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Quantitative analysis of nitrate and nitrite contents in vegetables commonly consumed in Delta State, Nigeria

I. Onyesom* and P. N. Okoh

Department of Biochemistry, PO Box 144, Delta State University, Abraka, Nigeria

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Plasma thiocyanate has been reported to be high among cassava-eating populations such as that in Nigeria because of the cyanide content of cassava. Thiocyanate, which is secreted into the stomach contents of animals, has been demonstrated to catalyse the formation of nitrosamines (potent carcinogens) in the stomach from secondary amines and nitrite. The main source of the nitrite precursor in this environment is vegetables, primarily eaten as the chief supplier of proteins. The present study attempts to analyse the levels of nitrate and nitrite in vegetables commonly grown and consumed in Delta State, Nigeria. The nitrate and nitrite contents in green vegetable (*Amaranthus* spp.), bitter leaf (*Vernonia amygdalina*), pumpkin (*Telfaria occidentalis*) and water leaf (*Talinum triangulare*) grown in different localities of the state were determined by standard analytical procedures. The results show that those vegetables grown in the industrialised urban centres of the state had higher nitrate (223 (SD 71) mg/kg dry weight; $P<0·05$) and nitrite (12·6 (SD 1·7) mg/kg dry weight; $P>0·05$) levels when compared with the same species (188 (SD 77) mg nitrate/kg dry weight and 10·9 (SD 1·1) mg nitrite/kg dry weight) cultivated in less industrialised suburbs. We conclude that frequent consumption of such vegetables whose nitrate and nitrite contents are high by cassava-eating individuals might put them at risk of developing stomach cancer and other possible results of nitrate and/or nitrite toxicity. In order to avoid an outbreak in our communities, appropriate agencies should monitor and regulate the release of chemicals into the environment. In the meantime, the cultivation and consumption of vegetables grown in industrialised areas of the state should be discouraged.

Thiocyanate: Vegetables: Nitrates: Cassava: Nitrites

Nitrates (NO_3^-) and nitrites (NO_2^-) are naturally occurring ions; both are products of oxidation of N by micro-organisms in plants, soil or water and, to a lesser extent, by lightening. Nitrate is the more stable form of oxidised N but can be reduced by microbial action to nitrite (Beatson, 1978). Vegetables tend to concentrate nitrate ions, especially if they are grown using a high application of N fertilisers. The concentration of nitrate in vegetables can vary considerably, and may be as much as 3–4 g/kg fresh weight, and these levels could have potential health impacts especially in cassava-eating populations (Okoh, 1992).

One of the toxicological implications of nitrate and nitrite ingestion in cassava-eating populations is that thiocyanate, which is present in high amounts in the stomach of such individuals, may act as a catalyst for the nitrosation of amines in the stomach to form carcinogenic nitrosamines (Mirvish, 1983; Maduagwu & Umoh, 1988; Okoh, 1992). A thiocyanate concentration of 1 mM reduces the optimal pH for the *in vitro* nitrosation of N-methylaniline from pH 3 to 1·5 and accelerates the reaction 550 times (Boyland & Walker, 1974). Although it is difficult to decide what would be the effective concentration of thiocyanate *in vivo*, it seems reasonable to conclude that if nitrosation is occurring, it will be accelerated in the stomach of animals or human consumers ingesting cyanide and high amounts of nitrites and nitrates in the diet.

Vegetables are important sources of protein in the diets of many Nigerians whose intake of animal protein is very low (Okoh, 1984). Yet vegetables are also the main source of nitrate and nitrite in the diet, accounting for about 75 % of the total intake (Corre & Breimer, 1979).

The objective of the present study was to determine whether the amount of nitrate and nitrite present in local vegetables commonly consumed in Delta State, Nigeria might be sufficient to cause the associated health problems, and attempt to offer advice to consumers.

