

## DETERMINATION OF NITRATE AND NITRITE LEVELS IN IMPORTED COW'S BUTTER AND THEIR PUBLIC HEALTH HAZARDS

By

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### SUMMARY

Forty random imported samples of natural pasteurized cow's butter (20 each of salted and unsalted samples) were collected from different supermarkets at Damietta City for analysis colorimetrically by using spectrophotometer to determine the level of nitrate and nitrite. The obtained results revealed that the mean values of nitrate in salted and unsalted samples were found to be  $0.795 \pm 0.124$  and  $0.352 \pm 0.062$  mg/kg respectively, while the average values of nitrite in salted and unsalted samples were  $0.011 \pm 0.0002$  and  $0.012 \pm 0.0002$  mg/kg respectively. Presence of nitrate and nitrite levels in butter may be ascribed to various sources rather than preservatives. Although butter must be free from these components, the obtained concentrations may favor carcinogenic nitrosamines formation which constitutes a hazard for human health. The public health hazards have been discussed.

### INTRODUCTION

In Egypt, large quantities of milk products are imported from different countries. Their amounts are imported to satisfy the requirements of Egyptian peoples specially infants and children. Price data reported by **Ministry of Foreign Trade, Egypt, (2002)** indicated that the total cost for importation of milk; milk products; eggs of birds and natural honey during 2001 was \$143 million. Based on this information, chemical investigation of different imported dairy products is highly required specially heavy metals and preservatives.

Butter was one of the first dairy products manufactured by humans and has been traded internationally since the 14<sup>th</sup> century (**Anderson, 1986** and **Varnam & Sutherland, 1994**). From ancient times through the latter part of the 1800s, cream was obtained from milk by gravity separation. In the 1850s, creameries began producing butter on a small scale. Large-scale manufacture only became possible after development of the mechanical cream separator in 1877s (**Varnam & Sutherland, 1994**).

Butter is the food product usually made exclusively from milk, cream or both, butter contains about 80% milk fat, 17% moisture, 1% carbohydrate and protein and 1.2-1.3% sodium chloride. Many countries allow sodium chloride and lactic cultures as the only non-milk additives in butter (**Milner, 1995**). Some countries allow neutralization of cream and addition of natural coloring agents to adjust for seasonal variation in colorant in the cream (e.g. annatto, carotene, and turmeric). Other countries allow the use of potassium sorbate and sodium benzoate as preservatives. However, countries such as United States; United Kingdom; France and Luxembourg prohibit preservatives in butter (**Kornacki et al., 2001**). Addition of 0.1% potassium sorbate inhibited growth of coliforms and molds, the inhibitory effect was enhanced when 2% salt was added along with 0.1% potassium sorbate (**Kaul et al., 1979**). The last observation is that butter is a good source of vitamin A (**Al-Ashmawy, 2002**).

Nitrates were approved by U.S. Food and Drug Administration in food including milk and milk products (**Dziezak, 1986**). Although nitrates later reduced to nitrites that have occasionally been added to milk as a preservatives or deodorizers (**Wood, 1932**). Nitrates have relatively low toxicity to man and animals, but it may be converted to more toxic nitrite during storage and technological processes (**WHO, 1977**). Nitrites as a preservative are dangerous owing to their action on blood haemoglobin by oxidation to methaemoglobinemia, infant cyanosis "blue baby disease" (**ECETOC, 1988**). The contents of nitrates and nitrites in milk and milk products have been studied by many investigators (**Stephny et al., 1978; Bertlson, 1979 and Trif et al., 1992**).

Recently, it has become common practice to incorporate nitrates and nitrites in processed food as preservatives and color stabilizers. Therefore, nitrates are used at the farm for the control of sporeforming bacteria in milk and milk products (**Siva et al., 1994**). Also in several European countries nitrates permitted to be add to raw milk (**Poulsen, 1980**). Furthermore, nitrate and nitrite may reach to milk products through original raw milk for manufacture and other sources.

Therefore, it is very important from the public health of view to determine the levels of nitrate and nitrite in imported natural, pasteurized cow's butter at Damietta City.

## MATERIALS AND METHODS

### **Sampling:**

Forty samples (20 each of salted and unsalted natural, pasteurized cow's butter samples) were collected from different supermarkets at Damietta City during summer season 2003. the samples were collected from their original packages; labeled and transferred to the laboratory for analysis. Analysis was carried out according to the method described in **A.O.A.C. (1990)**.

### **Preparation of samples:**

#### **A-Softening of samples:**

20 gm of each sample were thoroughly mixed with 180 ml distilled water in water bath at 40 °C for 15 minutes.

