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EFFECTS OF NATAMYCIN AS BIOPRESERVATIVE ON PEANUT BUTTER QUALITY

BY

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ABSTRACT

The antifungal activity of natamycin against *Aspergillus flavus* was studied by determinations of the minimal inhibitory concentration (MIC) for natamycin on mold growth.

The results indicated that natamycin exhibited a high degree of antifungal activity against *A. flavus*, with average MIC values ranging from 21 to 26 ppm. Growth, sporulation and production of aflatoxins of *A. flavus* were affected by the addition of natamycin and the effect was most pronounced when the natamycin was added at a levels of 24 and 26 ppm at pH 5.7. The natamycin as antifungal compound was not affected by heat treatment.

A. flavus was inoculated onto peanut butter containing variable amounts of natamycin and stored at 4 and 25°C for 20 wk. The initiation time of growth was paralled to the concentrations of natamycin and temperatures of storage. Natamycin at concentration of 26 ppm completely inhibited the growth of yeast and mold during storage. The results for treated samples reflect the effect of natamycin on delaying the increase of TBA number and all organoleptic tests were significant when compared with the control.

The results give basis to recommend natamycin for use as biopreservation control agent in the food industry.

INTRODUCTION

Peanuts, corn and cotton seed are often invaded before harvest by *Aspergillus flavus* Link. This fungi produce aflatoxins, potent carcinogens and cyclopiazonic acid (CPA), which is toxic to a variety of animals and has been implicated in human poisoning (Rao and Husain, 1985; Peraica *et al.*, 1999; Pildain *et al.*, 2004). Aflatoxins have been found as contaminants in agricultural and food products, being peanut and their derivative products such as peanut butter and oil (Blesa *et al.*, 2003).

Mycotoxicoses, which can occur in both industrialized and developing countries, arise when environmental, social and economic conditions combine with

meteorological conditions (humidity, temperature) which favour the growth of moulds (Bakirci 2001; Galvano *et al.*, 2001; Aycicek, *et al.*, 2005). Large amounts of food and feed are lost every year due to spoilage by moulds and yeasts. Biopreservation, i.e. the use of microorganisms as preservatives instead of chemicals, has gained increased interest (Lind, *et al.*, 2005). Several techniques are used for the preservation of food and feeds. Drying, freeze-drying, cold storage, modified atmosphere storage and heat treatments are all means of physical methods of food preservation (Farkas, 2001). Several chemical additives also function as preservatives, even though the exact mechanisms or targets often are not known (Davidson, 2001). The organic acids acetic, latic, propionic, sorbic and benzoic acids are used as food preservatives (Brul and Coote, 1999; Gab-Alla and Sheriff, 2002).

The antibiotic natamycin, produced by *Streptomyces natalensis* is effective against yeasts and moulds and a common preservative on surfaces of hard cheese (Davidson, 2001). Natamycin is usually applied as a surface treatment, particularly to the surface of hard cheeses and dry fermented sausages and has also been used to treat the surface of smoked cured meat. Natamycin is also used as direct addition to yoghurt, bakery products, fruit juice, wine (Thomas and Broughton, 2001; Aideia and Sherif, 2004).

The aim of this study was to evaluate antifungal agent (natamycin) as biopreservative on peanut butter quality during storage.

MATERIALS AND METHODS

Strain cultivation and maintenance

Aspergillus flavus strain was obtained from the mycotoxin laboratory National Research Center, Doki, Cairo, Egypt. *A. flavus* was maintained on sabouraud dextrose slants, and subcultured monthly. The sabouraud dextrose agar was composed of 40 g dextrose, 10 g bacteriological peptone and 16 g bacteriological agar per liter, adjusted to final pH 5.6 and was autoclaved at 121°C for 15 min (oxid, England).

A. flavus spore suspension was prepared as described by Osman (2004). *A. flavus* was grown on sabouraud dextrose slant agar at 28°C for 10 days. The spores were harvested in sterile phosphate buffered diluent (pH 7.2 containing 0.05%, v/v Tween 80). The buffer solution (30 ml) was added to the slants and the spores were loosened by gently bruching with a sterile inoculating loop. Mycelial debris was removed from spore suspension by filtering twice through several layers of sterile damp cheesecloth. The concentration of the spores in the suspension was determined on sabouraud dextrose agar medium using the spread plate technique and incubation at 30°C for 4 days.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) for natamycin on mold growing on sabouraud dextrose agar containing natamycin according to methods described by Brothers and Wyatt (2000) as follows:

One ml of 24 hr activated cultures was serially diluted and one ml from each dilutions (in duplicates) was transferred into Petri dishes. Different concentrations of natamycin from 0 to 24 ppm were thoroughly mixed with sterilized medium (10 ml), then poured into aforementioned Petri dishes. The dishes were incubated at 30°C for 72 hr. the viable colonies were counted and inhibition percent was calculated and referred to the viable count at 0.0% natamycin.