Materials and methods

Collection of samples

Four types of vegetables, pumpkin (*Telfaria occidentalis*), green vegetable (*Amaranthus* spp.), bitter leaf (*Vernonia amygdalina*) and water leaf (*Talinum triangulare*) grown on artificial fertiliser- or manure-free soils, were used in the present study. Sixty-four samples were collected from four different farms in Delta State, Nigeria. Four samples of each vegetable were collected from each farm located in Abraka, Obiaruku, Orazi and Enerhen. The pumpkin samples were randomly collected from different heads in the farm. In the case of the other vegetables that were planted in ridges, samples

* **Corresponding author:** Dr I. Onyesom, email onyesominno@yahoo.co.uk

were randomly collected from plants in different ridges. The vegetables were assembled and taken immediately to the laboratory for analysis of their moisture, nitrate and nitrite contents. Sampling was done on 7 January 2003 and repeated on 7 February 2003. A total of eight different collections of each vegetable were taken per location. Each collection was divided into two parts. One part was crushed fresh and prepared for nitrate assay, while the other part was sliced into convenient pieces and allowed to stay at room temperature for about 4 h before being prepared for nitrite analysis (Atawodi *et al.* 1991). The time lag is necessary for sufficient reduction of nitrate to nitrite.

Determination of moisture content

This was determined by drying each sample in an oven at 105°C to constant weight. The difference in weight was then expressed as percentage moisture content.

Preparation of oven-dried sample digest for nitrate and nitrite analyses

About 0·25 g dried sample was weighed out and placed in a 50 ml conical flask. A solution (5 ml) containing 3·3 ml nitric acid and 1·7 ml perchloric acid was added to the flask under a fumehood. This was allowed to stand overnight at room temperature. The vegetable samples in the flasks were digested in a block digestor at 150°C for about 1·5 h. The temperature was increased to 230°C and digestion was continued for another 30 min. The temperature was then reduced to 150°C, and 50 % HCl (1 ml) was added and the mixture was heated for another 30 min. Each sample was then washed into a 50 ml volumetric flask and made up to mark with water to obtain the digest.

Nitrate assay

The nitrate contents in the vegetables were determined by the phenoldisulfonic acid method (Association of Official Analytical Chemists, 1975). A portion of the digest (5 ml) was measured into a beaker and evaporated to dryness. Phenoldisulfonic acid (2 ml) was then added and later diluted with 20 ml distilled water. Ammonia solution (7 ml) was then added until a yellow permanent colour developed. The yellow-coloured solution was transferred into 50 ml volumetric flask and made up to mark with distilled water. Absorbance was measured at 410 nm using a spectrophotometer (Spectronic 21; Milton Roy Co., Rochester, NY, USA).

Nitrite assay

The nitrite contents in the vegetables were determined by the Nessler's Reagent method (Bassett *et al.* 1986). A portion of 5 ml digest was measured into a beaker and 1 ml Nessler's reagent was added. The volume was made up to 25 ml with distilled water and absorbance of the resulting orange–brown colloidal product was read at 525 nm using a spectrophotometer (Spectronic 21; Milton Roy Co.). The AnalaR Grade chemicals used for both assays were supplied by BDH (Poole, Dorset, UK).

Statistical analysis

The nitrate and nitrite contents in the same type of vegetables collected from different locations, and the contents in the four different vegetables collected from the same location, were compared separately using ANOVA, and the level of statistical significant difference was established at $P < 0{\cdot}01$.

Results

Data obtained from the analyses of the nitrate and nitrite contents in vegetables grown in different locations, and commonly consumed in Delta State, Nigeria are shown in Table 1.

The nitrate contents of *Amaranthus* spp. (green vegetable) and *Talinum triangulare* (water leaf) grown in Orazi and Enerhen were significantly higher ($P < 0{\cdot}01$) than those grown in Abraka and Obiaruku, but *V. amygdalina* (bitter leaf) and *Telfaria occidentalis* (pumpkin) are the vegetables that accumulate the highest levels of nitrate and nitrite. The quantitative differences in the vegetables grown in Enerhen and Orazi are likely to be biologically significant when compared with those cultivated in Obiaruku and Abraka. The amounts of nitrate in bitter leaf and pumpkin were within the range (133–499 mg/kg) observed to bear strong correlation with the incidence of stomach cancer (Risch *et al.* 1985). Although, there are yet to be any epidemiological or descriptive data in our environment, the present data suggest a measure of cancer risk.

