

## Short Communication

# Determination of nitrate, nitrite, N- nitrosamines, cyanide and ascorbic acid contents of fruit juices marketed in Nigeria

Okafor P.N\*. and Nwogbo .E.

Department Of Biochemistry, Michael Okpara University Of Agriculture, Umudike, P.M.B. 7267 Umuahia Abia State Nigeria.

Accepted 17 June, 2005

The nitrate, nitrite, N- nitrosamines and ascorbic acid content as well as the levels of cyanide in eight brands of fruit juices and twelve brands of sachet water commonly marketed and consumed in Nigeria were estimated. The mean values of nitrate ranged from  $2.29 \pm 0.05$  to  $16.50 \pm 1.21$  mg/L for the juices and  $0.64 \pm 0.21$  to  $7.56 \pm 3.10$  mg/L for sachet water. While the mean concentration of nitrite ranged from  $6.84 \pm 0.47$  to  $12.03 \pm 1.07$  and  $0.12 \pm 0.02$  to  $4.42 \pm 0.33$  mg/L for juices and sachet water, respectively. Four out of the eight brands of juices analyzed contained detectable levels of nitrosamines ( $2.75 \pm 0.47$  to  $45.70 \pm 3.07$   $\mu\text{g/L}$ ) while none of the samples contained any detectable amount of cyanide. Ascorbic acid concentrations of the samples varied from 100 to 400 mg/L. The results are discussed from nutritional and toxicological points of view.

**Key words:** Nitrate, nitrite, N-nitrosamine, cyanide, contamination, fruit juices.

## INTRODUCTION

The increasing incidence of various forms of cancer in the world at large and in Nigeria in particular has been attributed to the levels of certain chemicals in our foods and drinks. Among these chemical carcinogens are N-nitrosamines and their precursors, nitrite and secondary amines. Various levels of carcinogenic volatile N-nitrosamines, nitrites and secondary amines are present in a wide variety of foods such as cured meat, smoked fish, dried malt and beer (Harvery and Fazio, 1985; Ostersahl, 1988). Considerable attention has been paid to the occurrence and formation of N-nitrosamines and their precursors in animal and human foods following outbreaks of liver disorder, including cancer, in various farms in Norway in the early 1970s (Sen and Baddoo, 1993). These nitrosamines are believed to be formed due to the interaction of various nitrosating agents (e.g.

nitrite, nitrogen oxide) and amines in the foods. Most N-nitrosamines have been shown to be carcinogenic in laboratory animals (Preussmann and Stewart, 1984) and there is concern about health hazard to man who consumes precursors of N-nitrosamines.

That nitrite is known to be a precursor of toxic and carcinogenic N-nitrosamines has been reported (Bassir and Maduagwu, 1978) and induces cancer in experimental animals (Sen and Baddoo, 1997; Mirvish, 1995). After ingestion, residual nitrite can form traces of certain N-nitroso compounds in stomach (where the pH < 7) on reacting with secondary amines which might also be present in the ingested food (O'Neil et al., 1984). Nitrite can also interact with haemoglobin by oxidation of ferrous ion ( $\text{Fe}^{2+}$ ) to ferric state ( $\text{Fe}^{3+}$ ) preventing or reducing the ability of blood to transport oxygen a condition known as methaemoglobinaemia (Philips, 1971; Tannenbaum, 1984; Jones, 1993). Nitrate on the hand can be reduced to nitrite *in vivo*.

Increasing attention has been focused on estimating the extent of human exposure to N-nitroso compounds

\*Corresponding Author E-Mail: [pnokafor@yahoo.com](mailto:pnokafor@yahoo.com).

**Table 1.** Nitrate, nitrite, N-nitrosamines, ascorbic acid and cyanide content of eight brands of fruit juices marketed in Nigeria.

Product <sup>n</sup>	NO <sub>3</sub> (mg/L)	NO <sub>2</sub> (mg/L)	N-nitrosamines (µg/L)	Ascorbic acid (mg/l)	Cyanide (mg/L)
Brand A	8.63± 1.24 <sup>a</sup>	6.88±1.53 <sup>c</sup>	ND	400	ND
Brand B	9.10±1.02 <sup>a</sup>	6.84±1.47 <sup>c</sup>	ND	320	ND
Brand C	7.86±1.23 <sup>a</sup>	6.09±0.97 <sup>c</sup>	15.81± 2.04 <sup>g</sup>	114	ND
Brand D	16.50±2.21 <sup>e</sup>	7.09±1.43 <sup>c</sup>	3.43±0.50 <sup>f</sup>	100	ND
Brand E	7.87±1.94 <sup>a</sup>	5.50±1.24 <sup>c</sup>	45.70±3.07 <sup>h</sup>	240	ND
Brand F	2.29±0.52 <sup>b</sup>	12.03±1.07 <sup>d</sup>	ND	300	ND
Brand G	4.43±0.87 <sup>b</sup>	10.86±2.65 <sup>d</sup>	ND	320	ND
Brand H	3.44±0.71 <sup>b</sup>	7.15±1.37 <sup>c</sup>	2.75±0.47 <sup>f</sup>	260	ND

\*Means with the same superscript are not statistically significant (  $p=0.05$ ).

