NITRATE AND NITRITE IN LOCALLY PRODUCED CHEESES AT DAMIETTA GOVERNORATE

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ABSTRACT

A total of 40 random samples of locally manufactured Damietta cheese and hard cheese (20 samples each) were collected from different outlets at Damietta governorate. The collected samples were analyzed for determination of nitrate and nitrite concentrations by using spectrophotometer. The results revealed presence of nitrate and nitrite at various levels in all examined cheese samples. The concentrations of nitrate exceeded the permissible limit (50ppm) in 13 samples of Damietta cheese and 16 samples of hard cheese. While, 3 samples of Damietta cheese and 5 samples of hard cheese contained nitrite level above the permissible limit (2ppm). Also, the calculated daily intake of nitrate and nitrite for adult person from consumption of these cheese types were compared with Acceptable Daily Intake (ADI) recommended by FAO/WHO. The public health significance of the obtained results and recommendations were discussed.

INTRODUCTION

Recently, it has become common practice to incorporate nitrate and nitrite for processed foods as preservatives and colour stabilizers. At the farm level, nitrate may be used for control of sporeforming bacteria in milk and milk products **(Silva et al.,1994)**. Consequently, it may reach to dairy products from milk used for manufacturing. Many researches documented that, nitrate and nitrite had a great toxicological effects. So, its presence in foodstuffs have received great attention. Moreover, excessive intake of nitrate and nitrite in the diet may cause toxic effects **(Chan,1996)**. Therefore ,due to lack in work done on these salts, the present paper deals with both salts.

MATERIALS AND METHODS

*** Collection of samples :**

A total of 40 random samples of locally manufactured Damietta cheese (soft cheese) and hard cheese (20 samples each) were collected from different outlets at Damietta Governorate. All samples were labelled and taken to the laboratory for analysis.

*** Extraction of the samples:**

The extraction of cheese samples was carried according to **Hanning et al. (1988)**. 10 g of each cheese samples were thoroughly mixed in a blender with 150 ml dist. water then transferred into 200 ml volumetric flask. The mixture was warmed to 50°c in a water bath, then 10 ml of zinc sulphate solution (12%) and 10 ml of NaOH solution (0.5N) were added to each flask. The contents were mixed and held at 50°c for 10 minutes. The sample solution was decanted through Whatman filter paper No. 42 and sufficient amount of filtrate (100ml) was collected in volumetric flask for subsequent analysis.

*** Determination of nitrate :**

Nitrate in clear filtrate of cheese samples was estimated by Brucine method according to **A.P.H.A (1960)**.

a- Reagents

- Sulfuric acid solution 64% by volume (64 ml conc. H_2SO_4 mixed with 3 ml distilled water).
- Brucine solution : 5 g of brucine were dissolved in 100 ml of acetic acid.
- Conc. hydrochloric acid.
- Stock nitrate solution : 0.7218 g anhydrous potassium nitrate was dissolved in 1,000 ml distilled water (the solution contain 100 mg /L N).
- Standard nitrate solution : 100 ml stock nitrate solution were added to 1,000 ml distilled water (1 ml=0.01 mg N=0.0443 mg nitrate ions).

b- Procedure :

In a flask of 100 ml capacity, 1 ml of the clear filtrate was added to 10 ml of 64% H_2SO_4, then 0.1 ml of brucine solution 5% and finally one drop of conc. Hcl was added to the mixture. The

mixture was heated in a boiling water bath for 10 minutes till the appearance of yellow colour. The blank was set and the sample was measured using spectrophotometer at wave length 410 mμ. The concentration of nitrate was estimated from a constructed standard curve.

• Determination of nitrite :

Nitrite in clear filtrate of cheese samples was measured by diazotization method recommended by A.P.H.A **(1985)**

a- Reagents:

- Sulphanilic acid: 60 mg sulphanilic acid were completely dissolved in 70 ml hot distilled water. After cooling 20 ml conc. Hcl were added then the mixture was diluted to 100 ml with distilled water.
- Naphthylamine hydrochloride : In distilled water to which 1ml conc. Hcl has been added 600 mg of 1-napthylamine hydroehloride were dissolved then diluted to 100 ml distilled water.
- Sodium acetate buffer : was prepared by dissolving 16.4g $NaC_2H_3O_2$ in nitrite free water and diluted to 100 ml.
- Sock nitrite solution: 1.233 g sodium nitrite were dissolved in dist. water then diluted to 1,000 ml (1ml = 250 μg N).
- One ml of chloroform was added to the solution as preservative.
- Intermediate nitrite solution: 50ml of the stock nitrite solution were added to 250 ml nitrite free water (1ml= 50μgN).
- Standard nitrite solution: 10ml intermediate nitrite solution were added in 1,000 ml with nitrite free water (1ml=0.5μg N).

b- Procedure :

To 50 ml of the clear filtrate, one ml sulphanilic acid reagent was added. After 3-10 minutes one ml 1-naphthylamine hydrochloride reagent and one ml sodium acetate buffer solution were added and thoroughly mixed. The mixture was left 10-30 minutes till the appearance of reddish purple colour. The sample measured using spectrophotometer at wave length 510 mμ against a reagent blank. The concentration of nitrite was estimated from constructed calibration curve.

