PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 10 (12): 1190-1194, 2011
ISSN 1680-5194
© Asian Network for Scientific Information, 2011

Cyanide, Nitrate and Nitrite Content of Some Leafy Vegetables and Fruits Commonly Consumed in the South-East of Nigeria

Friday O. Uhegbu[1], Ifeanyi Elekwa[1], Emeka E.J. Iweala[2] and Ijeoma Kanu[3]
[1]Department of Biochemistry, [3]Department of Microbiology,
Abia State University, P.M.B. 2000, Uturu, Nigeria
[2]Department of Biological Sciences, (Biochemistry and Molecular Biology Unit)
Covenant University, P.M.B. 1023, Ota, Ogun State, Nigeria

Abstract: The cyanide, nitrate and nitrite levels of some leafy vegetables and fruits commonly consumed in the South East of Nigeria were determined by standard analytical procedures. All the samples showed the presence of cyanide, nitrate and nitrite of varying concentrations. The mean value of cyanide ranged from 5.04±0.20 mgHCN/kg to 12.96±3.53 mgHCN/kg for vegetables; 2.79±0.16 mgHCN/kg to 6.07±0.16 mgHCN/kg for fruits. While the mean concentration of nitrate and nitrite ranged from 2.64±0.38 mg/kg to 4.80±0.56 mg/kg and 0.97±0.15 mg/kg to 1.97±0.74 mg/kg respectively for vegetables. The mean concentration of nitrate and nitrite of the fruits is in the range of 14.74±4.12 mg/kg to 72.76±14.51 mg/kg and 0.97±0.20 mg/kg to 4.86±0.76 mg/kg respectively. This study highlights the need to study the toxicological implications of chronic low level exposure to cyanide, nitrate and nitrite from vegetables and fruits.

Key words: Vegetables, fruits, nutrition, cyanide, nitrate, toxicity

INTRODUCTION

The presence of substances in plants which may be harmful to the consumer is dependent on environmental conditions, crop fertilization programmes, age, post harvest handling and various methods of preparation (Kemdirim *et al*., 1995; Okolie and Omoigborule, 1999; Siritunga and Sayre, 2007).

Cyanides as Hydrogen Cyanide (HCN), Potassium Cyanide (KCN) and Sodium Cyanide (NaCN) are found in a number of foods and plants. They are produced by certain bacteria, fungi and algae (Phambu *et al*., 2007; Siritunga and Sayre, 2007). Traces of cyanide can be demonstrated in almost all plants and found in the form of cyanogenic glycoside. The enzyme linamarase, hydrolyse the cyanogenic glycoside to yield cyanide (Onyesom and Okoh, 2006). Some plants also absorb cyanide from the soil (Jorgensen *et al*., 2005).

Fitzgerald *et al*. (2002); Oboh and Ekporigin (2004) reported that the pips of several berries and stones of several plum species (almond, cherry and apricot) contain considerable amounts of cyanogenic glycosides. Naturally, cyanide occurs in a variety of vegetables, fruits and grains. Dietary exposure may occur as a result of high intake of the products of some nutritive plants, eg. Cassava, some fruits like almonds, apricots, peach pits, are various means through which man gets exposed to cyanide (Oboh and Ekporigin, 2004; Onyesom and Okoh, 2006).

Nitrate and nitrite occur naturally in food and water as a consequence of the nitrogen cycle whereby nitrogen is fixed by bacteria. Nitrogen is absorbed by plants in the form of either ammonium (NH_4^+) or nitrate (NO_3^-) and its accumulation is influenced by a series of factors that are depending on the species, cultivar, age and soil conditions. Once nitrate is absorbed by plants, it has to be reduced by the enzyme nitrate reductase to ammonium and assimilated via glutamate (Prakasa and Puttanna, 2000).

The concentration and amount of nitrates levels in plants will vary depending on the type of vegetable, the temperature that it is grown at, the sunlight exposure, soil moisture levels and the level of natural nitrogen in the soil (Corre and Breimer, 1979). The agronomic practice of large application of nitrogenous fertilizers to obtain heavier yields and improper disposal of human and animal waste may lead to accumulation of nitrate in food plants (Walker, 1990). Nitrate concentrations may be different for organically grown and conventionally grown lettuces (and probably other vegetables). Nitrates are natural constituents of plants and are present in large quantities in many vegetables. Our major intake of nitrates in foodstuffs comes from vegetables (Corre and Breimer, 1979).