\*n = 25 where n is the number of samples of each brand analyzed with an average of three determinations per sample.

\*ND - Non-detectable within the limit of analytical method used.

and their precursors following the demonstration by Sander (1966) that ingested nitrite and secondary amines could react *in vivo* to produce carcinogenic nitrosamine. More recently, the European Commission's Scientific Committee for Food (SCF) considered the implications for human health of nitrate and nitrite in food and has set Acceptable Daily Intake (ADIs) for nitrate and nitrite (SCF, 1995). In our previous work high levels of nitrate and nitrite were demonstrated in some fruit juices and water marketed and consumed in Nigeria (Okafor and Ogbonna, 2003). Whether N-nitrosamines occur together with nitrate and nitrite in these juices, since processing conditions have been shown among other factors to lead to nitrosamine formation and to influence the concentration of nitrate and nitrite in foods (Huarte-Meridicoa et al., 1997) is not yet known. This forms the basis of this present paper. The concentration of ascorbic acid in these fruit juices will also be determined since this compound is a known inhibitor of N-nitrosamine formation. Moreover, the possible contamination of these juices with cyanide will be determined. Poisoning of Chilean grapes with cyanide has been reported (Khan Swerdlow and Jurank, 2001) suggesting that no food or drink is invulnerable to such contamination.

## MATERIAL AND METHODS

### Juices and sachet water

Sample of 8 brands of fruit juices (some were imported) commonly marketed and consumed in Nigeria were purchased from retail outlets and were used in this study. The fruit juices include apple juice, pineapple juice, mixed fruits and others. Samples of twelve brands sachet water (previously analyzed for nitrate and nitrite; Okafor and Ogbonna, 2003), were purchased from retail outlets and brought for analysis.

### Nitrite, nitrite and n-nitrosamine determination

Nitrite was determined by the spectrophotometric method as described by Follett and Ratcliff (1963). Nitrate was determined

after reduction to nitrite using cadmium column (Follett and Ratcliff, 1963), while nitrosamine was determined after decomposition to nitrite by UV irradiation. Coloured samples were cleared with animal charcoal. For colour formation, nitrite was reacted with sulphanic acid and N (1-naphthyl) ethylenediamine hydrochloride and the purple colour that developed after 20 min was read spectrophotometrically at 520 nm.

### Determination of cyanide and ascorbic acid content of samples

Cyanide was determined spectrophotometrically according to the method of Esser et al. (1993) and ascorbic acid content by titrimetric method using 2, 6-dichlorophenol indo-phenol.

## RESULTS AND DISCUSSION

The result from this study show that all the fruit juices analyzed contained detectable amount of nitrate, nitrite and ascorbic acid, non-detectable amount of cyanide while sample from only four brands contained N-nitrosamines (Table 1). The mean nitrate and nitrite levels in the juices ranged from 2.29±0.52 to 16.50±2.21 and 6.09±0.97 to 12.03±1.07 mg/L, respectively. For sachet water, the mean values for nitrate and nitrite ranged 0.64±0.21 to 7.56±3.10 and 0.12±0.02 to 4.42±0.33 mg/L, respectively (Table 2). The concentration of nitrate in the samples fall below the WHO's Acceptable Daily intake (AD1) which is set at 5 mg/kg body weight. However, the risk to human with respect to methaemoglobinaemia and conversion of nitrate to nitrite in oral cavity and stomach leading to the possible formation of nitrosamines cannot be ignored. Chronic ingestion of small dose of nitrate can also cause dyspepsia, mental depression, and headache (Magee, 1983).

