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The Occurrence of nitrate in Milk and Some Dairy Products

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Abstract:

The levels of nitrate in 45 of raw milk samples and 58 samples of soft cheese in Assiut vicinity were investigated. The mean levels in raw milk samples were 52.87±26.947 and 70.48 ±29.854 mg/l respectively, for samples collected during cold and warm months.

Kareish cheese of street vendors and from Arab-Elmadabigh region, where sewage water is used for irrigation and drinking of animals, contained 67.143 (22.0 -132.0), and 103.2(50.0-228.0) mg/l of nitrate, while Lab white soft cheese contained 75.57 (zero – 120.0) mg/l . The high level of nitrate content in both raw milk and soft cheese was much higher than the national and international recommended levels which lead to nitrosamines formation which constitutes a hazard to human health. Thus the use of such preservatives in milk and cheese must be strictly controlled to prevent the possible formation of the carcinogenic nitrosamines.

Introduction

Nitrate and nitrite occur widely in human and animal food stuffs. Such chemicals are added to foods such as meat and certain types of cheese internationally for their preservative effects. The content of nitrate is very high not only in processed food but also in natural ones (Kyriakidis *et al*, 1997) . The occurrence of nitrate in food may be considered hazardous because nitrates can be reduced to nitrite before ingestation, in saliva and in the gastrointestinal tract (Anonymous, 1981 and Kyriakidis et al. 1997). Nitrite may react in the stomach with secondary or tertiary amines and amides present in foods such as cheese or meat to form N- nitroso compounds which are potentially carcinogens. Generally, potassium or sodium nitrates are added to cheese milk to prevent the growth of gas- producing bacteria, causing blowing of the cheese, i.e. coliforms, at the beginning of the maturation period and *Clostridia butyricum*, which cause late blowing by virtue of their antimicrobial properties also, it can control undesirable color and taste. (Korenekova *et al.*2000). The nitrate may naturally be present in milk and the level of it depends on the quality of feeding materials (i.e. water, feeds) of cows. In many areas, water used in agriculture has relatively high concentrations of nitrates owing to pollution by fertilizers and different domestic

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effluents (Kyriakidis *et al*, 1997). Codex Standard for Cheese (2003) considers as safe and permits the use of sodium nitrate singly or in combination with potassium nitrate in different varieties of cheese in amount up to 50 mg/ kg, expresses as $NaNo_3$. An acceptable daily intake for nitrate of 3.7 mg nitrate / kg body weight was established by EU Scientific Committee for Food (1995). However, the low concentration of nitrate and nitrite in cheese does not cause health hazard for the consumer. Cheese production techniques when not correctly applied and failure to provide hygienic condition cause defects in cheese .For this reason, small farms may use nitrate to prevent cheese losses caused by microorganisms.

Common source of nitrate include fertilizers and manure, animal and feedlots, municipal wastewater and sludge, septic systems and N- fixation from atmosphere by legumes, bacteria and lightning.

The maximum contaminant level for US Environmental Protection Agency (EPA) of nitrate (NO_3) in drinking water was 45 mg/l which might cause methemoglobinemia in infants or blue baby syndrome. Methemoglobinemia in infant blood can not change back to hemoglobin, which normally occurs in adults. Also intakes of high level of nitrate are linked to gastric problems due to the formation of nitrosamines (carcinogenic compounds). Ruminant animals are susceptible to nitrate poisoning because bacteria present in the rumen convert nitrate to nitrite.(Self and Waskom , 2008)

The objectives of this study was to investigate the nitrate content in raw milk and white soft cheese (Kareish and Domiati) mostly consumed in Assiut Governorate and to evaluate their compliance with international regulations.

Materials and Methods

The investigated milk was raw milk samples collected from individual cows during cold and warm months over one year from the herd of the Faculty of Agriculture, Assiut University. Samples were collected in clean dry and sterile jars with sewer top. Samples of white soft cheese (Domiati type) were obtained from the laboratory of Dairy Department, Faculty of Agriculture, Assiut University. Samples of Kareish cheese were house made, collected from Arab- Elmadabigh region near Assiut city, where waste water is used for irrigation and animal drinking, and from street vendors at Assiut vicinity. The incidence of nitrate in milk and soft cheese samples were determined according to Thomas, et al. (1980).