Nitrates and nitrites may accumulate in plants tissues and are very dangerous substances for human health, leading to different health disturbances like (methemoglobinemia, changes in vitamin level, thyroxin production and negative influence on reproduction) (Zhong *et al*., 2002). The concentration of nitrate in vegetables can vary considerably, reaching sometimes

Corresponding Author: Friday O. Uhegbu, Department of Biochemistry, Abia State University, P.M.B. 2000, Uturu, Nigeria

as much as 3-4 g/kg fresh weight and these levels could have potential health impacts (Chung *et al*., 2003).
The present study is therefore designed to assess the cyanide, nitrate and nitrite level in some vegetables and fruits commonly consumed as part of daily diet of the people of South East Nigeria.

MATERIALS AND METHODS

Plant materials: The fresh experimental vegetables and fruits were bought from Eke Okigwe market as sold on 20th of April 2010. The vegetables and fruits were identified and authenticated at the Department of Plant Science and Biotechnology Abia State University, Uturu. The voucher specimens were deposited in the herbarium of the Plant Science and Biotechnology Department, Abia State University, Uturu.

Extraction of cyanide: The samples were washed with distilled water and then ground into a paste in a mortar and used for wet (fresh) analysis. 15 g of the sample paste was weighed into a 1000 ml flask and 200 ml distilled water added and allowed to stand for 4 h. The resulting mixture was then vacuum distilled and the distillate collected in 20 ml of 0.5 M NaOH solution.

Determination of cyanide: The HCN content of the samples were determined using the alkaline titration method of Association of Official Analytical Chemists (AOAC, 2005). The distillate was diluted to 250 ml and an aliquot of 100 ml was titrated by adding 8 ml of 6 N NH_4OH solution and 2 ml of 5% KI solution. This was then titrated against 0.02N $AgNO_3$ solution to get a light turbid end point. The titre values were then used to calculate the cyanide concentrations in mgHCN/kg.

$$(1 \text{ ml of } 0.02 \text{ N } AgNO_3 = 1.08 \text{ mgHCN})$$

Determination of nitrate and nitrite

Treatment of sample: The vegetable and fruit samples was chopped and ground in a porcelain mortar with 80 mL of double distilled water until fine homogeneous slurry was formed. The slurry was then centrifuged. A spatula full of mercuric chloride was added to the supernatant as a deproteinizer. The mixture was allowed to stand for 15 min and then filtered through Whatman No. 32 filter paper to obtain a clear sample extract.

Determination of nitrate: Nitrate was determined essentially by the colorimetric method of Harper (1924) as modified by Bassir and Maduagwu (1978). To 25 mL of each clarified sample solution, 1 mL of nitrate-free Ag_2SO_4 (4 g/L) was added to remove any interfering chloride ions. Precipitated chloride was removed by filtration. Loss of nitrate was prevented by the addition of 0.2 g of magnesium oxide to 1 mL of filtrate. The optical density of the yellow nitrophenolic colour developed was measured in an ELL photoelectric colorimeter using a blue filter. Double distilled water was used as blank and levels of nitrate were extrapolated from a standard curve prepared from 1-mL aliquots of potassium nitrate standard solutions containing 0.0-20.0 µg nitrate N/mL.

Determination of nitrite: The nitrite content of the clarified extracts solutions were determined by the method of Montgomery and Dymock (1961). Absorbances of the pink-coloured solutions developed were measured at 550 nm using a reagent blank. Levels of nitrite were extrapolated from a standard curve prepared from 1-mL aliquots of Na_2NO_3 standard solutions containing 0.0-2.0 µg nitrite N/mL.

Statistical analysis: Data collected were statistically analyzed for differences between samples by the use of students' t-test. Values for $p<0.05$ were considered statistically significant.

