

Pharma Sci-Study of Storage Conditions Effect (Light-Heat) on Natamycin Content and Stability in Some Dairy Products (Cheese-Yoghurt)- Al-Haj Saeed Nihad- Damascus University

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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. 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. Natamycin is a natural antimitotic polyene characterized by the presence of a large macro cyclic lactone ring containing a series of conjugated double bonds and one or more sugar residues. Natamycin has an empirical formula of $C_{33}H_{47}NO_{13}$ and a molecular weight of 665.75. It is produced by *Streptomyces natalensis* and is commonly employed in dairy-based food products to prevent yeasts and moulds contamination. 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) 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. 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). 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.

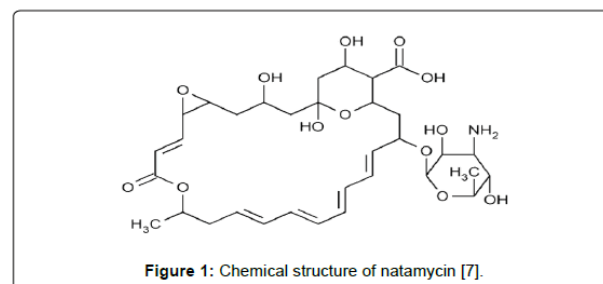


Figure 1: Chemical structure of natamycin.

The stability of natamycin is affected by extreme pH values, light, heat, oxidants, chlorine, and heavy metals. 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 between 5.0 and 9.0 when stored in the dark. Aqueous suspensions of natamycin are more stable than aqueous solutions. 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.

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. 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](#). Oxidation of natamycin proceeds more readily at higher temperatures. Oxidation was prevented by ascorbic acid, hydroquinone, and Gallic acid. The antimitotic 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. 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. The polyene macrolide antibiotics are very light sensitive antimicrobial substances.

Photolytic oxidation clearly poses a problem in the handling and storage of the polyene antibiotics. The photolysis of natamycin is a very rapid upon light exposure to a xenon lamp with a spectrum similar to that of natural sunlight.

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) 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.

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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