Discussions

Table 1 Changes in natamycin content and the percentage of natamycin content of different types of cheese and yoghurt products stored at refrigeration conditions ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$, dark place) within 6 months.

Stability of natamycin in cheese and yoghurt products under refrigeration conditions is presented in Table 1 and the result is graphically represented in Figures 2 and 3. There was a significant negative correlation between storage time and natamycin content.

Storage time (months)	Natamycin content of different types of dairy products (mg/400 g) for cheese and (ml/250 ml) for yoghurt				The percentage of natamycin content of different types of dairy products (mg/400 g) for cheese and (ml/250 ml) for yoghurt			
	Processed	Cream	Common	Flavored	Processed	Cream	Common	Flavored
0	4.8	5	2.12	2.25	100	100	100	100
1	4.76	4.98	2.09	2.19	99.2	99.86	98.65	97.22
2	4.73	4.96	2.04	2.09	98.66	99.21	95.92	93.15
3	4.67	4.92	1.95	1.98	97.35	98.35	92	88.35
4	4.6	4.86	1.9	1.9	95.81	97.17	89.52	84.5
5	4.48	4.79	1.84	1.81	93.44	95.98	86.81	80.33
6	4.36	4.74	1.78	1.72	91	94.91	84	76.5

Table 1: Changes in natamycin content and the percentage of natamycin content of different types of cheese and yoghurt products stored at refrigeration conditions ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$, dark place) within 6 months.

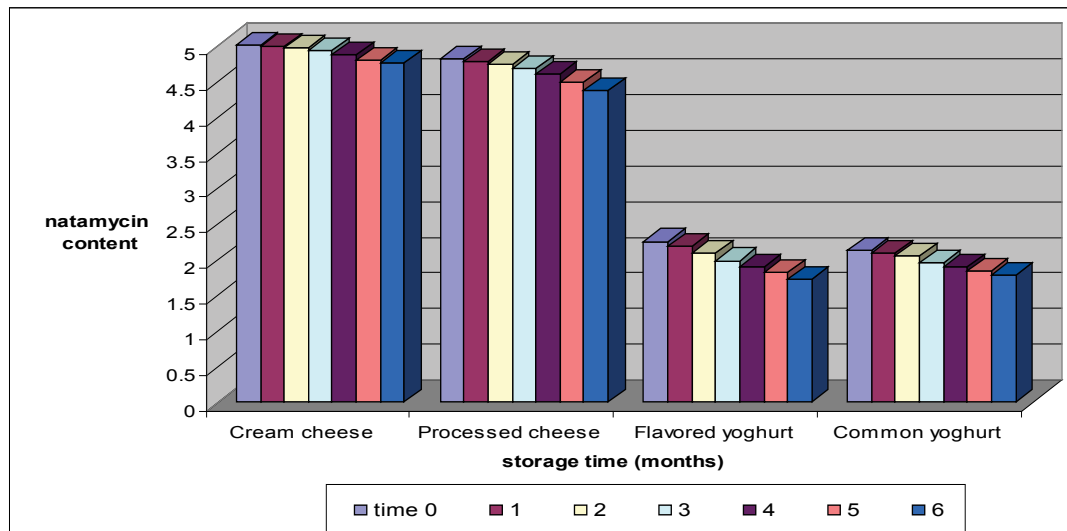


Figure 2: Changes in natamycin content of different types of dairy products stored at refrigeration conditions ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$, dark place) within 6 months.

