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A Study on the Aetiological Factors of Bilharzial Bladder Cancer in Egypt—2 Nitrosamines and their Precursors in Egyptian Dairy Products

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Abstract—*Milk, yoghourt and four types of cheese consumed by Egyptians have been analysed for the presence of nitrosamines, and that formed after deliberate chemical nitrosation. Two, three, seven out of ten samples of milk, cottage cheese, white cheese and six out of 9 samples of salted cheese respectively were contaminated with nitrosamines at* $g \times 10^{-9}$ *level. Deliberate chemical nitrosation produced increasing amounts of both volatile and nonvolatile nitrosamines especially in blue cheese and salted cheese (cottage cheese stored in high concentrations of sodium chloride). The daily consumption of salted cheese and drinking water with high nitrate content, beside a heavily infected urinary bladder due to bilharzial infestation, may contribute to the observed high incidence of bladder cancer among Egyptian farmers.*

INTRODUCTION

THE CORRELATION between *N*-nitrosamine levels and morbidity rates in certain environments could throw some light on the aetiology of cancer.

Nitrosamines could be easily formed from their precursors, nitrite and amines in organisms [1]. Therefore the occurrence of free amines in the human environment will be also important as the presence of nitrosamines.

Detection of nitrosamines in a number of foodstuffs had been reported [2–4]. Cheese contains amines, if consumed together with foodstuffs rich in nitrite, toxic doses of N-nitroso compounds could occur in the organism [5, 6]. Milk and its products have been investigated for nitrosatable amines, since they are widely consumed by all age-groups and classes of many populations [7].

Data presented in this paper deal with the analysis of Egyptian dairy products consumed by different Egyptian population. Results will be correlated with the high incidence of bladder cancer among Egyptian farmers for whom diary products represent a major source of their protein diet.

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MATERIALS AND METHODS

Samples of pasteurised milk, yoghourt, white cheese (full cream), cottage cheese (defatted), salted cheese (cottage cheese stored for 1 to 2 yr in high concentrations of NaCl) and blue cheese were investigated. A total of 10 samples of each type have been analyzed.

Extraction of nitrosamines

Total and volatile nitrosamines were extracted with dichloromethane (DCM) before and after chemical nitrosation in 0.1 M acetate buffer, pH 3.0 (unless otherwise stated) in the presence of 0.1 M sodium nitrite according to the scheme on p. 295.

DCM extracts were washed with 50 ml 5% K_2CO_3 dried overnight on anhydrous Na_2SO_4 and concentrated to 1 ml.

Estimation of nitrosomines

The method of Eisenbrand and Preussman [8] was adapted and data were calculated as *N*-nitrosopyrrolidine (mol. wt 100).