Natamycin produced by Danisco comp. DK. 8220 Brabrand, Denmark and obtained from Amson International Trading Company, El-Mohandesin, Giza, Egypt.

Effects of natamycin on mold growth and aflatoxin production at different pH

Different concentrations of the natamycin (0, 18, 21, 24 and 26 ppm) were thoroughly mixed with sterilized sabouraud dextrose broth medium (100 ml) and inoculated with *A. flavus* (from spore suspension) to a level of 6.1×10^5 spores ml^{-1} at different pH (4.7, 5.7 and 6.7) for each concentrations. The flasks were incubated at 27°C for 35 days (the end of the experiment) with daily examination for visible fungal growth and spore initiation at the end of the experiments (35 days) flasks showing visible fungal growth were autoclaved at 121°C for 15 min to help the release of aflatoxin from the spore mycelia (Osman, 2004) and kept at -18°C until analysis for aflatoxins.

Effects of heat treatments on the antifungal activity against *A. flavus*

Sabouraud dextrose broth medium at pH 5.7 were mixed at concentration 24 ppm of natamycin and divided into four treatments. One left without any heat treatment as control, one pasteurized at 60°C for 30 min, one steamed at 100°C for 15 min, and one autoclaved at 121°C for 15 min and followed by cooling to 20°C, then the flasks were inoculated with *A. flavus* to level of 6.1×10^5 spores ml^{-1}. The count of viable *A. flavus* spores was counted on sabouraud dextrose agar medium using the spread plate technique and incubation at 30°C for 4 days (American Public Health Association, 1993).

Effect of natamycin on growth of mold in peanut butter

Peanuts were obtained from Ismailia city (2004 season), shelled manually and roasted at 160°C for 60 minutes according to (Gab Alla and Gad, 2001).

Peanuts butter was prepared using the following formula: roasted peanut (92%), glycerol (2%), dextrose (2%), NaCl (1%) and peanut oil (3%). The roasted peanut was mixed with all ingredients in a kitchen mixture to produce homogenous peanut butter. The mixture was then pasteurized at 70°C for 15 min., and cooled. The prepared peanut butter was divided into two portions for treatments. One portion was divided into three parts and adjusted pH to 4.7 and 6.7 (using $NaHPO_4$) then every part divided into five treatments and mixed with natamycin at levels of 0, 18, 21, 24 and 26 ppm. All treatments were inoculated with *A. flavus* to a level of 6.1×10^5 spores ml^{-1}. The second portion was also divided by the same method but without inoculated with *A. flavus*. All samples were packed in sterile containers and stored at 4 and 25°C .

Samples from each treatment were taken at specified time intervals throughout storage for analysis.

Analysis

The inoculated treatments were examined daily for visible fungal growth (growth initiation and spore initiation. Mold and yeast counts were enumerated in uninoculated treatments on potato dextrose agar (APHA, 1993). Thiobarbituric acid (TBA) value was determined as described by Pearson (1981). Extraction and determination of aflatoxins according to the method described by (AOAC, 1995) using a high performance liquid chromatography system (HPLC, Agilent, Technologists 1601 California, Avenue Palo AHO, CA 94304, USA).

Sensory evaluation

All samples (uninoculated) were subjected to sensory evaluation at 0, 10 and 20 week of storage at 4 and 25°C for flavour (50) colour (25) and texture (25). Statistical analysis were applied by carrying out analysis of variance followed by the multiple comparisons using LSD at $p < 0.05$ according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

MIC values of natamycin

The minimum inhibitor concentrations (MIC) of natamycin against *A. flavus* is shown in Fig. (1). Generally the reduction of viable cells gradually increased according to the increase in the natamycin concentration, which was added to the growth medium. *A. flavus* gave total inhibition at concentrations of 24 ppm. Thomas and Broughton (2001) attributed the activity of natamycin against both moulds and yeast due to irreversible binding of natamycin to ergesterol disrupts the cell membrance, increasing its permeability and causing leakage from the eventually leading to cell death, in which the ergesterol is an essential component of the cell membranes that is present in all yeasts and fungi. MIC value determinations rely on discrimination of growth or none growth of the target organism.