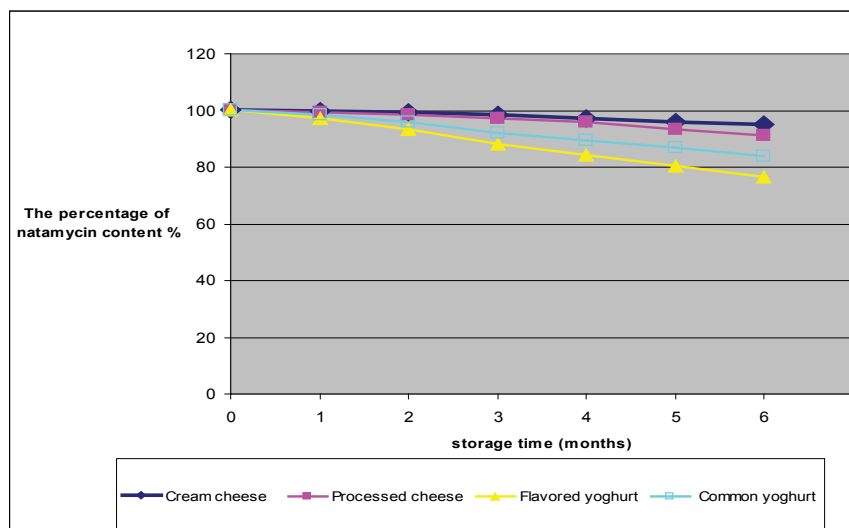


Figure 3: Changes in the percentage of natamycin content of different types of dairy products stored at refrigeration conditions ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$, dark place) within 6 months.

The percentage of natamycin content in processed and cream cheese was 91.0% and 94.91% respectively. And the percentage of natamycin content in common and flavored yoghurt was 84% and 76.5% respectively. These results indicated a little influence on the content of natamycin under refrigeration conditions ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$) in a dark place.

Effects of storage temperature on natamycin content of the different sources without exposure directly to sunlight are shown in Table 2 and the result is graphically represented in Figures 4 and 5. The result indicated a rapid loss in natamycin content along the storage time when exposed to high temperatures compared to the room temperature which occur a little influence on natamycin content.

After 3 months of exposure to room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$), the percentage of natamycin content in processed cheese decreased to (86.5%). In cream cheese, the percent decreased to (88.25%). In common yoghurt, the percent decreased to (84.75%). In flavored yoghurt, the percent decreased to (80.35%).

After 3 months of exposure to high temperature ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}$), the percentage of natamycin content in processed cheese decreased to (52.44%). In cream cheese, the percentage decreased to (55.15%). In common yoghurt, the percent decreased to (42%). In flavored yoghurt, the percent decreased to (36.20%).

By comparing the pervious results, we found a significant effect of temperature on the percentage of natamycin content in cheese and yoghurt products. The temperature increase, the percentage of natamycin content decreased.

Effects of sunlight at room temperature on natamycin content in cheese and yoghurt products is shown in Table 3 and the results are graphically represented in Figures 6 and 7. After 3 months of exposure to sunlight at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$), the percentage of natamycin content in processed cheese decreased to (65.0%). In cream cheese, the percent decreased to (68.25%). In common yoghurt, the percent decreased to (54.21%). In flavored yoghurt, the percent decreased to (49.11%).

Natamycin content of different types of cheese and yoghurt products exposed to different temperatures (25°C ± 2°C , 40°C ± 2°C) within 3 months								
Storage time (months)	Processed cheese		Cream cheese		Common yoghurt		Flavored yoghurt	
	25 ± 2°C	40 ± 2°C	25 ± 2°C	40 ± 2°C	25 ± 2°C	40 ± 2°C	25 ± 2°C	40 ± 2°C
0	4.8	4.8	5	5	2.12	2.12	2.25	2.25
1	4.58	3.96	4.84	4.26	2.01	1.57	2.1	1.53
2	4.38	3.18	4.62	3.72	1.89	1.24	1.96	1.11
3	4.15	2.52	4.41	2.76	1.8	0.89	1.81	0.81
The percentage of natamycin content of different types of cheese and yoghurt products exposed to different temperatures (25C+2C , 40C+2C) within 3 months								
Storage time (months)	Processed cheese		Cream cheese		Common yoghurt		Flavored yoghurt	
	25 ± 2°C	40 ± 2°C	25 ± 2°C	40 ± 2°C	40 ± 2°C	25 ± 2°C	40 ± 2°C	25 ± 2°C
0	100	100	100	100	100	100	100	100
1	95.42	82.45	96.75	85.21	67.98	93.44	74	94.45
2	91.33	66.25	92.44	74.5	49.35	87.25	58.44	89
3	86.5	52.44	88.25	55.15	36.2	80.35	42	84.75

Table 2: Changes in natamycin content and the percentage of natamycin content of different types of cheese and yoghurt products exposed to different temperatures (25°C ± 2°C, 40°C ± 2°C) within 3 months.