RESULTS

The leafy vegetables contain cyanide in the range of 5.04±0.20 mgHCN/kg for *Basella alba* as the lowest to 12.96±3.53 mgHCN/kg for *Gnetum africanum* as the highest. *Basella alba* 5.04±0.20 mgHCN/kg, *Amaranthus* sp 5.37±0.24 mgHCN/kg, *Telfaria occidentalis* 5.14±1.02 mgHCN/kg and *Laurea tarxicifolia* 5.40±0.18 mgHCN/kg show no significant ($p<0.05$) difference in their cyanide concentration (Table 1). The nitrate and nitrite concentrations of the vegetables are in the range of 2.64±0.38 mg/kg to 4.80±0.56 mg/kg and 0.97±0.15 mg/kg to 1.97±0.74 mg/kg respectively. *Amaranthus* sp has the lowest nitrate concentration of 2.64±0.38 mg/kg, while *Telfaria occidentalis* has the highest 4.80±0.56 mg/kg which is significantly ($p<0.05$) higher compared to the lowest. There is no significant ($p<0.05$) difference in nitrate level of the other vegetables. Nitrite level of *Gnetum africanum* 0.97±0.15 mg/kg is the lowest, while the highest level 1.97±0.74mg/kg is found in *Telfaria occidentalis*. The nitrite level of the vegetables are not significantly ($p<0.05$) different when compared.
Result on Table 2 shows that the fruits contain varying levels of cyanide. Cyanide levels occur in the range of 2.16±0.01 mgHCN/kg to 6.07±0.16 mgHCN/kg. *Dialium guineense* 2.16±0.01 mgHCN/kg as the lowest, while *Chrysophyllum albidum* 6.07±0.16 mgHCN/kg is the highest. The cyanide concentration 6.07±0.16 mgHCN/kg for *Chrysophyllum albidum* is significantly ($p<0.05$) higher than 2.16±0.01 mgHCN/kg for *Dialium guineense*. Cyanide concentration for *Persia Americana* 4.87±0.33 mgHCN/kg is also significantly ($p<0.05$) higher compared to cyanide concentration of *Primus malus* 3.39±0.23 mgHCN/kg and 2.79±0.16 mgHCN/kg of *Anarcardium occidentale*. Nitrate and nitrite level ranged from 14.74±4.12 mg/kg for *Dialium guineense*

Table 1: Cyanide, nitrate and nitrite concentration of leafy vegetables

Sample	Cyanide conc. [mgHCN/kg]	NO_3 [mg/kg]	NO_2 [mg/kg]
Basella alba	5.04±0.20	3.46±1.21	1.56±0.64
Amaranthus spp	5.37±0.24	2.64±0.38	1.73±0.13
Telfaria occidentalis	5.14±1.02	4.80±0.56	1.97±0.74
Gnetum africanum	12.96±3.53	2.78±0.86	0.97±0.15
Laurea tarxicifolia	5.40±0.18	2.79±1.54	1.86±0.53

*Values are mean±SD of triplicate determinations

Table 2: Cyanide, nitrate and nitrite concentration of fruits

Sample	Cyanide conc. [mgHCN/kg]	NO_3 [mg/kg]	NO_2 [mg/kg]
Dialium guineense	2.16±0.01	14.74±04.12	0.97±0.20
Persia Americana	4.87±0.33	72.76±14.51	1.79±0.54
Chrysophyllum albidum	6.07±0.16	54.65±07.48	2.90±1.74
Anarcardium occidentale	2.79±0.16	57.43±08.68	1.38±0.66
Primus malus	3.39±0.23	61.31±18.74	4.86±0.76

*Values are mean±SD of triplicate determination

as the lowest to 72.76±14.51 mg/kg for *Persia Americana* as the highest and 0.97±0.20 mg/kg for *Dialium guineense* as lowest and 4.86±0.76 mg/kg for *Primus malus* as the highest respectively.

DISCUSSION

Our results show that the leafy vegetables and fruits contain varying concentrations of cyanide, nitrate and nitrite as shown on Table 1 and 2. The leafy vegetables seem to have significantly ($p<0.05$) higher cyanide content compared to the fruits; except for *Chrysophyllum albidum* 6.07±0.16 mgHCN/kg. The fruits have significantly ($p<0.05$) higher nitrate and nitrite levels compared to the vegetables.