The initiation of growth is influenced by the inoculum size (Jensen *et al.*, 1987; Fernandez-Garayzabal and Genigeorgis, 1990; Lind *et al.*, 2005). We used 61 x 104 cell ml-1 in the examination of MIC of natamycin. This level was based on previous experience, considered a suitable inoculum size with regard to experimental repoducibility. Another inoculum size may have given slightly different results which is important to remember when the MIC are referred to in future projects.

Effects of natamycin on growth of *A. flavus* and aflatoxin production in nutrient broth medium at different pH

Natamycin was mixed with nutrient broth medium inoculated with *A. flavus* spores and incubated at 27°C exhibited inhibitory effect on *A. flavus* spores at concentration 24 ppm (there were no fungal growth or aflatoxin production). *A. flavus* showed a visible fungal growth after 2 days in control sample at different pH, while showed visivle fungal growth after 11 and 21 days at concentration of natamycin 18 and 21 ppm at pH 4.7, on the other hand *A. flavus* showed a visible fungal growth after 18 and 31 days at pH 5.7 and 15 and 25 days at pH 6.7 at the same concentrations of natamycin (Table, 1).

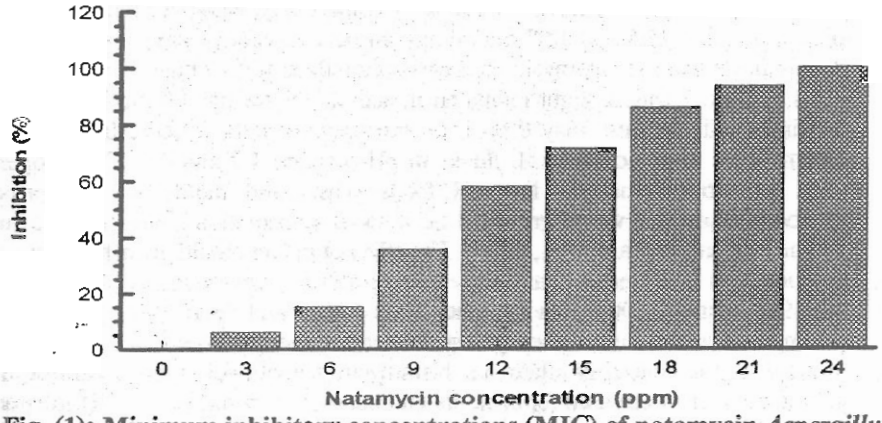

Fig. (1): Minimum inhibitory concentrations (MIC) of natamycin *Aspergillus flavus*

Table (1): Effects of natamycin on growth of *A. flavus* and aflatoxin production in broth medium at different pH

Natamycin concentration (ppm)	*A. flavus*		Aflatoxins (ng/ml)				
	1	2	B_1	B_2	G_1	G_2	Total
			pH 4.7				
0	2	5	32.1	55.1	27.9	18.7	133.8
18	11	21	8.3	8.9	13.6	6.7	37.5
21	21	30	5.9	6.1	8.2	2.1	22.3
24	-	-	ND	ND	ND	ND	-
26	-	-	ND	ND	ND	ND	-
			pH 5.7				
0	2	5	31.9	55.2	28.0	19.1	134.2
18	18	25	5.6	9.1	12.3	5.6	32.6
21	31	b	2.3	3.9	5.0	1.3	12.5
24	-	-	ND	ND	ND	ND	-
26	-	-	ND	ND	ND	ND	-
			pH 6.7				
0	2	5	31.6	56.0	27.3	19.0	133.9
18	15	23	8.0	8.2	13.4	5.9	35.5
21	25	30	4.8	5.6	6.4	1.9	18.7
24	-	-	ND	ND	ND	ND	-
26	-	-	ND	ND	ND	ND	-

1= growth initiation (days)
2= spore initiation (days)
b= no growth or sporulation detected after incubation period
ND = not detectable