For the children under 10 kg body weight, the levels of nitrite from those fruit juices are higher than the WHO's (1978) recommended AD1 of nitrite which is stipulated at 0.2 mg/kg body weight. The concentrations of nitrite in brands B, E, J and L of sachet water (442±0.33,

**Table 2.** Levels of nitrate, nitrite, N-nitrosamine and cyanide in 12 brand of sachet water.

Product	NO <sub>3</sub> (mg/L)	NO <sub>2</sub> (mg/L)	N-nitrosamine (µg/L)	Cyanide (mg/L)
Brand A	2.32 ± 0.47	0.50 ± 0.05	ND	ND
Brand B	6.55 ± 0.93	4.42 ± 0.33	ND	ND
Brand C	1.22 ± 0.41	0.88 ± 0.07	ND	ND
Brand D	0.64 ± 0.21	0.12 ± 0.02	ND	ND
Brand E	7.56 ± 3.10	3.48 ± 1.22	ND	ND
Brand F	3.10 ± 0.55	1.65 ± 0.67	ND	ND
Brand G	4.27 ± 0.63	1.88 ± 0.42	ND	ND
Brand H	2.65 ± 1.21	1.02 ± 0.33	ND	ND
Brand I	1.93 ± 0.12	0.86 ± 0.03	ND	ND
Brand J	7.56 ± 3.00	2.52 ± 0.50	ND	ND
Brand K	2.47 ± 0.73	1.44 ± 0.33	ND	ND
Brand L	2.87 ± 1.33	2.10 ± 0.89	ND	ND

\*n = 25, where n is the number of samples of each brand with an average of three determinations.

\*ND = non-detectable within the limit of analytical method used.

\*Neg = Negligible levels detected.

3.48±1.22, 2.52±0.50 and 2.10±0.89 mg/L, respectively) are higher than the WHO's (1978) recommended ADI of nitrite. The levels of nitrite in the total volume pack (330 ml) for the brand with least level of nitrite (5.50±1.24 mg/L) will be 1.82 mg and 3.97 mg for brand with the highest concentration (12.03±1.07 mg/L). Children of 15 to 20 kg of body weight consuming one serving of these products will be ingesting about 45.5 to 60.7% respectively of ADI (for brand with least nitrite level) and 99.25 to 132.33% of ADI (for brand with highest level of nitrite). Furthermore, the consumption of two or more servings of these products or one serving along with supplement sources of nitrite in diet (such as vegetables, legumes, or even some water sources) could have the daily intake increased above ADI value as recommended by WHO. Thus, some of these fruit drinks could be said to be unsafe for children and infants. Children might be especially susceptible to the toxic effects of these compounds as they have low body weight, immature enzymatic system (especially for xenobiotic metabolism) and low gastric acidity (a good condition for N-nitrosamine formation). In this connection, infant illness and death from nitrite induced methaemoglobinemia has been reported in place like Provincial Children's Hospital, Krakow, Poland (Lutynski et al., 1996) and South Dakota (Johnson and Kross, 1990). The results from this work is consistent with earlier report of Grijspaardt-Vink (1994) that exposure to nitrate and nitrite from foods and drinks has been found to be much higher than when initial ADI were developed.

Nitrosamines were also detected in some of the samples together with nitrate and nitrite. The concentrations of nitrosamines measured in sample of four brands of those fruit juices ranged from 2.75±0.47 to 45.70±3.07 µg/L. That nitrosamines are parts of the chemical component of those drinks is a significant

finding in this study. N-nitrosamines are known toxicants as well as chemical carcinogens. Investigators have also demonstrated nitrosamine formation in food nitrite mixtures mainly in attempt to determine the extent of formation under human gastric conditions (Siddiqi et al., 1988; Atawodi et al., 1990). However it is important that the nature of the nitrosamines contained in those drinks be determined and the levels of the volatile, nitrosamines such as N-nitrosodimethylamine (NDMA) be quantified. It is only then, that the toxic and carcinogenic potential of these nitrosamines be ascertained.

The levels of ascorbic acid determined in those fruit juices ranged from, 100 to 400 mg/L. Ascorbic acid addition is common in manufacture of beverages, especially fruit juices. Ascorbic acid is used extensively in Food Industries not only for its nutritional value, but for its many functional quality as antioxidant and inhibitor of N-nitrosamine formation through reduction of nitrate to nitrogen oxide which will not be able to react with amines to form nitrosamines. We did not find any correlation between the ascorbic acid concentration of these drinks and that of N-nitrosamines.

None detectable levels of cyanide were observed in the fruit juice. It is interesting that cyanide was not detected in the juices, since the main cyanide metabolite (thiocyanate) catalyses N-nitrosamine formation. Poisoning of Chilean grapes with cyanide has been reported (Khan et al, 2001) suggesting that no food or drink is invulnerable to such contamination.

In this work we have demonstrated the presence of high concentration of nitrate and nitrite in fruit juice commonly consumed in Nigeria as well as N-nitrosamine contamination of some of those drinks. However, a more analytical method such as thermal energy analyzer (TEA) needs to be used to ascertain the exact amount of the N-nitrosamines and hence estimation of the potential

toxic effect of such amount.