The mean soil nitrate value for the soil samples collected from industrialised areas was shown to be significantly higher ($P < 0{\cdot}01$) when compared with the mean value for soil samples obtained from rural centres. Thus, municipal and industrial activities in urban centres, especially oil exploration, might increase the degree of pollution and, hence, contribute to the higher soil nitrate levels (Table 2).

Discussion

Our major intake of nitrates in foodstuffs comes from vegetables. Nitrates are natural constituents of plants, and are present in large quantities in many vegetables. Vegetables such as beets, celery, lettuce, radishes and spinach contribute about 85–90 % of an adult's dietary intake of nitrate, with nitrate levels ranging from 1·7 to 2·4 g/kg food (Corre & Breimer, 1979).

The most important factors that favour large accumulations of nitrate in vegetables include nitrate-rich soil caused by high levels of fertilisation (Grunderson, 1981), and environmental chemicals (Miroslav & Vladimir, 1999). The vegetables were cultivated in artificial fertiliser- (or manure)-free soils. Therefore, the results of the present investigation seem to further confirm that environmental chemicals may be a strong determining factor responsible for the nitrate and nitrite distribution and accumulation in the vegetables, since those grown in urban centres had the highest levels. The municipal and industrial activities (especially oil exploration and exploitation) in an area may have probably spun (released) chemicals into the immediate environment (Miroslav & Vladimir, 1999), and this could probably be implicated for the significant increase ($P < 0{\cdot}01$) in nitrate and nitrite contents in vegetables grown in Enerhen and Orazi compared with those

Table 1. Nitrate and nitrite contents in vegetables collected during two sampling periods from different locations in Delta State, Nigeria (Mean values and standard deviations)

Vegetable	Location	Nitrate (mg/kg dry wt)		Nitrite (mg/kg dry wt)	
		Mean	SD	Mean	SD
Green vegetable (*Amaranthus* spp.)	Abraka	118	7	10·1	0·5
	Obiaruku	123	9	10·9	0·1
	Orazi	163	7	11·4	0·3
	Enerhen	177	11	11·9	0·5
	All locations	145	9	11·1	0·4
Bitter leaf (*Vernonia amygdalina*)	Abraka	277	5	10·9	0·4
	Obiaruku	285	12	13·4	0·1
	Orazi	315	16	14·2	0·3
	Enerhen	292	5	14·0	0·2
	All locations	292	10	13·1	0·3
Pumpkin (*Telfaria occidentalis*)	Abraka	230	12	10·9	0·5
	Obiaruku	237	12	11·1	0·2
	Orazi	269	13	13·8	0·2
	Enerhen	274	23	14·5	0·6
	All locations	253	13	12·6	0·4
Water leaf (*Talinum triangulare*)	Abraka	113	8	9·85	0·3
	Obiaruku	117	8	9·99	0·5
	Orazi	144	10	10·6	0·9
	Enerhen	147	5	10·5	0·2
	All locations	130	8	10·2	0·5

For details of procedures, see p. 902–903.

grown in Abraka and Obiaruku. The contents of soil nitrate were not determined presumably because nitrate levels in soil might vary hugely from one site to the next, and from 1 month to the next even within the same area depending on the amount of released contaminants and recent fertilisation. Thus, soil nitrate level may not correlate with vegetable level, partly because of the tendency of vegetables to bioaccumulate nitrate. However, Table 2 shows that soils from urban areas (where there is more oil exploration and industrial activities) have higher nitrate levels.