#### **B-Extraction of samples:**

Extraction of fat from each sample was carried out, where the liquid mixture was mixed by swirling and warmed to 60 °C in water bath, then 10 ml of zinc sulfate solution (12%) and 10 ml of NaOH (0.5N) were added, the content was mixed and held at 60 °C for 10 minutes. Zinc hydroxide that formed is the most satisfactory precipitating agent for milk fat and protein removal (**Garrison, 1935 and Manning et al., 1968**). After cooling to room temperature, the solution was decanted through Whatman No. 42 and sufficient amount of filtrate collected (100 ml) in a volumetric flask for subsequent analysis.

### **Determination of nitrate and nitrite:**

These are based on the well-known, selective, diazotization-coupling reaction in acidic media to form an azo dye or other nitrosoderivatives (**Kamm et al., 1965 and Manning et al., 1968**).

### **Procedure:**

#### **1-Determination of nitrate (according to APHA, 1960) as the following:**

In a flask of 100 ml capacity, 1 ml of the clear filtrate was added to 10 ml of 64% H<sub>2</sub>SO<sub>4</sub>, then 0.1 ml of brucine solution 5% in acetic acid and finally one drop of conc. HCL was added to the mixture. The mixture was heated in boiling water bath for 10 minutes till the appearance of yellow color. The blank was set and the samples were measured colorimetrically using spectrophotometer [**Jenway 6105-England**] at wavelength 410 nm. The concentration of nitrate was estimated from a constructed standard calibration curve. The obtained results were recorded.

## 2-Determination of nitrite (according to APHA, 1985) as the following:

To 50 ml of the clear filtrate, one ml sulphamic acid reagent was added. After 3-10 minutes one ml  $\alpha$ -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 color. The samples were measured colorimetrically using spectrophotometer [Jenway 6105-England] at wavelength  $510 \text{ nm}$  against a reagent blank. The concentration of nitrite was estimated from a constructed standard calibration curve. The obtained results were recorded.

## RESULTS AND DISCUSSION

Nitrates have been used extensively in Europe and Canada for prevention of the bacterial growth as *Bacterium coli* and sporeforming bacteria in milk and milk products (**Davis, 1955** and **Fedorov & Bondarenko, 1955**). Nitrates have relatively low toxicity to man and animals, but it may be converted to more toxic nitrites during technological processes and storage (**WHO, 1977**).

Recorded data in tables (1&2) revealed that all examined salted natural pasteurized cow's butter samples, proved to be polluted with nitrates, the mean concentration was  $0.795 \pm 0.124 \text{ mg/kg}$  with maximum concentration of 2.570 and minimum concentration of 0.310 mg/kg, while 15 (75%) of unsalted natural pasteurized cow's butter samples polluted with nitrates, the mean concentration was  $0.352 \pm 0.062 \text{ mg/kg}$  with maximum concentration of 0.930 and minimum concentration of 0.221 mg/kg, although these results lie within the permissible limit (50 mg/kg in milk and milk products) that mentioned by **Stephny et al., (1978)** but the only allowable preservative is sodium chloride salt which must not exceeded 3% to enhance the flavour and improves the keeping quality of butter (**Al-Ashmawy, 2002**).

Nitrites are more toxic than nitrates to man and animals, out of 20 salted natural pasteurized cow's butter samples, 16 (80%) have nitrite levels were found to be ranged from 0.004 to 0.030 mg/kg with mean value  $0.011 \pm 0.002 \text{ mg/kg}$  (table, 1) while 19 (95%) of unsalted natural pasteurized cow's butter samples, were contained nitrite concentrations with mean value  $0.012 \pm 0.002 \text{ mg/kg}$  with maximum concentration of 0.033 mg/kg (table, 2) according to the permissible limit of **Bertelsen, (1979)**(2 mg/kg milk and milk products) may investigation was recorded under limits.

In available literatures there is no data overlap fairly with may findings for salted and unsalted natural pasteurized cow's butter.

Bovine milk in the natural state has been reported to be free from material traces of nitrates and nitrites (**Davis and Macdonald, 1952**). The presence of nitrates and nitrites in milk and manufactured dairy products are generally contributable to other sources. These compounds reach milk via drinking water and feedstuffs that were given to dairy animals (**WHO, 1977** and **Yeraham et al., 1977**). In addition to post secretary pollution of milk and milk products from incompletely removed water used for washing and rinsing of containers (**Przybylowski et al., 1989**). Washing of butter granules during working, with water sources polluted by nitrates (**Varnam & Sutherland, 1994**). Most of municipal water supplies contain parts per million levels of nitrates (**Davis & Macdonald, 1953; APHA, 1965** and **Kamm et al., 1965**). Chemical impurity of salts used in producing slurry (**Varnam & Sutherland, 1994**).