A dilute of 40 ml of liquid milk or 8 g of white soft cheese, to 150 ml, warmed to 50 ^{0}C, clarified by the addition of 10 ml of 12% ($ZnSO_4$) solution and 10 ml of 0.5 N (NaOH), maintained at 50 ^{0}C for 10 minutes, cool, diluted to 200 ml, filtered and nitrate was determined colorimet-

rically by method described by Thomas, *et al.* (1980). The dilutions were applied to the Spectro UV-VIS RS Spectrophotometer and measured at 530 nm wave length. To convert to nitrate ion (NO_3) was multiplied by 4.428 Thomas, *et al.* (1980).

Statistical analysis:

Data were programmed in a computer using statistical computing software (SAS 1989) for statistical analysis.

Results and discussions

Nitrates have low toxicity to man and animals, but it may be converted to more toxic nitrates during storage and technological processes (WHO 1977). The level of nitrates and nitrites in milk and milk products varies considerably depending on the degree of environmental contamination, seasonal factors, whether pasture or grazing was used , methods used for the primary treatment and processing of milk , milk processing technology and analytical methods used (Shidlovskaya and Knyazeva1995).

The recorded data in Table (1) indicated that the mean concentrations of nitrate in raw milk during the cold and the warm months were 52.87±26.947 mg / l (ranged from 0.0 – 142.0) and 70.48±29.854 mg /l (ranged from 0.0 to 198.0).These results lie within the permissible limits of Stephany *et al.* (1978) 50mg /kg. Hence, it reflects clearly that water and fodder of dairy animals are subjected to nitrates pollution due to these compounds reach the milk production by dairy animals via drinking water and fodder. (W.H.O 1977). However higher values were stated by Trif et al. (1992).They observed that the mean values of nitrates in raw and pasteurized milk were 102.57 and 127.07 mg/l respectively. On the other hand our findings were higher than those obtained by Tsyganenko, *et al,* (1991) 27.2 mg/l. Deryagina, et al. (1993) 20 mg/kg and Baranova, *et al* (1998)3.21 mg/l.

Also lower values were found by Shidlovskaya(1986) 1.78 mg/kg and El-Hoshy S.M.(1994) 3.9 mg/kg in examined raw milk samples. There was a significant variation in nitrate content in raw milk samples between cold and warm menthes by using students "t" test Table (5). These results were in agreement with Peichevskii and Mikhailova(1993), who observed a considerable monthly variation in nitrate and nitrite content in raw milk, also nitrate contents were generally highest in the spring / summer period and lowest in autumn winter .

Approximately 21.74 % of cold months milk samples ranged from 100-150 mg/ l. On the other hand approximately 9.1 % of warm month's milk samples ranged from 150-200 mg/l (Table 2) (Fig 1). These may be attributed to both external sources such as contamination by nitrate fertilizers, forage and agricultural drinking water.

The mean values of nitrates in Kareish cheese samples

Table (1): The incidence of nitrate content in raw milk samples during cold and warm months.

Observation	Cold months*			Warm months **			General means
	Min.	Max.	Mean	Min.	Max.	Mean	
Nitrates (mg/L)	zero	142	52. 87± 26. 947	zero	198	70. 48± 29. 854	61. 675

***Average of 23 Samples.**
**** Average of 22 Samples.**

Table (2): The intervals of nitrate content in raw milk samples during cold and warm months.

Intervals	Cold months		Warm months	
	No	%	No	%
Zero-50	13	56.52	10	45.455
50-100	5	21.74	5	22.73
100-150	5	21.74	5	22.73
150-200	-	Zero	2	9.091
Total	23	100%	22	100%

Fig (1): The intervals of nitrate content in raw milk samples during cold and warm months.

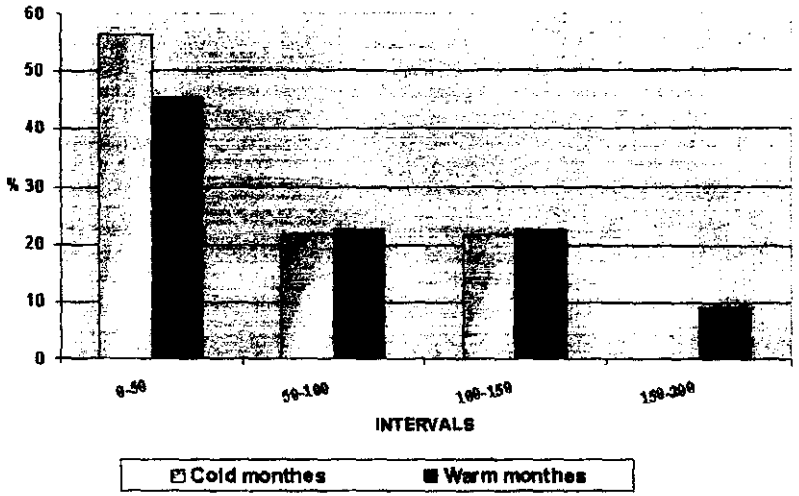

Table (3): Concentration of nitrate content in Kareish and Domiati cheese samples Collected from Assiut Vicinity (mg/kg).