Total aflatoxin production at concentration of 18 ppm natamycin were 37.5, 32.6 and 35.5 mg/ml at pH 4.7, 5.7 and 6.7 respectively, while at concentration of 21 ppm were 22.3, 12.5 and 18.7 mg/ml as compared to control sample (Table, 1). The data indicate that the natamycin performs optimally at pH 5.7 but was still effective at pH 4.7 and 6.7 with a slight reduction in activity, below pH 4.7 and above pH 6.7, antifungal effectiveness may drop from the previous data it could be noticed that natamycin is more active on *A. flavus* in pH between 4.7 and 6.7. This property is very interesting, since in low pH foods yeasts and molds are the principal microorganisms that would grow and cause some deterioration of organoleptic quality and/or food safety (Faid *et al.*, 1995). The effect of pH on mould growth and aflatoxin production is a matter of controversy among different investigators (Karunaratue *et al.*, 1990; Osman, 2004). On the other hand Aideia and Sherif (2004) reported that natamycin has no direct action against aflatoxin or their synthesis, but overall control of fungi lead to control of aflatoxins. Natamycin is commonly used to control fungal growth on agar media used for bacterial enumeration or strain isolation (Mohamed *et al.*, 2005).

Effect of heat treatment on the antifungal activity on the growth of *A. flavus*.

The effect of heat treatment on the antifungal activity of natamycin was studied at concentration of 24 ppm which had been heat treated under different conditions and inoculated with *A. flavus* as shown in Fig. (2). The data showed that natamycin remain nearly stable at pasteurized and outoclaved temperatures while the effectiveness is reduced (Slight reduce) by exposure to steaming at 100°C for 15 min. This indicated that the natamycin as antifungal compound was not affected by heat treatment.

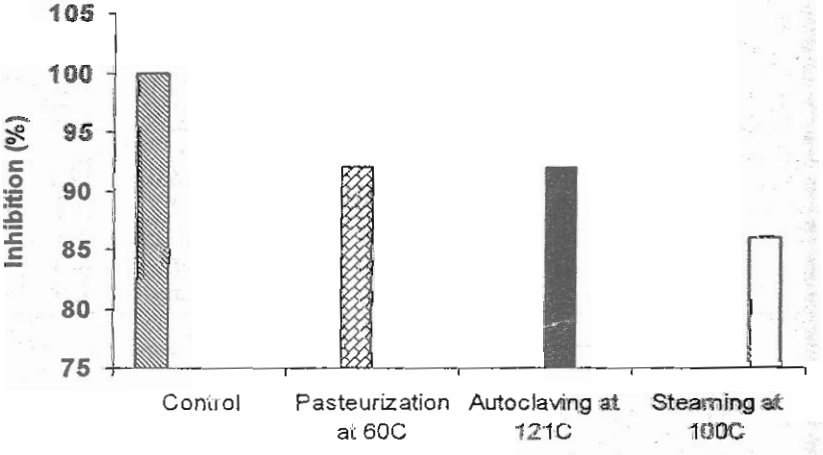

Fig. (2): Effect of different heat treatment on the antifungal activity (24 ppm natamycin) on the growth of *A. flavus*

Effect of natamycin on growth of *A. flavus* in peanut butter during storage:

Mould growth and spore initiation were not detected in samples treated with 26 ppm natamycin for 20 wk at pH 5.7 and 6.7 and 17 wk at pH 4.7

compared to control sample during storage at 4°C (Table, 2). The same trend observed during storage at 25°C but for a short time (Table, 3). Mohamed *et al.* (2005) concluded that natamycin concentration up to 200 ppm were not sufficient to control fungal growth when plates were incubated at 28°C. This might be due to the poor stability of natamycin during incubation period at 28°C. The findings revealed that 26 ppm of natamycin prevented the mould growth for 20 week in peanut butter at pH 5.7 during storage at 4 or 25°C. These results are agreement with that reported by Var *et al.* (2004) who reported that no mould growth were detected in kashar cheese samples produced by combined application of natamycin and packaging materials during five month ripening period. The shredded cheese may also be coated with an antimycotic agent to inhibit the growth of molds on the surface of the shreds. Natamycin is the preferred choice, since it is for more effective against molds and yeast than sorbates and propionates (Hamilton-Miller, 1974; Elayedath and Barringer, 2002).

