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Estimation of Nitrite, Nitrate and N-Nitrosamines in Selected Food Samples

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Abstract: Determination of nitrite, nitrate and N-nitrosamines in different types of milk and yogurt were carried out using colorimetric and GLC methods. This study shows that the average contents of nitrite and nitrate in milk were 0.045 µg/ml and 2.96 µg/ml respectively. Unidentified nitrosamines were observed in samples analyzed and neither DMN or DEN were detected. Selection of soft drink samples contained 3.9 µg/ml and 0.43 µg/ml of DMN and DEN respectively, water of industrial areas were observed to contained relatively high concentration of DMN (3.4 µg/ml) and DEN (0.25 µg/ml).

Key words: Nitrite, nitrate, nitrosamines, Dimethylnitrosamine (DMN), Diethylnitrosamine (DEN), toxic groups

INTRODUCTION

N-nitroso compounds are one of the most toxic groups of organic chemical. With the recent concern being shown over the possible occurrence of carcinogenic nitrosamines in drinks and food (Hawksworth *et al.*, 1974; Ward *et al.*, 2005 Lijinsky, 1999). It was essential to determine the levels of these compounds in a selective locally manufactured dairy products and soft drinks in Iraq, as a part of an intensive survey for the nitrosamines in the largely and commonly consumed foods and drinks.

Dimethylnitrosamine (DMN) AND diethylnitrosamine (DEN) are the most potent carcinogens of the N-nitrosamines (Waston, 1983). DMN was detected in powder milk at levels ranging from 0-4.5 µg/kg (Lijinsky, 1999).

Nitrite, nitrate and amines are precursors for the formation of nitrosamines and that potential exist for the nitrosation to occur in the human gastrointestinal tract (Choi, 1985). Besides, the possible production of methemoglobinemia in infants from ingestion of nitrate or nitrite containing foods or drinks (Choi, 1985; Anonymous, 1981; MAFF, 1987). Nitrites are formed in nature by the action nitrifying bacteria as an intermediate stage in the formation of nitrate (Ali *et al.*, 2006). The fact that most water supplies contain higher nitrate level than milk the reasoning behind several early methods developed to indirectly adulteration of milk by added water (Waston, 1983).

MATERIALS AND METHODS

The samples were locally manufactured products. Analytical grad solvents were used for extraction. Dimethylnitrosamine and diethyl nitrosamine were purchased from (MERCK). Griess reagent (equal volumes of sulphanilic acid 1% and naph-ethylamine 0.18% in 30% acetic acid) was prepared and stored in a cold and dark place to be mix.

The denitrosating mixture contained HBr solution (46% corrected to 4% with glacial acetic acid and acetic anhydride 20% of the final mixture). Water samples were obtained from the soft drink.

Estimation of nitrite and nitrate: Samples of freshly sterilized, boiled and stored opened containers, milk (100) and different brands of yogurts (10-20 g) were deproteinated and extracted by zinc sulphate, potassium hexacyanoferrate and buffer solution (Ali *et al.*, 2006). The nitrite content of the test samples were estimated by a colorimetric spectrophotometrically after quantitative reduction to nitrite with a cadmium column (Waston, 1983; Ali *et al.*, 2006).

Analysis of N-Nitrosamines

Extraction: Extraction of nitrosamines from milk or yogurt was carried out according to the reported method (Coker *et al.*, 1991) using acetone for deproteination. The aqueous residue was then saturated with NaCl and extracted with ethyl acetate and extracts were pooled together, dried by anhydrous sodium sulphite and concentrated by a rotary evaporator at 40°C. Water samples (200 ml) were extracted with dichloromethane (3 x 50 ml) and treated similarly (Ward *et al.*, 2005). Soft drink samples were extracted similarly except that the extracts were passed over activated charcoal to remove the coloring materials, dried and then concentrated. A gas chromatograph equipped with a flame ionization detector was used for detection quantification of nitrosamines. The column (130 cm x 105 mm) was packed with 60/80 carbopack-B (4%) carbowax 20 and 0.8% koH and operating temperature for injector, column and detector were 160, 180 and 250°C respectively.

Chemical and TLC detection of the nitrosamines: The nitrosamines were calorimetrically determined using a

Table 1: Level of nitrite and nitrate in milk, expressed as Maximum and minimum range (coefficient of variation, CV %)

Storage time (days)	No. of samples	Nitrite		Nitrate	
		µg/g Range	CV (%)	µg/g Range	CV (%)
1	16	0.017-0.07	44	2.30-3.5	12
2	16	0-0.6	85	2.77-3.8	8
3	16	0-0.25	97	2.50-4.3	11
Boiled	16	0-0		3.10-5	16

Table 2: Levels of Nitrite and Nitrate in different brands of yogurt, expressed as maximum and minimum range (coefficient of variation, CV%)

Storage time (days)	No. of samples	Nitrite		Nitrate	
		µg/g Range	CV (%)	µg/g Range	CV (%)
1	16	0-0.13	100	0.52-1.44	31
2	16	0-0.35	59	0.47-1.8	35
3	16	0-0.03	85	0.34-0.7	26
4	16	0-0.11	90	0.37-98	32

Table 3: Levels of Nitrosamines in water and selected soft drinks, expressed as maximum and minimum range (coefficient of variation, CV%)