Following the ingestion of cyanogenic glycosides, cyanide ions are rapidly absorbed from the Gastro-Intestinal Tract (GIT). The poisoning of hydrocyanic acid is as a result of its affinity for metal ions such as copper and iron. Hence the CN^- radical is a strong poison due to its inhibition of the enzyme cytochrome oxidase at the terminal step of the electron transport chain (Oluwole and Onabulu, 2004; Lehininger, 2005). Cyanide is a highly toxic element and has been implicated as a causative agent in certain diseases (Kamalu, 1995).

The ingestion of cyanide or cyanogenic glycoside can trigger off a lot of toxic manifestations (Eka, 1998; Onabolu *et al*., 2001). Ingestion of acute and innocuous levels of cyanide is known to cause instant death and a number of debilitating neurological disorders respectively (Oluwole *et al*., 2002; Oluwole and Onabulu, 2004). The fatal oral dose to humans is believed to be only 60-90 mgHCN/kg fresh weight or 0.5-3.5 mgHCN/kg body weight. The WHO recommended safe level is 10 mg of HCN/kg body weight (FAO/WHO, 1991). It has been suggested that cyanide exposure from cassava diet is the cause of Tropical Ataxia Neuropathy (TAN) seen in Nigeria (Oluwole *et al*., 2003). Repeated injections of cyanide can produce neurological changes in animals, while repeated oral ingestion of sub-lethal doses can also lead to chronic neurological problems (Famuyiwa *et al*., 1995). In humans, two neurological syndromes resulting from chronic exposure to cyanide have been recognized: Leber's disease and tobacco amblyopia, which are caused by hydrocyanic acid contained in tobacco smoke (Famuyiwa *et al*., 1995; Oluwole *et al*., 2003). A strong association has also been established between Epidemic Spastic Paraparesis (ESP) and a high dietary intake of cyanide and low intake of sulfur needed for cyanide detoxification (Oboh and Ekporigin, 2004). The disease [ESP] mainly affects women and children. It manifests as an acute disease permanently crippling the victim from one day to the next by damaging nerve tracts in the spinal cord that transmits signal for movement.

Occurrence of intoxication symptoms of cyanide depend upon the rate of the increase of cyanide concentration of the tissues. The decisive factor is determined by the kind of cyanide compound, the way of intoxication, the digested dose and the ability of the organism to detoxify it (Hahs, 1988). Upon uptake of higher doses, survival time up to three hours can be observed. Small non lethal dose may cause headache, irritation of the mucus membrane of the eye and throat (Pitchumoni *et al*., 1988; Kamalu, 1995). Most of the clinical symptoms of hydrocyanic acid poison can be explained on the basis of its affinity for metal ions such as copper and iron. It combines with hemoglobin to form cyanohaemoglobin which is not an oxygen carrier. It also reversibly combines with the copper of the cytochrome oxidase to inhibit its function as an oxidative enzyme in electron transfer (Lehininger, 2005).

The consumption of foods containing cyanogens could result in acute or chronic cyanide toxicity. The former is fatal, resulting to a high rate of mortality and morbidity, while the later has been associated with some disease conditions (Pitchumoni *et al*., 1988; Kamalu, 1995). Nonetheless, the cyanide concentrations of the experimental leafy vegetables and fruits assessed in this study do not pose any considerable danger to consumers, because their average cyanide content is below the estimated maximum sub-lethal dose of cyanide: 20 mgHCN/kg (FAO/WHO, 1991; Onabolu *et al*., 2001). Also, since the vegetables are processed (cooking, steaming, heating, boiling) before consumption, the cyanide levels could be further reduced (Kemdirim *et al*., 1995). But with improper processing methods for the leafy vegetables and the fruits which are eaten raw and fresh, cyanide intake could increase. With possible bioaccumulation, cyanide levels in consumers' tissue may increase, followed by its attendant toxic effects.