Cheese type	Min.	Max.	Mean
Kareish cheese * House made (Street vendors)	**22**	**132**	**67.143 (C)**
Kareish cheese ** House made (Arab El-Madabigh region)	**50**	**228**	**103.2 (A)**
Domiati cheese * (Lab. Mad)**	**Zero**	**120**	**75.57 (B)**

*Average of 14 samples. **Average of 37 samples.
***Average of 7 samples.

collected from street vendors and Arab – Elmadabig region were 67.143 (from 22.0 to 132.0) and 103.2 (from 50.0 to 228.0) respectively (Table 3) . Nearly similar concentrations were reporteded by El- Hoshy (1994) 62.5 mg/kg. However lower values of nitrates in cheese samples have been reported by Garcia, *et al.* (1983) 27.65 and Borawska, *et al.* (1997) 50.0mg/kg (rennated cheese). However higher values have been reported by Diraman (1993) (0.19-191.64 ppm).The variation could be due to the age of the cheese as nitrates decrease during ripening and storage Zerfiridis and Manolkidis (1981). However the high recorded values of nitrate could be attributed to not fully ripened cheese and undue addition of nitrates to the cheese milk Topcu, et al. (2006).

In case of Domiate cheese samples (Labe. made) the mean concentration was 75.57 (from zero to 120.0). These finding run parallel to those obtained by Trif et al. (1992) and El-Hoshy (1994) who found that the mean values of nitrates in soft cheese and full cream soft cheese were 62.5 and 71.20 mg/kg respectively.

Also approximately 14.3% of Kareish cheese samples (street vendors) ranged from 100-150 mg /kg. However 5.41% of Kareish cheese samples (Arab Elmadabigh) ranged from 200-250 mg/kg. Meanwhile, 14.29% of Domiati cheese samples ranged from 100-150, (Table4, Fig2).

There was a very significant variation in nitrate content between groups of cheese samples owing to sampling location, Table (6). These may be attributed to the presence of nitrate naturally in milk and the pollution of feeding materials (i.e. water, feeds) by fertilizers and different domestic effluents Topcu, et al. (2006). According to Turkish Food Codex (2002) the maximum permitted residual value for nitrate was 10 mg/kg for cheese.

This work indicated that milk and cheese containing nitrates above the permissible limits which constitute a major risk for human health. The excessive dose of these chemicals may increase the possibility of nitrosamines formation by reaction with secondary amines (El- Hoshy 1994), in this respect White (1975) recommended that the acceptable daily intake of nitrates and nitrites were 99.8 mg and 11.22 mg / person respectively. Therefore, the preventive measures for minimizing the pollution of milk and milk products with nitrates and nitrites including,

-Prevention of environmental pollution and hygienic disposal of industrial effluents.

-Animal feed in polluted area as well as drinking water should be controlled.

-Regular examination of milk and milk products and their load for nitrates and nitrites should be evaluated according to the international guide lines.

Table (4): The intervals of nitrate content in Kareish and Domiate cheese samples Collected from Assiut Vicinity (mg/kg).

Intervals	Kareish cheese * House made (Street vendors)		Kareish cheese ** House made (Arab El-Madabigh region)		Domiate cheese *** (Lab. Mad)	
	No	%	No	%	No	%
Zero-50	**5**	**35.7**	**22**	**59.46**	**1**	**14.29**
50-100	**7**	**50.0**	**10**	**27.03**	**5**	**71.43**
100-150	**2**	**14.3**	**2**	**5.41**	**1**	**14.29**
150-200	**-**	**-**	**1**	**2.70**	**-**	**-**
200-250	**-**	**-**	**2**	**5.41**	**-**	**-**
Total	**14**	**100**	**37**	**100**	**7**	**100**

*Average of 14 samples.
**Average of 37 samples.
***Average of 7 samples.