Table (2): Effect of natamycin on growth of *Aspergillus flavus* in peanut butter at different pH degrees during storage at 4°C for 20 week

A. flavus growth	Natamycin concentration (ppm)				
	0	18	21	24	26
			pH 4.7		
Growth initiation (days)	7	38	61	88	113
Spore initiation (days)	15	50	72	101	130
			pH 5.7		
Growth initiation (days)	7	49	88	115	-
Spore initiation (days)	15	56	93	-	-
			pH 6.7		
Growth initiation (days)	7	43	78	113	-
Spore initiation (days)	15	52	86	131	-

Table (3): Effect of natamycin on growth of *Aspergillus flavus* in peanut butter at different pH degrees during storage at 25°C for 20 week

A. flavus growth	Natamycin concentration (ppm)				
	0	18	21	24	26
			pH 4.7		
Growth initiation (days)	2	18	31	62	81
Spore initiation (days)	5	29	52	86	94
			pH 5.7		
Growth initiation (days)	2	28	49	89	-
Spore initiation (days)	5	36	57	115	-
			pH 6.7		
Growth initiation (days)	2	18	33	65	106
Spore initiation (days)	5	31	56	88	113

Yeast and mold counts of peanut butter containing natamycin during storage

Total yeast and mold of peanut butter containing natamycin up to 24 ppm decreased at the storage period progressed then slightly increased for both microorganisms until the end of storage periods at 4 or 25°C. The decrease of growth at the end of incubation periods was paralled to the temperatures of incubation. Natamycin at concentration of 26 ppm completely inhibited the growth of yeast and mold during storage at 4 or 25°C (Table, 4 and 5). The presence of natamycin and thus their ability to inhibite the yeast and mould might explain the decline of total number Elayedath and Barringer (2002). Natamycin a product that is safe for humans and utilized in food technology was proposed by Pedersen (1992) to replace cycloheximide in agar media. The concentration used vary over on extended range from 21 to 500 ppm (Gomez *et al.*, 1995; Edelstein and Edelstein, 1996; Stack *et al.*, 2002; Mohamed *et al.*, 2005).

Table (4): Yeast and mold counts of peanut butter containing natamycin at different pH degrees during storage at 4°C

Storage period (wk)	Natamycin concentration (ppm)				
	0	18	21	24	26
		pH 4.7			
0	$1.6 \pm 0.17 \times 10^2$	$1.1 \pm 0.17 \times 10^2$	$0.9 \pm 0.33 \times 10^2$	-	-
5	$3.3 \pm 0.06 \times 10^2$	$0.4 \pm 0.11 \times 10^2$	$0.3 \pm 0.08 \times 10^2$	-	-
10	$2.5 \pm 0.33 \times 10^2$	$0.5 \pm 0.13 \times 10^2$	$0.6 \pm 0.08 \times 10^2$	-	-
15	$2.3\ 0.08 \times 10^3$	$0.7 \pm 0.28 \times 10^2$	$0.6 \pm 0.08 \times 10^2$	-	-
20	$3.7 \pm 0.26 \times 10^3$	$1.4 \pm 0.01 \times 10^2$	$0.9 \pm 0.13 \times 10^2$	-	-
		pH 5.7			
0	$1.5 \pm 0.07 \times 10^2$	$0.9 \pm 0.28 \times 10^2$	$0.6 \pm 0.13 \times 10^2$	-	-
5	$3.3 \pm 0.06 \times 10^2$	$0.3 \pm 0.32 \times 10^2$	$0.3 \pm 0.08 \times 10^2$	-	-
10	$0.8 \pm 0.11 \times 10^2$	$0.5 \pm 0.05 \times 10^2$	$0.3 \pm 0.08 \times 10^2$	-	-
15	$2.1 \pm 0.08 \times 10^3$	$0.7 \pm 0.01 \times 10^2$	$0.4 \pm 0.23 \times 10^2$	-	-
20	$3.5 \pm 0.26 \times 10^3$	$1.1 \pm 0.13 \times 10^2$	$0.7 \pm 0.08 \times 10^2$	-	-
		pH 6.7			
0	$1.5 \pm 0.07 \times 10^2$	$1.1 \pm 0.26 \times 10^2$	$1.0 \pm 0.11 \times 10^2$	-	-
5	$3.4 \pm 0.08 \times 10^2$	$0.3 \pm 0.27 \times 10^2$	$0.7 \pm 0.29 \times 10^2$	-	-
10	$0.7 \pm 0.11 \times 10^2$	$0.6 \pm 0.01 \times 10^2$	$0.5 \pm 0.09 \times 10^2$	-	-
15	$2.2 \pm 0.07 \times 10^3$	$0.8 \pm 0.22 \times 10^2$	$0.5 \pm 0.01 \times 10^2$	-	-
20	$3.6 \pm 0.26 \times 10^3$	$1.2 \pm 0.03 \times 10^2$	$0.9 \pm 0.28 \times 10^2$	-	-