Results in Table 1 and 2 show the vegetables and fruits contain varying levels of nitrate and nitrite. Nitrite is able to be produced endogenously. In humans, saliva is the major site for the formation of nitrite with about 5% of dietary nitrate converted to nitrite in the mouth (Gangolli *et al*., 1994). The toxic effects of nitrate are due to its endogenous conversion to nitrite. The range of nitrate conversion is 5-7% for normal individuals and 20% for individuals with a high rate of conversion (JECFA, 2002). Nitrite has been implicated in a variety of long term health effects. Nitrite may also combine with secondary or tertiary amines to form N-nitroso derivatives (Uhegbu and Maduagwu, 1995). Certain N-nitroso compounds have been shown to produce cancers in a wide range of laboratory animals (Zhong *et al*., 2002). That nitrite is known to be a precursor of toxic and carcinogenic N-nitrosamines has been reported (Bassir and Maduagwu, 1978; Uhegbu, 1997) and induces cancer in experimental animals (Mirvish, 1995; Sen and Baddoo, 1997). 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 catalyze the formation of nitrosamines (potent carcinogens) in the stomach from secondary amines and nitrite. (Onyesom and Okoh, 2006). The main source of the nitrite precursor in this environment is vegetables, primarily eaten as the chief supplier of proteins.

The Acceptable Daily Intake (ADI) of nitrate and nitrite set by European Commission's Scientific Committee for Food (ECSCF) is 3.7 mg/kg body weight and 0.06 mg/kg body weight, respectively (Zhong *et al*., 2002).

The concentration of nitrate and nitrite observed in this study is lower than the Allowable Daily Intake (ADI) for nitrate is 0-3.7 mg/kg body weight per day (expressed as nitrate ion) (JECFA, 2002) and may not constitute any health hazard. But with possible bioaccumulation toxic levels may be attained.

ACKNOWLEDGEMENT

The authors acknowledge the assistance of Dr Bob Ezuma of the Department of Plant Science Abia State University, Uturu, who identified the fruits and vegetables used in this work. We also appreciate the contributions of Mr Uche Arukwe of the Biochemistry Laboratory, who helped in the various analytical processes.

REFERENCES

AOAC, 2005. Official methods of analysis of the Association of Analytical Chemists.Horowotz, W and GW Latin [Eds] 18th Edn., pp: 14.

Bassir, O. and E.N. Maduagwu, 1978. Occurrence of nitrate, nitrite, dimethylamine and dimethynitrosamine in some fermented Nigerian beverages. J. Agric. Food Chem., 26: 200-203.

Chung, S.Y., J.S. Kim, M. Kim, M.K. Hong, J.O. Lee, C.M. Kim and I.S. Song, 2003. Survey of nitrateand nitrite contents of vegetables grown in Korea. Food Addit. Contam., 20: 621-628.

Corre, W.J. and T. Breimer, 1979. Nitrate and Nitrite in Vegetables. Wageningen, The Netherlands: Centre for Agricultural Publishing and Documentation.

Eka, O.U., 1998. The chemical composition of yam tubers. In: Advances in yam research, the Biochemistry and technology of yam tubers. Vol. 1 Osuji G (Ed). Biochemical society of Nigeria. ASUTECH. Enugu. Nigeria, pp: 51-57.

Famuyiwa, O.O., A.O. Akanji and B.O. Osuntoku, 1995. Carbohydrate tolerance in patients with tropical ataxic neuropathy-a human model for chronic cyanide intoxication. Afr. Med. Med. Sci., 24: 151-157.

FAO/WHO, 1991. Food Standard programmes. Cordex Alimentaris Commission XII supplement 4 FAO Rome Italy.

Fitzgerald, T.D., P.M. Jeffers and D. Mantella, 2002. Depletion of host-derived cyanide in the gut of the eastern tent caterpillar, *Malacosoma americanum*. J.Chem. Ecol., 28: 257-268.

Gangolli, S.D., P.A. van den Brandt and V.J. Feron, 1994. Nitrate, nitrite and *N*- nitroso compounds. Eur. J. Pharmacol. Environ. Toxicol. Pharmacol. Section; 292: 1-38.

Hahs, R., 1988. Cassava toxicity and food security. Tryck konkat Uppsala, Sweden, pp: 1-2.

Harper, H.J., 1924. The accurate determination of nitrate in soils by phenol disulfonic acid method. Ind. Eng., 16: 180-183.

JECFA (FAO/WHO Expert Committee on Food Additives), 2002. Evaluation of certain food additives. Fifty ninth report of the joint FAO/WHO Expert Committee on Food Additives. Geneva: World Health Organization.