• Calculation of daily intake of nitrate and nitrite :

To estimate daily intake of nitrate and nitrite from consumption of Damietta cheese (soft cheese) and hard cheese, mean data analyzed in the present work were combined with consumption data based on daily average cheese intake equal to 100 g of soft cheese and 80 g of hard cheese for adult person (**Nutrition Institute, Cairo, A.R.E, 1996**). Comparison of Acceptable Daily Intake (ADI) value of nitrate and nitrite recommended by **FAO/WHO (1996)** with daily intake of nitrate and nitrite from consumption of examined cheese types was calculated.

RESULTS AND DISCUSSION

Regarding to the results recorded in table (1), the concentration of nitrate in Damietta cheese samples was ranged from 35.44 to 150.62 ppm with a mean value of 87.62 ppm. In the examined samples of hard cheese, the minimum concentration of nitrate was 44.3 ppm and the maximum concentration of nitrate was 221.5 ppm with a mean value of 134.22 ppm. Also, it was found from the results that 13 samples of Damietta cheese and 16 samples of hard cheese have nitrate concentration above the permissible limit (50 ppm) recommended by Ministry of health, Egypt (1995). There was a highly significant difference at level ($P \leq 0.01$) between nitrate concentrations in both cheese types.

The occurrence of nitrate with high levels in the examined cheese types may be attributed to its presence with high concentrations in the original milk used for cheese making. The presence of nitrate in raw milk of lactating animals may be due to consumption of contaminated feed stuffs and water (**Yeruham et al., 1997**). Also, nitrate plays a certain role in the post-secretory contamination of milk, as in some cases, the cheese may be contaminated from residual water after washing and rinsing of collector containers (**Przybylowski et al., 1989**).

Moreover, the main source of contamination is the addition of nitrate salt for preservation of cheese (**WHO, 1992; Kozhev and Kozhev, 1994 and Massey, 1997**). Nitrate is added to control the production of gas and undesirable flavours by bacteria in a permissible range of cheese. In sevesal countries, nitrate permitted to be added to milk used for production of solid, semisolid and mould cheeses at level of 150-200 mg/L potassium nitrate (**Poulsen,1980**). In cheese making a supplementation of nitrate (15g/100kg) yields a contamination of 56 ppm immediately after addition, 3 weeks later the nitrate content was 30 ppm in the normally observed range (**Goodhead et al., 1976**).

The results recorded in table (2) showed that the nitrite concentration in Damietta cheese was

ranged from 0.42 to 2.30 ppm with mean value of 1.07 ppm. While nitrite level in hard cheese samples ranged from 0.52 to 5.26 ppm with a mean concentration of 2.11 ppm. In the other hand, 3 samples of Damietta cheese and 5 samples of hard cheese were contained nitrite concentration above the permissible limit (2.0 ppm). There was a highly significant difference at level ($P \leq 0.01$) between nitrite concentrations in both cheese types.

The presence of nitrite in cheese may be attributed to addition of nitrite salt as preservatives **(Dziezak, 1986)** or may be due to reduction of nitrate by microbial action **(MAFF, 1987 and Massey, 1997)**. The supplementation of nitrate to bulk milk (15g/100kg) yielded less than 1.0 ppm nitrite in cheese immediately after processing, dropping to 0.5 ppm after 4 - 5 weeks **(Goodhead et al., 1976)**. This is attributed to the unstable nature of nitrite which appear transiently as they are further transformed to other reductive products **(Warminska - Radyko et al., 1976)**.

Results recoded in table (3) revealed that the calculated daily intake of nitrate for adult person was 8.762 mg from consumption of Damietta cheese (100 gram/day) and 10.737 mg from consumption of hard cheese (80 gram/day) and these quantities representing 3.38 % and 4.14 % of Acceptable Daily Intake (ADI) recommended by **FAO/WHO (1996)**, respectively. While, the calculated daily intake of nitrite for adult person was 0.107mg from consumption of Damietta cheese (100 gram/day) and 0.168 mg from consumption of hard cheese (80 gram/day) and these quantities representing 2.54 % and 4.0 % of Acceptable Daily Intake (ADI) recommended by FAO/WHO(1996), respectively. From the results obtained in table (3), it was clear that the contribution of Damietta cheese and hard cheese to daily ingestion of nitrate and nitrite is some what significant and may constitute a possible health hazardous effect for man.