The presented data is the mean ± standard deviation of three replicate experiments

TBA values of peanut butter during storage

TBA values for control and treated samples increased at any given time of storage at 4 and 25°C except treatments 4 and 5 had less TBA values compared to anothers samples (Fig. 3 and 4). The results for treated samples reflect the effect of natamycin on delaying the increase of TBA comparison with control sample. The increase of TBA values at the end of storage time might be due to the increase of lipolytic activity as a result of yeast and mold growth which encourage lipid oxidation. These results are in good agreement with that reported by Aidieia and Sherif (2004) concluded that natamycin could be used as a safe preservative against mould and yeast in kareish cheese and other dairy product.

Table (5): Yeast and mold counts of peanut butter containing natamycin at different pH degrees during storage at 25°C

Storage period (wk)	Natamycin concentration (ppm) 0	18	21	24	26
			pH 4.7		
0	$1.6 \pm 0.17 \times 10^2$	$1.6 \pm 0.7 \times 10^2$	$1.1 \pm 0.17 \times 10^2$	-	-
5	$3.3 \pm 0.06 \times 10^2$	$1.2 \pm 0.12 \times 10^2$	$0.3 \pm 0.23 \times 10^2$	-	-
10	$8.5 \pm 0.33 \times 10^2$	$0.8 \pm 0.24 \times 10^2$	$0.7 \pm 0.08 \times 10^2$	-	-
15	$2.3\ 0.08 \times 10^3$	$2.1 \pm 0.23 \times 10^2$	$1.1 \pm 0.16 \times 10^2$	$0.7 \pm 0.23 \times 10^2$	-
20	$3.7 \pm 0.26 \times 10^3$	$5.6 \pm 0.01 \times 10^2$	$2.1 \pm 0.05 \times 10^2$	$0.9 \pm 0.11 \times 10^2$	-
			pH 5.7		
0	$1.5 \pm 0.07 \times 10^2$	$1.2 \pm 0.13 \times 10^2$	$0.9 \pm 0.01 \times 10^2$	-	-
5	$3.3 \pm 0.06 \times 10^2$	$1.0 \pm 0.11 \times 10^2$	$0.2 \pm 0.23 \times 10^2$	-	-
10	$8.8 \pm 0.11 \times 10^2$	$0.9 \pm 0.27 \times 10^2$	$0.2 \pm 0.12 \times 10^2$	-	-
15	$2.1 \pm 0.08 \times 10^3$	$1.6 \pm 0.15 \times 10^2$	$0.8 \pm 0.03 \times 10^2$	$0.3 \pm 0.16 \times 10^2$	-
20	$3.5 \pm 0.26 \times 10^3$	$4.6 \pm 0.16 \times 10^2$	$1.3 \pm 0.17 \times 10^2$	$0.9 \pm 0.21 \times 10^2$	-
			pH 6.7		
0	$1.5 \pm 0.07 \times 10^2$	$1.4 \pm 0.31 \times 10^2$	$0.9 \pm 0.11 \times 10^2$	-	-
5	$3.4 \pm 0.08 \times 10^2$	$1.1 \pm 0.82 \times 10^2$	$0.5 \pm 0.11 \times 10^2$	-	-
10	$8.7 \pm 0.11 \times 10^2$	$0.9 \pm 0.11 \times 10^2$	$0.8 \pm 0.07 \times 10^2$	-	-
15	$2.2 \pm 0.07 \times 10^3$	$2.3 \pm 0.22 \times 10^2$	$1.3 \pm 0.15 \times 10^2$	$0.6 \pm 0.21 \times 10^2$	-
20	$3.6 \pm 0.26 \times 10^3$	$6.0 \pm 0.13 \times 10^2$	$1.9 \pm 0.21 \times 10^2$	$1.1 \pm 0.08 \times 10^2$	-

The presented data is the mean ± standard deviation of three replicate experiments