## REFERENCES

- Atawodi SE, Maduagwu EN, Preussmann R, Spiagallader, B (1991). Potential of endogenous formation of volatile nitrosamines from Nigeria vegetables and spices. *Cancer Lett.* 57:219-222.
- Bassir O, Maduagwu EN (1978) Occurrence of nitrate, nitrite, dimethylamine and dimethylnitrosamines in some fertilized Nigerian beverages. *J. Agric. Food Chem.* 26(1): 200-203.
- Esser AJA, Bosveld M, Van Der Grift III RM, Voragen AGJ (1993). Studies on the quantification of specific cyanogens in cassava products and introduction of a new chromogen. *J. Sci. Food Agric.* 63(3): 287 - 296.
- European Commission Scientific Committee for Food (1995) Opinion on Nitrate and Nitrite (expressed on 22 September, 1995) Annex 4 to Document 111/5611/95.
- Follett IA, Ratcliff PN (1963) Determination of nitrite and nitrate in meat products. *J. Sci. Fd. Agric.* 14:138 - 144.
- Grijspaardt -Vink C (1994). An European view of nitrates in vegetables. *Food Technol.* 48 (7): 31.
- Havery DC, Fazio T (1985) Human exposure to nitrosamines from foods. *Food Technol.* 39(1): 80 - 83.
- Huarte - Mendicoa, J.C., Astiasaran, I, and Bello, J. (1997). Nitrate and nitrite levels of fresh and frozen broccoci. Effect of freezing and cooking. *Food Chemistry* 58 (1): 39-42.
- Johnson LJ, Kross BC (1990). Continuing importance of nitrate contamination of ground water and wells in rural areas. *Am. J. Ind. Med.* 18(4): 449-456.
- Jones T (1993). Poison: Nitrate/ nitrite. *In Practice*, 15:146-149.
- Khan AS, Swerdlow DL, Jurank DD (2001). Precaution against biological and chemical terrorism directed at food and water supplies. *Public Health Rep.* 116:3-14.
- Lutynski R, Steczek Wojdyla M, Wojdyla Z, Kroch S (1996). The concentrations of nitrate and nitrite in food product and environment and occurrence of acute toxic methaemoglobinaemia. *J. Przegląd Lekarski* 53(4): 351 - 355.
- Mirvish SS (1995). Role of N-nitroso compounds (NOC) and nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to Noc. *Cancer Lett.* 93:17-48.
- O'Neil IK, Von Borstel RC, Miler CT, Long G, Bartech H (1984). N-nitroso compounds. Occurrence, biological effects and relevance to human cancer. Lyon, France: International Agency for Research on Cancer.
- Okafor PN, Ogbonna UI (2003). Nitrate and nitrite contermination of water sources and fruit juices marketed in South-Easter Nigeria. *J. Food Comp. Anal.* 16: 213-218.
- Osterdahl BC (1988). Volatile nitrosamines in foods on the Swedish market and estimation of their daily intake. *Food Addit. Contam* 5(4): 587-585.
- Phillips WEJ (1971). Naturally occurring nitrate and nitrite in foods in relation to infantile methemoglobinaemia. *Food and Cosmetics Toxicol.* 9:219-228.
- Preussmann R, Stewart BW (1984). N-nitroso Carcinogens. In: *Chemical Carcinogens*, 2<sup>nd</sup> ed. CE Searle, ACS Monograph 182. American Chemical Society Washington DC, USA pp. 643 -822.
- Sen NP, Baddoo PA, Seaman SW (1993). Nitrosamine in cured pork products packaged in elastic rubber nettings: An update. *Food Chem.* 47: 387 -390.
- Sen NP, Baddoo PA (1997). Trends in the levels of residual nitrite in Canadian cured meat products over the past 25 years. *J. Agric. Food. Chem.* 45: 4714-4718.
- Sander J (1968) Nitrosamunsynthase durch Barkterian ( Nitrosamine synthesis by bacterial) *Z. Physiol. Chem.* 349: 432.
- Siddiqi M, Tricker AR, Prussmann R (1988). Formation of N-nitroso compounds under stimulated gastric condition from Kashmir foodstuffs. *Cancer Lett.* 39: 259-265.
- Tannenbaum SR (1984). A policy perspective on safety. Nitrite and nitrate. Nuthry, NJ Roche 1984.
- WHO's (1978). Nitrates, nitrite and N-nitroso compounds: *Environ. Health Critena* 5: 107.