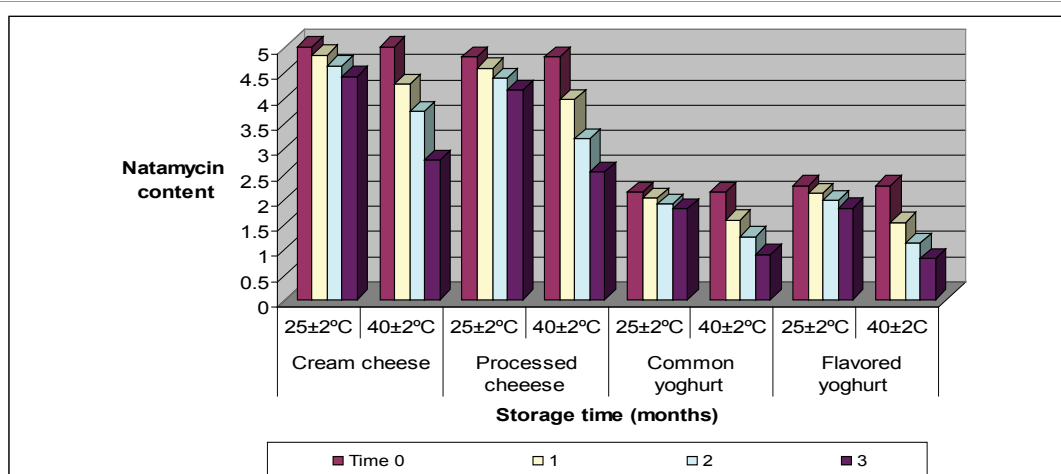


Figure 4: Changes in natamycin content of different types of cheese and yoghurt products exposed to different temperatures (25°C ± 2°C, 40°C ± 2°C) within 3 months.

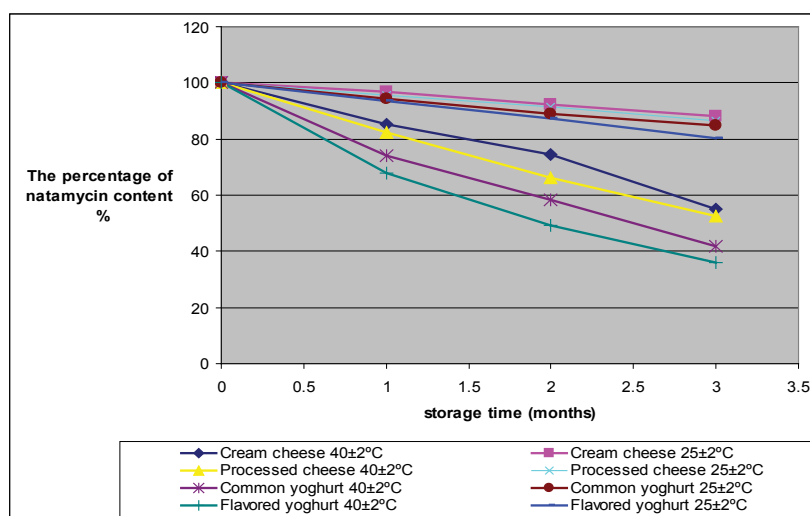


Figure 5: Changes in the percentage of natamycin content of different types of cheese and yoghurt products exposed to different temperatures (25°C ± 2°C, 40°C ± 2°C) within 3 months.