Nitrate contents of milk and dairy products were sometimes abnormally high because of industrial thermal processing increase the nitrates like (**Guingamp and Linden, 1980**).

The level of nitrites in butter may be probably a sequence of microbial activity, this hold the view reported by **Wolff & Wasserman, (1972)**, who found that nitrite reducing bacteria may increases nitrite level in some food products by reduction of nitrate.

This work clarifies that the natural pasteurized cow's butter containing nitrate and nitrite levels which constitute a risk for human and the possibility of nitrosamines formation (potentially cancerous substance) by reaction with secondary and tertiary amines (**Haworth & Hill, 1971; Gray et al., 1979; Scanalan, 1983 and Bernal et al., 1999**). Nitrosamines were over all the most toxic and carcinogenic compounds (**Magee & Barnes, 1967**). Therefore, strict control measures on using these chemicals should be adopted in order to minimize the hazards for human health specially infants and children who are more sensitive than adults to these compounds.

Table (1): Statistical analytical results of nitrate and nitrite concentrations in examined imported salted natural pasteurized cow's butter samples (n= 20).

Preservatives	Salted natural pasteurized cow's butter				
	Positive samples		Concentrations (mg/kg sample)		
	No.	%	Minimum	Maximum	Mean $\pm$ S.E.
Nitrate (NO <sub>3</sub> )	20	100.00	0.310	2.570	0.795 $\pm$ 0.124
Nitrite (NO <sub>2</sub> )	16	80.00	0.004	0.030	0.011 $\pm$ 0.0002

Table (2): Statistical analytical results of nitrate and nitrite concentrations in examined imported unsalted natural pasteurized cow's butter samples (n= 20).

Preservatives	Unsalted natural pasteurized cow's butter				
	Positive samples		Concentrations (mg/kg sample)		
	No.	%	Minimum	Maximum	Mean $\pm$ S.E.
Nitrate (NO <sub>3</sub> )	15	75.00	0.221	0.930	0.352 $\pm$ 0.062
Nitrite (NO <sub>2</sub> )	19	95.00	0.005	0.033	0.012 $\pm$ 0.0002

#### Public health significance:

Chemical pollution with special reference to nitrates and nitrites in milk and milk products possess a public health hazards, generally milk contain nitrates and nitrites should not be directed to industrial manufacturing, Nitrates were converted in the digestive tract into toxic nitrites which reduce the capacity of blood to carries oxygen (**Arms, 1989**). Moreover, infants and children are more sensitive than adults to these compounds.

Toxicological effects of nitrates and nitrites in different mammalian species are well documented and includes carcinogenesis; hepatotoxicity; impairment of reproductive

function; endocrine disturbances; growth retardation; destruction of vitamin A; methaemoglobinemia (MetHb) and impairment of certain defense mechanisms (**Jahries et al., 1986**).

Milk and milk products account for 5% of average human dietary intake of nitrates and nitrites (**Shildlovskaya and Knyazeva, 1995**).

Nitrites as preservatives in food can be dangerous owing to their action on blood haemoglobin, and to possible formation of nitrosamines (potentially cancerous substance) (**Bernal et al., 1999**).

Most of the nitrosamines compounds are carcinogenic and in addition some exhibit mutagen C-embryopathic and teratogenic effects (**Druckrey et al., 1967** and **Magee and Barnes, 1967**). Absorbed nitrate is excreted mostly unchanged in the urine, but some reduced by bacteria to nitrite and which was taken up in the blood to react with haemoglobin forming methaemoglobinemia (infant cyanosis or “blue baby disease”) (**ECETOC, 1988**). Methaemoglobinemia (infant cyanosis or “blue baby disease”) in infants caused by high concentration of nitrates intake (**Davis and Cornwell, 1985**).

Methaemoglobinemia symptoms are seen when about 20% of haemoglobin is converted into methaemoglobin, death occurring when the level reaches about 80% (**London et al., 1967**).

Infants suffered from “blue baby disease” may seem healthy but showed intermittent signs of blueness around the mouth, the hands and feed, also they may had episodes of breathing trouble, some cases an infant may had a peculiar lavender color and showed little distress. Blood samples from such cases appear chocolate brown and don’t turn pink when exposed to air, by increase the methaemoglobin level infants expressed a market lethargy, excessive salivation and unconsciousness. Convulsions and death may occur at extreme methaemoglobin levels (**Skipton and Hay, 1998**).

Exogenous and endogenous nitrosamines exposure in food may play a role in development of hepatocellular carcinoma (HCC) (**Mitacek et al., 1999**).

