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Some Anion Levels in Fresh Vegetables in Maiduguri, Borno State, Nigeria

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Abstract: Levels of nitrate, nitrite, phosphate and sulphate concentrations of edible parts of some fresh vegetable samples obtained from Alau dam and Gongula in Maiduguri, Borno State, Nigeria were determined. The anions were determined from samples of spinach, lettuce, cabbage, carrot, onion, tomato, beans, groundnut and garlic during their harvesting stages. The anions were determined by Uv-Visible spectrophotometry. The results showed no significant difference between anion levels in vegetable from the two sampling areas $p<0.05$. The values obtained were below the tolerance levels recommended by World Health Organization (WHO). Therefore, the consumption of these vegetables as food does not pose health hazard to human and animal as a result of these anions.

Key words: Vegetable, anions, Maiduguri, Borno State

INTRODUCTION

The anthropogenic activities aimed at enhancing food production may facilitate accumulation of undesirable substances in plants and affect the quality of soil and water resources adversely. Excessive amounts of nitrogenous fertilizers are applied to crops, considering that it is a reasonable insurance against yield losses and their economic consequences. However, when input of nitrogen exceeds the demand, plants are no longer able to absorb it and nitrogen then builds up in the soil, mostly as nitrates (Nosengo, 2003). This causes imbalance of nutrients in the soil and increases the nitrate, nitrie and sulphate level in groundwater supplies (NAAS, 2005) which influences the nitrate content of plants (Dapoigny *et al*., 2000; Vieira *et al*., 1998) especially the leafy vegetables. Nitrate has long been one of the highly emotive anions, always being talked about, whether with pride or with horror (Hill, 1999). There is conflicting evidence regarding the potential long-term health risks associated with nitrate, sulphate and phosphate levels encountered in the human diet. Reduction in dietary nitrate is a desirable preventive measure (Santamaria, 2006) stands undisputed.

Vegetables are the major source of the daily intake of nitrate by human beings, supplying about 72 to 94% of the total intake (Dich *et al*., 1996). Therefore, the European Union prescribed, almost a decade ago, the maximum limits for nitrate, nitrite and sulphate in lettuce and spinach, which became the foundation stone for the subsequent European Commission Regulation (No. 1822/2005). Investigations have indicated that a high nitrate accumulation in plants results in nitrite production, which is converted into Nitric Oxide (NO) which, together with O^{-}_{2}, could be rapidly catalyzed by nitrate reductase into peroxynitrite (ONOO-) which is highly toxic to plants (Durner and Klessig, 1999; Lamattina *et al*., 2003). Therefore, high nitrate, nitrite and sulphate accumulation in plants is harmful to human health (Ikemoto *et al*., 2002; Ishiwata *et al*., 2002) as well as to plant growth (Reddy and Menary, 1990; Cantliffe, 1973). The factors responsible for nitrate accumulation in plants are mainly nutritional, environmental and physiological. Nitrogen fertilization and light intensity have been identified as the major factors that influence the nitrate content in vegetables (Anjana *et al*., 2006). Many nutrients, such as chloride, calcium, potassium, sulfate and phosphorus, are involved in the nitrate accumulation process in plants. The nitrate content varies in various parts of a plant (Maynard *et al*., 1976; Santamaria, 2006) and with the physiological age of the plant (Maynard *et al*., 1976; Santamaria, 2006). A reduction in nitrate content can add value to vegetable products already very popular for their nutritional and therapeutic properties (Santamaria, 2006). Therefore, it is important to adopt appropriate strategies and determine the role of individual physiological factors in the process in order to limit accumulation of nitrate, nitrite, sulphate and phosphate in

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vegetables, optimize the use of nitrogen fertilizer and reduce the potential degradation of soil and water resources.

Nitrogenous phosphatic and sulphatic fertilizers, mainly of the nitrate, phosphorous variety, are used widely in vegetable agriculture, resulting in accumulation of nitrate, sulphate and nitrate in plants, if the rate of its uptake exceeds the rate of its reduction to ammonium (Luo *et al.*, 1993). As suggested by McCall and Willumsen (1998) high rates of nitrate application increase the plant nitrate content without increasing the yield. Therefore, growers who apply excessive fertilizers to ensure that nitrogen is not limiting for plant growth are unlikely to achieve any gain in terms of yield, but increase the nitrate content of crops to the levels potentially toxic to humans.

Various classes of vegetables are grown in many parts of Borno State, Northeast Region of Nigeria. They are heavily cultivated and consumed as food (Bokhari and Ahmad, 1985). To enhance the yield of these vegetables, it has been established that in addition to the common nutritive element (N, K, P) as fertilizer and manures are occasionally added to the soil to improve yields. Nitrogenous, phosphatic and sulphatic fertilizers and manures are commonly employed; there are therefor the possibilities of over applications of these fertilizers and manures. Hence, the uptake and storage of nitrate, nitrite phosphate and sulphate by these vegetables is very likely since these salts are soluble and mobile in ground water.