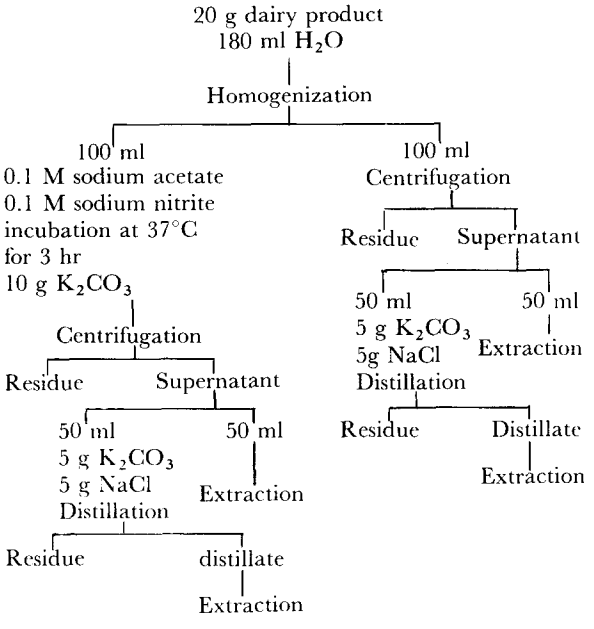
RESULTS

Nitrosamines (NAs) content of milk and dairy products are shown in Table 1. NAs

Table 1. *The level of endogenous nitrosamines in the Egyptian dairy products*

Sample No.	Fresh milk		Yoghourt		Cottage cheese		White cheese		Salted cheese		Blue cheese	
	Total	Volatile	Total	Volatile	Total	Volatile	Total	Volatile	Total	Volatile	Total	Volatile
1	9.7	ND	ND	ND	ND	ND	39	ND	70.0	40.0	ND	ND
2	ND	ND	ND	ND	86.8	ND	115.8	ND	40.0	ND	ND	ND
3	ND	ND	ND	ND	ND	ND	57.9	38.6	20.0	ND	ND	ND
4	ND	ND	ND	ND	ND	ND	38.6	ND	77.0	ND	ND	ND
5	ND	ND	ND	ND	ND	ND	57.9	38.6	39.0	39.0	ND	ND
6	ND	ND	ND	ND	135	ND	ND	ND	20	20	—	—
7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	—	—
8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	—	—
9	3.2	ND	ND	ND	ND	ND	159.9	ND	ND	ND	—	—
10	ND	ND	ND	ND	40	20	115.8	ND	—	—	—	—
Mean	6.5	ND	ND	ND	87.3	20.0	67.6	37.6	41.3	33.0	0.0	0.0
%	20.0	0.0	10.0	0.0	30.0	10.0	80.0	20.0	66.6	33.3	0.0	0.0

Results expressed as $g \times 10^{-9}$ nitrosamines calculated on the basis of *N*-nitrosopyrrolidine.
Mean and per cent is calculated for positive samples only.



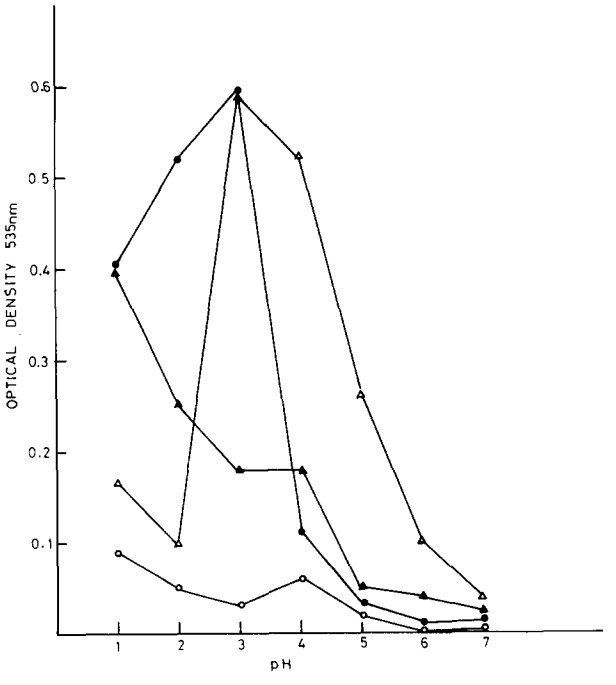


Fig. 1. Effect of pH *on the rate of chemical nitrosation of Egyptian dairy products.*
Salted cheese, ●—●
White cheese, ▲—▲
Cottage Cheese, ○—○
Blue cheese, △—△

could not be detected in all 5 samples of blue cheese and the 10 yoghourt samples tested. Two milk samples were contaminated with NAs at a low level (3.2 and 9.7 $g \times 10^{-9}$). A higher level of contamination has been detected in 3 out of 10 samples of cottage cheese (range 40–135 $g \times 10^{-9}$), 7 out of 10 samples of white cheese (range 38.6–159.9 $g \times 10^{-9}$) and 6 out of 9 samples of salted cheese (range 20–77 $g \times 10^{-9}$). On the other hand volatile NAs have been only detected in two white cheese and three salted cheese (range 20–40 $g \times 10^{-9}$).

The optimal pHs for chemical nitrosation of different dairy products were investigated over a wide range of pH values (Fig. 1).

Amines present in white and cottage cheese were nitrosated optimally at pH 1 and 4 while the blue and salted cheeses at pH 3.0.