Nitrate (No_3) and nitrite (No_2) are natural components of many agricultural products. These compounds are widely used in food industries and can react with amines and amides to produce nitrosamines and nitrosamides in gastric fluid. These compounds readily induce tumor of the liver, oesophagus, kidneys, intestine, central nervous system and lymphoid system of laboratory animals **(Shields and Harris,1991)**. The principle route of nitrate exposure for people is food. Nitrate have relatively low toxicity to man and animals, while nitrites are more toxic to man and animals **(Bertelsen, 1979)**. The major clearance of nitrate from the blood occurs in the kidneys and renal clearance of nitrate to be in the range 50-90% of an oral dose **(Hartman, 1982)**. On the other hand, excessive intake of nitrate and nitrite in the diet may cause toxic effects since methaemoglobinaemia was produced by oxidation of hemoglobin by nitrite **(Chan, 1996)**. Moreover, exceeding doses of nitrite may increase the possibility of formation of most toxic and carcinogenic nitrosamines **(Walker, 1990 and Shields and Harris, 1991)**.

CONCLUSION AND RECOMMENDATION

From this study, it can be concluded that the examination of Damietta cheese and hard cheese indicate the presence of nitrate and nitrite at various levels in all examined samples. This may be due to addition of such chemicals as preservatives and colour stabilizers or from contamination of the original milk used for manufacture of such products. Nitrate concentration exceeded the recommended limits in most cheese samples, while nitrite exceeded the permissible limit in few samples. Moreover, the daily intake of nitrate and nitrite for adult person from consumption of Damietta cheese and hard cheese is some what significant in-comparing to ADI recommended by FAO/WHO(1996).

In order to protect the Egyptian human health from the hazardous effect of these chemicals, a regular and representative monitoring of preservatives in milk and all types of cheeses is recommended. Ascorbic acid could be applied with success in minimizing the concentration of nitrate and nitrite in the milk and food (**Scanlan, 1983 ; Shidlovskaya et al., 1990 and Walker, 1990**).

Table (1): Nitrate concentration (ppm) in examined cheese samples.

Type of samples	No. of + ve samples	Min.	Max.	Mean ± S.E	Permissible limit**
Damietta cheese	20	35.44	150.62	87.62 ± 9.85*	50
Hard cheese	20	44.30	221.50	134.22±13.78*	50

n = 20

* Highly significant differences at level (P≤ 0.01).

** Ministry of Health, Egypt (1995).

Table (2) : Nitrite concentration (ppm) in examined cheese samples.

Type of samples	No. of + ve samples	Min.	Max.	Mean ± S.E	Permissible limit**
Damietta cheese	20	0.42	2.30	1.07±0.13*	2.0
Hard cheese	20	0.52	5.26	2.11±0.29*	2.0

n = 20

* Highly significant differences at level (P≤ 0.01).

** Balcerska et al (1997).

Table (3) : Comparison of Acceptable Daily Intake (ADI) value of nitrate and nitrite with the calculated daily intake from cheese types.

Type of preservative	ADI[a] mg/70kg Person	Mean concentration (mg/kg) in present study		Calculated daily intake of presevative from consumption of cheese types			
				Damietta cheese (100g/day)[b]		Hard cheese (80g/day)[b]	
		Damietta cheese	Hard cheese	mg/day/person	%	mg/day/person	%
Nitrate	259	87.62	134.22	8.762	3.38	10.737	4.14
Nitrite	4.2	1.07	2.11	0.107	2.54	0.168	4.0

(a) FAO/WHO, Joint Expert Committee on food Additives, (1996).
(b) Daily consumption of soft cheese and hard cheese for adult person according to Nutrition Institute, Cairo, A.R.E. (1996).

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الملخص العربى

أجريت هذه الدراسة لاستبيان تركيز المواد الحافظة (النترات والنيتريت) فى عينات عشوائية من الجبن الدمياطى (٢٠ عينة) والجبن الجاف (٢٠ عينة) المنتجة محلياً والمجمعة من الأسواق المختلفة فى محافظة دمياط. وقد أوضحت النتائج وجود هذه المواد الحافظة بتركيزات مختلفة فى جميع العينات، وأوضحت النتائج أيضاً وجود مادة النترات بتركيز أعلى من الحدود المسموح بها فى عدد ١٣ عينة من الجبن الدمياطى وفى عدد ١٦ عينة من الجبن الجاف ووجود مادة النيتريت بتركيز أعلى من الحدود المسموح بها فى عدد ٣ عينات من الجبن الدمياطى وفى عدد ٥ عينات من الجبن الجاف. وأظهرت النتائج إنخفاض المأخوذ اليومى من النترات والنيتريت مقارنة بالمأخوذ اليومى المسموح به عالمياً عند إستهلاك الإنسان البالغ لهذه الأنواع من الجبن، وهذا وقد ناقشت الدراسة أخطار هذه المواد الحافظة على الصحة العامة وطرق الوقاية منها.