Samples	No.	DMN (µg/L)		DEN (µg/L)	
		Range	CV (%)	Range	CV (%)
Water	10	1.8-5.0	47	0.17-0.33	32
Carbonated cola	12	4.3-7.3	19	0.18-0.52	36
Conc. Orange juice	10	2.5-5.1	27	0.22-0.64	32
Conc. Lemon juice	10	2.1-6.0	34	0.18-0.62	37
Conc. Apple juice	12	1.6-4.8	39	0.30-0.81	37
Conc. Grapes juice		2.9-5.6	25	0.24-0.59	29
Conc. Apricot juice	10	2.3-5.6	31	0.19-0.66	40

denitrosating agent together with Griess reagent as previously described (Coker *et al.*, 1991). N-Nitrosamines were chromatographed on silica G plates developed in a cold and dark places using hexane: ether: dichloromethane (6:5:3) as the solvent system. The chromatograms were irradiated under UV light (254 nm) for one minute, sprayed with griess reagent to give purple spots (Coker *et al.*, 1991) and the Rf values of the nitrosamines were determined.

RESULTS AND DISCUSSION

The levels of nitrite and nitrate in milk and yogurt were relatively low and fall within acceptable limit (Lijinsky, 1999). But this could be added up to the amounts present in other sources. However, storage of the opened milk containers at 4°C resulted in an increases in nitrate level and reduction of the nitrite content table. Boiled milk samples, on the other hand, contained higher level of nitrate. The mechanism responsible for this increase are somewhat complicated. Apparently, conditions such as temperature are suitable to convert some nitrogen dioxide to nitrate ions (Ayanaba *et al.*, 1973). Nitrate level may also be increased due to microbial activity (Ayanaba *et al.*, 1973).

The percentage recoveries of DMN and DEN in the samples under investigate were 76% and 74% respectively. GLC analysis indicated the presence of

unidentified nitrosamine in milk and yogurt of a higher retention time (170 sec) compared to standard DMN (133 sec). Chemical and TLC detection confirmed the presence of a nitrosamines in the samples. The Rf values of the minimum detection limit for DMN was 0.55, while it was 0.36 for DEN. The minimum detection limit for DMN was 0.01 µ. It has been noticed that stored milk contained higher amounts of the unidentified nitrosamine. Further work is undergoing to identify the type and levels of this unidentified nitrosamine. DEN was not detected in the samples analysed. Anonymous (1981) has reported the presence of diisopropyl nitrosamine (5-15 ppb) in some dairy products. Ali Topcu *et al.* (2006) on the other hand, detected DMN in milk powdered above a level of 1 µ/g.

This survey shows that the nitrosamines occur in relatively high concentration in water and the selected soft drinks (Table 3). All the samples analysed contained DMN and DEN. The mean values of DMN and DEN in water were 3.4 µ/L and 0.23 µ/L respectively, where as the selected soft drink samples contained 3.9 µ/L and 0.43 µ/L of DMN and DEN respectively.

Contamination of water by carcinogenic nitrosamines could either arise from the formation of these contaminate in soil due to bacterial processes, or from their addition to water from plant, animal or industrial waste (Ayanaba *et al.*, 1973). Obviously, it was not

possible to remove the nitrosamines from water during the water treatment (Ward *et al.*, 2005). Table 3 shows that the levels of nitrosamines in water and selected soft drinks are closely similar which indicated that the sources of nitrosamines in soft drink are mainly from water used during processing.

REFERENCES

- Ali Topcu, Aylin Ayaz, libilge Saldami and Mine Urthagul, 2006. Determination of nitrate and nitrite content of Turkish cheese. *Afr. J. Biotechnol.*, 5: 1411-1414.
- Anonymous, 1981. The health effects of nitrate, nitrite and N-nitroso compounds. Committee on Nitrate and Alternative Curing Agents in Food. National Research Council. National Academy Press, Washington, DC.
- Ayanaba, A., W. Vrstraete and M.J. Alexander, 1973. Possible microbial contribution to nitrosamine formation in sewage and soil. *J. Natl. Cancer Inst.*, 50: 811-813.
- Choi, B.C., 1985. N-nitroso compounds and human cancer: Molecular epidemiologic approach. *Am. J. Epidemiol.*, 121: 737-743.
- Coker, H.A.B., A.E. Thomas and A. Akintonwa, 1991. Determination of the total level of nitrosamine in select consumer products in Lagos area of Nigeria. *Biol. Environ. Contam. Toxicol.*, 47: 706-710.
- Hawksworth, G., M.J. Hill, G. Gordill and C. Ceullo, 1974. Possible relationship between nitrates, nitrosamines and gastric cancer in S.U. Columbia. *IRAC Sci. Publ.*, 9: 229-234.
- Lijinsky, W., 1999. N-nitroso compounds in the diet. *Mutate Res.*, 443: 129-138.
- MAFF, 1987. Nitrate, Nitrite and N-Nitroso compounds in food, ministry of agriculture, fishereies and food, the twentieth report of the steering group on food surveillance, No: 20, Her Majesty's Stationary Office, London.
- Waston, R.J., 1983. Trace Amounts of Nitrosamines in powdered milk and milk proteins. *J. Sci. Food Agric.*, 34: 893-895.
- Ward, M.H., T.M. deKok, P. Levallois, J. Brender, G. Gullis, B.T. Nolan and J. VanDerslice, 2005. Workgroup: Drinking-water nitrate and health-recent findings and research needs. *Environ Health Perspect*, 113: 1607-1614.