As shown in Table 2, deliberate nitrosation of milk and dairy products yielded large amounts of extractable NAs. Blue cheese showed the highest level of extractable NAs more than 60% of which were volatile. Salted and white cheese contain less NAs, only about 15% of which are volatile. The lowest level of NAs was detected in cottage cheese with more than 50% volatile. Compared with cheese, milk and yoghourt contain much less NAs and volatile NAs were detected in only one sample of each out of ten samples tested.

DISCUSSION

Analysis of food in different countries proved that NAs are present at a level of g $\times 10^{-9}$ in a variety of items [2–4, 9]. The presence of nitrite and amines in human diet may also represent a health hazard [10–13]. In the present investigation milk and dairy products in Egypt have been analysed for the presence of NAs and chemically nitrosatable amines. Milk, yoghourt, white and blue cheese are consumed mainly in towns, whereas cottage and salted cheese are consumed , as a main daily diet, by farmers. *N*-nitrosamines have not been detected in blue cheese whereas the majority of white cheese was contaminated with NAs. Deliberate chemical nitrosation produced very high amounts of NAs, indicating presence of plenty of amines available for nitrosation, especially in blue cheese. Fortunately, blue cheese is not a common diet and is consumed only by the richer class who drink tap water with low nitrate content. On the other hand cottage cheese and salted cheese are almost daily consumed by farmers. It was of interest to notice that storage of cottage cheese in salt increased the incidence of contamination with NAs and increased the amount of amines that could be nitrosated (Tables 1 and 2). Lactic fermentation bacteria, present in many foodstuffs and dairy products, produce organic amines [14]. Information on the level of nit-

Table 2. The level of nitrosamines, after chemical nitrosation of Egyptian dairy products

Sample No.	Fresh milk		Yoghourt		Cottage cheese		White cheese		Salted cheese		Blue cheese	
	Total	Volatile	Total	Volatile	Total	Volatile	Total	Volatile	Total	Volatile	Total	Volatile
1	193.0	23	67.6	ND	1042.2	617.6	3860	212.0	540.0	231.0	14861	10615
2	87.0	ND	193.0	ND	714.1	231.6	482.5	68.2	510.0	70.0	7334	4323
3	ND	ND	115.8	ND	443.9	289.5	2895.0	443.9	579.0	116.0	3551	386
4	28.8	ND	57.9	ND	965.0	482.5	38.6	ND	386.0	347.0	4632	2238
5	38.4	ND	77.2	ND	96.5	ND	67.6	ND	2026.5	97.0	3860	3474
6	36.6	ND	57.9	ND	115.8	96.5	308.8	38.6	1042.2	463.2	—	—
7	144.8	ND	38.6	38.6	502.0	ND	656.0	57.0	7237.5	250.9	—	—
8	ND	ND	57.9	ND	810.6	386.0	67.6	ND	1544.0	ND	—	—
9	29.3	ND	96.5	ND	386.0	39.0	96.5	57.9	3860.0	ND	—	—
10	ND	ND	96.5	ND	600.0	250.9	772.0	695.0	—	—	—	—
Mean	74.2	23.0	85.9	38.6	567.1	299.2	924.5	161.0	1814.1	225.0	6847.0	4207.0
%	70.0	10.0	100.0	10.0	100.0	80.0	100.0	70.0	100.0	77.8	100.0	100.0

Legends as Table 1.

rate, nitrite amines in foodstuffs and on the role of micro-organisms in NAs formation could help avoid the diet which could expose the human to high doses of NAs [15].

It had been reported by many investigators [16–18] that the appearance of *N*-nitrosamines in blood and milk of lactating animals is associated with exposure of these animals to *N*-nitrosamines.

The parent amines of three of the NAs characterised after the treatment of milk and cheese with high levels of nitrite, namely nitrosodimethylamine (NDMA) nitrosopyrrolidine (NPy) and nitrosopiperidine (NPip), have been reported to occur in normal urines [19].

Therefore, in bilharzial infested farmers, with associated urinary bacterial infection, who drink canal water, expected to contain high nitrate levels due to the wide-spread use of nitrate fertilizers, and plenty of amines in their diet from dairy products such as salted and cottage cheeses consumed daily, there will be a greater possibility for NAs formation in their bladders. This may represent a major factor associated with the induction of bilharzial bladder cancer among Egyptian farmers which needs further investigation.

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