Storage time (months)	Natamycin content of different types of dairy products (mg/400 g) for cheese and (ml / 250 ml) for yoghurt				The percentage of natamycin content of different types of dairy products (mg/400 g) for cheese and (ml/250 ml) for yoghurt			
	Processed	Cream	Common	Flavored	Processed	Cream	Common	Flavored
0	4.8	5	2.12	2.25	100	100	100	100
1	4.29	4.56	1.66	2.25	89.41	91.15	78	75.9
2	3.51	3.85	1.41	1.71	73.2	77	66.56	60.56
3	3.12	3.41	1.15	1.36	65	68.25	54.21	49.11

Table 3: Changes in natamycin content and the percentage of natamycin content of different types of cheese and yoghurt products exposed to sunlight at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) within 3 months.

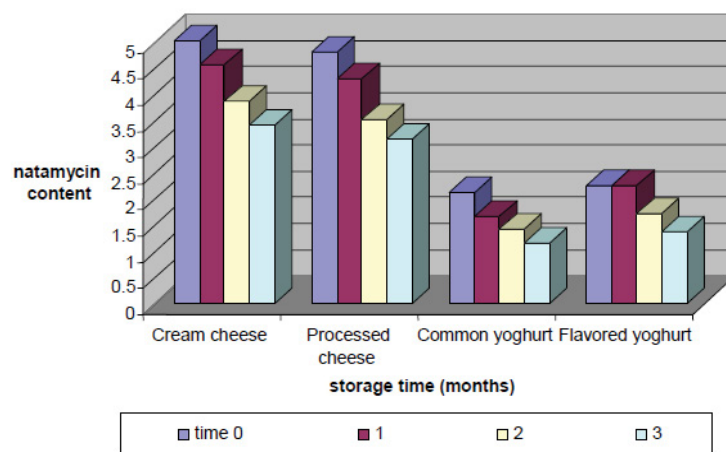


Figure 6: Changes in natamycin content of different types of dairy products exposed to sunlight at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) within 3 months.

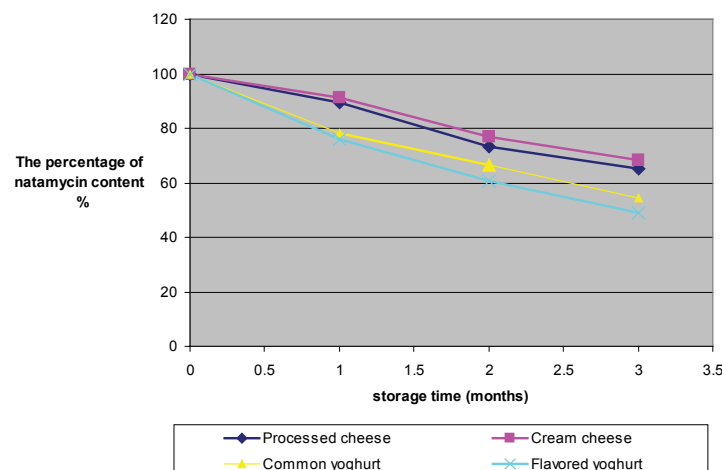


Figure 7: Changes in the percentage of natamycin content of different types of dairy products exposed to sunlight at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) within 3 months.

The results indicated a significant effect of sunlight on the percentage of natamycin content in cheese and yoghurt products. Amore reduction in natamycin content along the storage time when exposed to sunlight compared to products not exposed to sunlight at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$).

Conclusions

The results of the analysis showed that exposure to sunlight and temperature conditions significantly affected the stability of natamycin with the storage time. It was observed that natamycin was more stable when stored under refrigeration conditions ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$) in a dark

place. It is therefore recommended that refrigeration conditions would be ideal conservation conditions for cheese and yoghurt products containing natamycin. The results also showed that natamycin was relatively stable in cheese and yoghurt samples when stored at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) compared to products exposed to sunlight at room temperature. It is therefore recommended that the room temperature would be also a suitable storage condition for cheese and yoghurt products in shops away from sunlight.

However, it was observed that natamycin was less stable when exposed to high temperature ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}$). High temperatures would be a poor storage conditions for cheese and yoghurt products

containing natamycin in addition to exposure to sunlight. So, it is therefore recommended that dairy products containing natamycin should be kept in refrigeration conditions in a dark place away from sunlight and high temperatures.

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