Jorgensen, K., S. Bak, P.K. Busk, C. Sorensen, C.E. Olsen and B.L. Puonti-Kaerlas, 2005. Cassava plant with depleted cyanogenic glucoside content in leaves and tubers.Distribution of cyanogenic glucosides, their sites of synthesis and transport and blockage of the biosynthesis by RNA interference technology. Plant Physiol., 139: 363-374.

Kamalu, B.P., 1995. The adverse effect of long term cassava (*Manihot esculenta* Grantz) consumption. Int. J. Food Sci. Nutr., 46: 65-93.

Kemdirim, O.C., A.O. Chukwu and S.C. Achinewhu, 1995. Effect of traditional processing of cassava on the cyanide content of gari and cassava flour. Plant Foods Hum. Nutr., 48: 335-339.

Lehininger, A.L., 2005. Principles of Biochemistry. 13th Edn., Worth Publishers Inc., New York, pp: 483.

Montgomery, H.A.C. and J.E. Dymock, 1961. The determination of nitrite in water. Analyst, 86: 414-416.

Mirvish, S.S., 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.

Oboh, G. and M. Ekporigin, 2004. Nutritional evaluation of some Nigerian wild Seeds. Nahrung, 48: 95-98.

Okolie, N.P. and R.Q. Omoigborule, 1999. Differential effects of cooking in open cups and in sealed cellophane bags on the residual cyanide content of moi-moi, a processed legume product. Food Chem. Toxicol., 37: 741-743.

Oluwole, O.S., A.O. Onabulu, I.A. Cotgreave, H. Rosling, A. Persson and H. Link, 2002. Low prevalence of ataxia polyneuropathy in a community with high exposure to cyanide from cassava foods. J. Neurol., 249: 1934-1040.

Oluwole, O.S., A.O. Onabulu, I.A. Cotgreave, H. Rosling, A. Persson and H. Link, 2003. Incidence of endemic ataxia polyneuropathy and its relation to exposure to cyanide in a Nigerian community. J. Neurol. Neurosurg. Psychiatry, 74: 1417-1422.

Oluwole, O.S. and A.O. Onabulu, 2004. High mortality of subjects with endemic polyneuropathy in Nigeria. Acta Neuro. Sci., 150: 94-99.

Onabolu, A.O., S.O. Oluwole, M. Bokanga and H. Rosling, 2001. Ecological variation of intake of cassava food and dietary cyanide load in Nigerian communities. Public Health Nutr., 4: 871-876.

Onyesom, I. and P.N. Okoh, 2006. Qualitative analysis of nitrate and nitrite contents in vegetables commonly consumed in Delta State-Nigeria. Br. J. Nutr., 96: 902-905.

Phambu, N., A.S. Meya, E.B. Djantou, E.N. Phambu, P. Kita-Phambu and L.M. Anovitz, 2007. Detection of residual cyanide in cassava using spectroscopic Technique. J. Agric. Food Chem., 55: 10135-10140.

Pitchumoni, C.S., N.K. Jain, A.B. Lowenfels and E.P. DiMaqno, 1988. Chronic cyanide poisoning: Unifying concept for alcoholic and tropical pancreatitis. Pancreas, 3: 220-222.

Prakasa, K. and K. Puttanna, 2000. Nitrates, agriculture and environment. Curr. Sci., 79: 1163-1168.

Sen, N.P. and P.A. Baddoo, 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.

Siritunga, D. and R. Sayre, 2007. Transgenic approaches for cyanogen reduction in cassava. JAOAC Int., 90: 1450-145517.

Uhegbu, F.O. and E.N. Maduagwu, 1995. Occurrence of nitrosatable amines in some Nigerian medicinal plants. Bull Environ. Contam. Toxicol., 55: 643-649.

Uhegbu, F.O., 1997. Dietary secondary amines and liver hepatoma in Port Harcourt, Nigeria. Plant Foods Human Nutr., 51: 257-263.

Walker, R., 1990 Nitrates, nitrites and N-nitroso compounds: A review of occurrence in food and diet and the toxicological implications. Food Addit. Contam., 7: 717-768.

Zhong, W., C. Hu and M. Wang, 2002. Nitrate and nitrite in vegetables from north China: Content and intake. Food Addit. Contam., 19: 1125-1129.