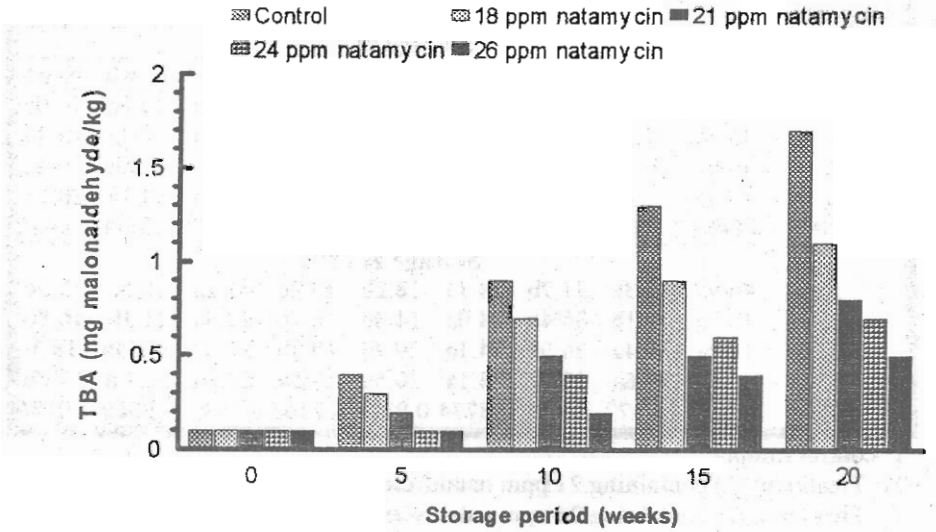

Fig. (3): TBA values of peanut butter containing natamycin at pH 5.7 during cold storage at 4°C

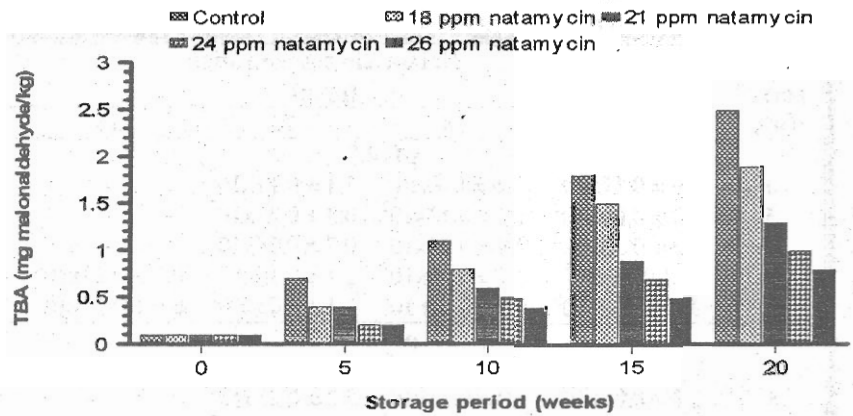

Fig. (4): TBA values of peanut butter containing natamycin at pH 5.7 during storage at 25°C

Sensory properties of peanut butter during storage

Data presented in Table (6) summarized the sensory evaluation of peanut butter made with and without different concentration of natamycin during storage at 4 and 25°C. It is obvious that there are not significant differences in flavour, color and texture between control and treatment at zero time.

Table (6): Sensory properties of peanut butter containing natamycin at pH 5.7 during storage at 4 and 25°C

Sensory properties	Flavour			Colour			Texture		
Treatments	Storage at 4°C								
	0 wk	10 wk	20 wk	0 wk	10 wk	20 wk	0 wk	10 wk	20 wk
T	46.9a	38.9b	35.9c	24.1a	19.8c	15.4c	23.2a	20.7c	16.0c
T_1	46.7a	44.7a	37.2c	24.0a	20.3bc	17.5bc	22.3a	21.8bc	18.3b
T_2	46.9a	46.1a	39.4ab	24.1a	21.ab	17.9b	23.4a	21.9ab	20.1a
T_3	46.5a	46.3a	41.6a	24.1a	21.9a	20.3a	24.0a	23.1a	20.3a
LSD	1.0605	2.3396	2.9326	0.8774	1.0506	2.1343	1.1142	1.2671	1.0430
	Storage at 25°C								
T	46.9a	36.3c	34.7b	24.1a	18.2b	15.2c	23.2a	18.5c	15.5c
T_1	46.7a	37.1b	35.4b	24.0a	14.0b	16.7b	22.3a	21.1b	16.8b
T_2	46.9a	39.4a	36.9a	24.1a	20.7a	19.2b	23.4a	21.6ab	18.3
T_3	46.5a	39.6a	37.8a	24.1a	20.5a	19.5a	24.0a	22.1 a	19.2b
LSD	1.0605	1.0177	1.425	0.8774	0.9320	1.2163	1.1142	0.9689	1.0186