Dietary nitrate is readily absorbed in the stomach and upper small intestine, and is distributed rapidly throughout the body. Roughly, 25 % is recirculated into the saliva, where approximately 20 % is reduced to nitrite by the oral microflora (Okonkwo *et al.* 1981). If the pH of the stomach is increased, the growth of nitrate-reducing bacteria is allowed and nitrates are converted to nitrites (Zhu *et al.* 1992). *In vivo*, nitrite production increases with age (Dykhuizen & Leifert, 1996) and is enhanced during bacterial infections causing diarrhoea (Zhu *et al.* 1992).

In the human stomach, nitrites can react with nitrosatable compounds, such as amides and amines, to form N-nitroso compounds (Walker, 1990; Janzowkski & Eisenbrand, 1995). Some N-nitroso compounds (for example, nitrosamines) are potent carcinogens in animal species (Hartman, 1983), and therefore can be carcinogenic in man (Risch *et al.* 1985). Some epidemiological studies linking intake of nitrate and nitrite with gastric cancer in man indicated a positive correlation (Dutt *et al.* 1987). Certain evidence for an association between the intake of nitrate (133–499 parts per million, i.e. 133–499 mg/kg, in vegetables) and the incidence of stomach cancer has been obtained in descriptive epidemiological

Table 2. Nitrate levels in soil samples (parts per million) collected from the Niger Delta area of Nigeria

Sample code	Rural areas†		Urban and crude oil-impacted areas‡	
	Obiaruku	Abraka	Sample code	Enerhen and Orazi
S3B	–	0·08	Maxwell 1	113
S1B	0·30	–	Maxwell 4	113
S4A	0·16	–	Maxwell 6	181
S3A	–	0·40	Maxwell 7	158
S1A	0·03	–	Maxwell 16	45
S4B	–	0·05	Maxwell 18	113
S5B	–	1·00	Maxwell 19	135
S2B	–	0·07	Maxwell 20	68
S4B	0·40	–	Maxwell 21	158
S2A	0·15	–	Maxwell 22	113
Mean	0·21	0·32		120*
SD	0·14	0·41		41·8

* $P < 0·01$.
† AN Kaizer, EO Adaikpoh and SE Odumuso (unpublished results).
‡ Iwegbue (2005).

studies conducted in Chile (Armijo *et al.* 1981). Since secondary amines and nitrite are present in a number of food sources (Lijinsky & Epstein, 1970), there has been increasing interest in the possibility that nitrosamines can be formed *in vivo*. Indeed, there is evidence that nitrosamines are formed in the stomach of rodents (Lijinsky *et al.* 1973).

It is now known that the nitrosation reaction is catalysed by some anions, particularly thiocyanate (Maduagwu & Umoh, 1988), and animals fed cassava- and sorghum-based diets containing cyanogenic glucosides convert the cyanide mainly to thiocyanate, which is secreted in large amounts into the stomach (Okoh *et al.* 1988; Okoh, 1992). These observations might increase the risk of a cassava-eating population to the carcinogenic activities of nitrates and nitrite in vegetables that they consume. A previous report (Eminedoki *et al.* 1994) has demonstrated that daily consumption of a cassava-based diet by human subjects increases the level of thiocyanate in their serum and urine. Therefore, the control of nitrate and nitrite intake may be important in Delta State, Nigeria, where plasma thiocyanate content is generally high because of the consumption of cassava-based diets, and especially now that the nitrate levels in *V. amygdalina* (bitter leaf), and *Telfaria occidentalis* (pumpkin) grown in the industrialised regions of the state are within the reference levels reported to be associated with an increased incidence of gastric cancer (Armijo *et al.* 1981; Dutt *et al.* 1987).

It is, therefore, important to conduct an environmental impact assessment in Delta State, so that appropriate agencies could start the monitoring and regulation of the release of chemicals into the environment. Meanwhile, the cultivation and consumption of vegetables, especially bitter leaf and pumpkin grown in Orazi, Enerhen, and probably in other municipal and highly industrialised areas of Delta State, Nigeria, should be discouraged in order to avoid the outbreak of nitrate toxicity and associated diseases arising from the increasing release of chemicals into the environment.

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