More risk of gastric cancer associated with high nitrate exposure (**Hill et al., 1973**).

Consumption of food and beverages containing nitrates; nitrites and N-nitrosodimethylamines were associated with increased risk of upper aerodigestive tract (laryngeal; esophageal and oral) cancer (**Rogers et al., 1995**).

Gastrointestinal cancers, mainly oesophageal, gastric, pancreatic and large bowel cancer, account for about 32% of all cancer deaths in England and Wales. Nutritional factors have been implicated in the cause of each cancer and probably act by promoting the effect of carcinogenic substances taken in diet or produced in the gut. Gastric cancer for example may be due to nitrosamine production in the stomach. This enhanced by readily available sources of nitrate and nitrite (**Cummings, 1978**).

N-nitroso compounds can induce colorectal cancer in humans (**Knekt et al., 1999**).

Nitrate and nitrite are significant in the development of endemic upper gastrointestinal (esophageal and gastric) cancer (**Turkdogan et al., 2003**).

Congenital malformations principally of the central nervous system, (CNS) were due to high nitrate intake (**Scragg et al., 1982**).

High nitrate levels in water and feed lead to reduced vitality and increased stillbirth, low birth weight and slow weight gain in man and livestock (**N.R.C., 1972**).

## CONCLUSION AND RECOMMENDATION

It can be concluded from the present investigation that analysis of imported natural pasteurized cow's butter indicates its pollution with nitrates and nitrites in most examined samples. Although, the only permissible preservative of butter is sodium chloride, this may be attributed to different sources of pollution: (i) from the original collected farm, bulk, tank, raw milk by addition as preservatives or via drinking water and feedstuffs which used by lactating cows; (ii) during manufacturing processes of butter through utensils; washing water at working; added salts and thermal effects may increase the levels of nitrate and nitrite.

In order to protect Egyptian human from the hazard effects of these chemicals in imported dairy products the following recommendations should be applied:

- Strict analysis of imported milk and milk products at different Egyptian ports and presence of these chemicals in such products above the recommended permissible limits should be refused and return to the original exported countries.
- Egyptian Standards must conclude the permissible limits of nitrate and nitrite in dairy products.
- Hazard Analysis and Critical Control Point (HACCP) are necessary to ensure the safety and prolong the shelf life of butter and spreads.

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## تقدير مستوى النيترات والنيتريت في الزبد البكري المستورد ومخاطرها على الصحة العامة

د. مدحت إبراهيم محمد عبد الله

باحث بمعمل فحوص الأغذية بمدينة دمياط البحري – معهد بحوث صحة الحيوان – مركز البحث الزراعية - مصر

### الملخص العربي

يعتبر الزبد من المنتجات الغذائية المرتفعة القيمة لاحتوائه على نسبة عالية من الدهون وعلى ذلك فإن قيمتها الحرارية أعلى بكثير من أي من منتجات الألبان الأخرى. هذا بالإضافة لما تحتويه من بعض الفيتامينات الهامة للجسم خاصة فيتامين (A) ويستعمل الزبد في الغذاء إما بمفرده أو مضافاً إلى أنواع عديدة من المأكولات. ولما كان لوجود المواد الحافظة في الألبان ومنتجاتها ومنها النيترات والنيتريت أثر ضار على صحة المستهلك لزم إجراء هذا البحث لاستبيان مدى تواجد هذه المركبات في الزبد الطبيعي البكري المستورد مع توضيح المصادر المختلفة لتواجدها في المنتج وقد جمعت ٤٠ عينة عشوائية من السوبر ماركت المختلفة بمحافظة دمياط (٢٠ عينة من كل من الزبد المملح وغير مملح) وذلك لتقدير مستوى النيترات والنيتريت باستخدام الإسبيكتروفوتوميتر.

وقد أوضحت النتائج أن متوسط تركيز النيترات nitrate في الزبد المملح وغير الملح على التوالي  $0.124 \pm 0.0795$  و  $0.352 \pm 0.062$  مجم/كجم. في حين أن متوسط تركيز النيتريت nitrite في الزبد المملح وغير الملح على التوالي  $0.012 \pm 0.0002$  و  $0.0002 \pm 0.00002$  مجم/كجم. كما قيمت النتائج طبقاً للمعايير الدولية والتي تسمح فقط باستخدام ملح الطعام أو ملح سوربات البوتاسيوم أو كلابهما معاً كمادة حافظة في الزبد وأن وجود تركيزات مختلفة من النيترات والنيتريت وإن كانت قليلة إلا أنها تعطي فرصة لتكون مواد مسرطنة مثل النيتروزامينات nitrosamines التي تشكل خطورة على صحة الإنسان وخاصة الأطفال.