T: control sample

T_1: Treatment (1) containing 21 ppm natamycin

T_2: Treatment (2) containing 24 ppm natamycin

T_3: Treatment (3) containing 26 ppm natamycin

A, b, c there is no significant difference ($p > 0.05$) between any two means have the same letter within the storage period at the same temperature

Regarding the effect of storage at 4°C and 25°C on sensory properties changes, the data showed that peanut butter stored at 4°C was more acceptable than that stored at 25°C. On the other hand, all organoleptic significant when compared with the control sample after 10 and 20 wk of storage at 4°C and 25°C. sensory panelists rated peanut butter treated with 24 and 26 natamycin as being more acceptable (are significantly higher) than control in all sensory characteristics under study after 10 and 20 wk of storage at 4°C or 25°C. This could be attributed to the inhibitor effect of natamycin in reducing the yeast and mold content. Elayedath and Barringer (2002) mentioned that the first appearance of mold was taken as the end of the shelf life as judged by a typical consumer. Similar results observed by Aideia and Sherif (2004) who reported that addition of natamycin to soft cheese like kareish cheese increase shelf life during refrigerated storage and keep safe for human consumption. Kilic *et al.* (2000) reported that some microbiological spoiling observed in cheese is a result of lipolytic and proteolytic activities of some microorganisms. Lipolyze is the formation of rancidity and off-flavour in cheese due to breaking of triglycerides with the effect of lipase enzyme into fatty acids and glycerine. On the other hand, proteolytic enzymes, not easily affected by heat, break proteins in the environment and they also produce unwanted taste and semell in the product. Yeasts can, on the one hand, contribute to taste, smell and aroma formation in ripening process of cheese, on the other hand, they can cause spoiling. They cause organoleptic change by hydrolyzing fat (Pereira-Dias *et al.*, 2000). The concentration of natamycin needed is less than that for other preservatives and natamycin does not penetrate the cheese and effect the flavour and natural microbiota of cheese (de Ruig and Van den Berg, 1985; Jay, 1998).

CONCLUSION

The effect of antifungal agent on the quality of peanut butter could be concluded that, the use of natamycin as a food preservative extends the following benefits to consumers and food manufacturers: 1- extends food shelf life by the control of spoilage due to yeast and mould contamination, 2- reduces product recalls resulting from spoilage. This not only protects the reputation of the food manufacturer but also cuts manufacturing costs, 3- satisfies the containing consumer demand for natural products, 4- imparts no adverse flavour to food unlike sorbates that often confer a bitter taste, 5- stonger cidal activity compared to sorbate, 6- prevents the formation of potentially carcinogenic mycotoxins. Even if mould growth is removed from the food surface where it tends to grow, the toxins and other chemicals it may had produced will already have migrated into the food.

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تأثير النتاميسين كعامل حفظ حيوى على جودة زبدة الفول السودانى

آمال جاب الله

قسم الصناعات الغذائية – كلية الزراعة – جامعة قناة السويس – الاسماعيلية

تم دراسة النشاط المضاد للفطريات للـ Natamycin على *Aspergillus flavus* بتقدير التركيز الادنى المثبط (MIC) للـ Natamycin على نمو الفطريات.

اوضحت النتائج أن الـ natamycin يعطى نشاط عالى مضاد للفطريات ضد *A. flavus* بمتوسط MIC تتراوح ما بين ٢١-٢٦ جزء فى المليون.

وجد أن نمو *A. flavus* وتكوين الجراثيم وانتاج السموم جميعها يتأثر باضافة الـ natamycin ، حيث كان التأثير واضح عند اضافته بمستويات ٢٤ ، ٢٦ جزء فى المليون على pH ٥,٧ ، كما وجد أن الـ natamycin كمركب مضاد للميكروبات لا يتأثر بالمعاملة الحرارية . تم تلقيح زبدة الفول السودانى المحتوية على كميات مختلفة من الـ natamycin بالـ *A. flavus* وتخزينها على ٤، ٢٥°م لمدة ٢٠ اسبوع . وتوازى وقت ابتداء النمو مع تركيزات النتاميسين ودرجة حرارة التخزين حيث تم تثبيط نمو الفطريات والخمائر بالكامل باستخدام ٢٦ جزء فى المليون من النتاميسين.

اوضحت نتائج المعاملات تأثير النتاميسين على تأخير زيادة رقم الـ TBA وكانت الفروق معنوية عند مقارنتها حسيا مع الكونترول. توصى النتائج باستخدام النتاميسين كعامل تحكم فى الحفظ الحيوى فى صناعة الغذاء