Fig (2): The intervals of nitrate content in Kareish and Domiate cheese samples Collected from Assiut Vicinity (mg/kg).

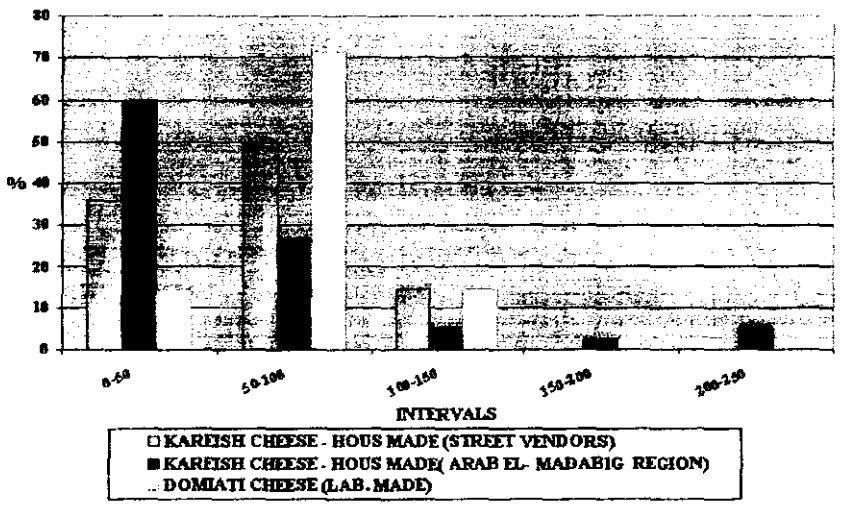

Table (5): Paired Samples Statistics.

Seasons	Mean	N	Std. Deviation	Std.Error Mean.	t.	d.f	Sing (2-tailed)
Cold samples	52.87	23	26.947	5.619	2.211*	22	0.038
Warm samples	70.48	23	29.854	6.225			

T.test

Table (6): One way anova.

	Sum of squares	d.f	Mean Square	F	Sig.
Between groups	4197.443	2	2098.722	7.242	0.002**
Within groups	15938.473	55	289.790		
Total	20135.916	57			

-Minimizing the use of phosphates and sludge for land fertilization as possible.

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تواجد النترات فى اللبن و بعض منتجات الألبان

طارق حمدى محمد

قسم الأغذية الخاصة - معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية.

تم اختبار تلوث اللبن الخام وبعض منتجات الألبان فى بعض مناطق محافظة أسيوط بالنترات فى 45 عينة من اللبن الخام و 58 عينة من الجبن الأبيض الطرى .حيث وجد ان مستوى النترات فى اللبن الخام خلال أشهر السنة الباردة و الدافئة يعادل 26.947± 52.87 و 29.854± 70.48 جزء في المليون على الترتيب وان هناك فروق معنوية بين عينات اللبن خلال الأشهر الدافئة و الباردة و قد اعزى وجود مستويات عالية من النترات فى عينات اللبن لاحتمال وجود تلوث للمياه وا للأعلاف المستخدمة فى سقاية وتغذية الحيوان – إضافة للاستخدام المفرط للأسمدة و المخصبات الزراعية .

اما بالنسبة للجبن القريش سواء المباع بالأسواق او ذلك المنتج فى منطقة عرب المدابغ حيث يتم استخدام مياه الصرف الصحى الغير معالجة فى الرى و سقاية الحيوان فوجد ان متوسط مستوى النترات به 67.143 (22الى 132 ملليجرام/ لتر) و 2 ،103 (50الى 228 ملليجرام /لتر) على نفس الترتيب . بينما كان مستواه فى الجبن الأبيض الدمياطى المصنع فى المعمل – قسم الالبان – كلية الزراعة- جامعة أسيوط 57، 75 (صفر الى 228 ملليجرام/ لتر) وان هناك فروق معنوية جدا بين المجموعات الثلاث راجع لاحتمال حدوث تلوث للمياه و الأعلاف المستخدمة فى سقاية وتغذية الحيوان واستخدام مياه الصرف الصحى و الزراعى الغير معالجة كما فى منطقة عرب المدابغ .

وقد أوضحت النتائج وجود مستويات عالية من النترات فى كل من اللبن و الجبن الأبيض طبقا للمعايير و الحدود الدولية مما يساعد على تكوين النيتريت و النيتروز امين الذى يعتبر احد مسببات السرطان.