In this study, levels of nitrate, nitrite, phosphate and sulphate in some fresh vegetables (spinach, lettuce, cabbage, carrot, onion, tomato, bean, groundnut and garlic) were determined from the agricultural area of Borno state, Nigeria.

MATERIALS AND METHODS

Sample and sampling: Edible parts of fresh vegetable samples were collected from two different areas on the outskirts of Maiduguri, namely; Alau Dam farming area along Bama Road and Gongula farm settlement. The vegetable samples were collected at the same periods at normal weather condition. Samples were collected from each sampling site for a period of 8 weeks. The samples were properly labeled on collection, placed in plastic bags and transported to the laboratory for subsequent analysis.

Determination of nitrate and nitrite: Levels of nitrate and nitrite analyzed in each of the vegetable samples were carried out in 8 replicates during the sampling periods. Vegetable sample solutions were prepared by chopping each sample into smaller sizes. A known amount (1 g) of the chopped sample was transferred into 100 mL flask and soaked with 50 mL of distilled water. The flask was capped and shaken for 30 min, then filtered into another 100 mL volumetric flask and the volume made to the mark with distilled water (Radojevic and Bashkin, 1999). Determination of nitrate and nitrite was done using Smart spectro Spectrophotometer (2000).

Determination of phosphate: Each of the vegetable samples except was chopped into small pieces. The chopped samples were then air-dried. The air-dried samples were ground and sieved with a sieve of mesh 1mm. A known amount (1 g) of each of the ground and sieved samples was weighed into acid-washed porcelain crucibles. The crucibles were labeled and 5 mL of 20% (w/v) magnesium acetate were added and evaporated to dryness. The crucibles were then transferred into the furnace and the temperature was raised to 500°C. The samples were ashed at this temperature for 4 h. Removed and cooled in a desiccators.

Ten milliliter of 6M HCl were then added to each of the crucible and covered, then heated on a steam bath for 15 min. The contents of each crucible were completely transferred into different evaporating basins and 1 mL of concentrated HNO_3 was added. The heating was made to continue for 1 h to dehydrate silica. One milliliter of 6M HCl was then added, swirled and then followed by the addition of 10 mL-distilled water and again heated on the steam bath to complete dissolution. The contents of the evaporating basins were cooled and then filtered through a Whatman No.1 filter paper into 50 mL volumetric flasks and the volumes made up to the marks with distilled water (Radojevic and Bashkin, 1999). Phosphate was determined using Hach Direct Reading 2000 Spectrophotometer.

Determination of sulphate: For sulphate determination, 5 mL of magnesium nitrate solutions were added to each of the ground and sieved samples in the crucibles. These were then heated to 180°C on a hot plate. When the samples were dried, the temperature was raised to 280°C. The heating process was allowed to continue until the colour of the samples changed from brown to yellow (Kenneth, 1990). The samples were then transferred to the furnace at a temperature of 500°C for 4 h. Magnesium nitrate was added to prevent loss of sulphur. The contents of each crucible were carefully transferred to different evaporating basins. Ten milliliter of concentrated HCl were added to each of them and covered with watch glasses. They were boiled on a steam bath for 3 min. On cooling, 10 mL of distilled water were added to each of the basins and the contents of each were filtered into 50 mL volumetric flasks and the volumes made up to the marks with distilled water (Radojevic and Bashkin, 1999). Sulphate was determined using Smart spectro Spectrophotometer (2000).

Results were subjected to analysis of variance using the General Linear Models (Proc GLM) procedure of the statistical analysis system program package. Proc Univariate procedure was carried out on residuals to support the assumptions of normality made by the researchers. Where a significant Fisher test was observed, treatment means were separated using the Least Significant Difference at $p<0.05$.

RESULTS AND DISCUSSION

Table 1 presents the concentrations of nitrate in some fresh vegetable samples analyzed between October and November 2005 in Alau dam and Gongula, in Maiduguri, Nigeria. From the table, it was observed that groundnut and spinach had high values of 900.78±6.87 and 873.31±25.26 μg g^{-1}, respectively in Alau dam and 904.60±16.36 and 869.13±23.5 μg g^{-1}, respectively in Gongula. Low values of 27.78 ± 2.36 μg g^{-1} in Alau dam and 29.93±2.14 μg g^{-1} in Gongula were noted for onions.

Results of the concentrations of nitrite in the vegetable samples are shown in Table 2. It was observed that, groundnut had the highest values of 195.44±11.15 μg g^{-1} in Alau dam and 199.42±15.40 μg g^{-1} in Gongula. Low values of 8.19±1.15 μg g^{-1} in Alau dam and 8.18±0.37 μg g^{-1} in Gongula were observed for lettuce.

Table 1: Concentration (μg g^{-1}) of nitrate in some fresh vegetables obtained from Alau Dam and Gongula, Maiduguri, Nigeria between the periods of October-November 2005

Vegetables	Alau-Dam	Gongula
Spinach	873.31^a±25.26	869.13^a±23.52
Lettuce	177.76^a±10.14	161.55^a±24.10
Cabbage	163.04^a±17.01	174.63^a±17.00
Carrot	247.45^a±11.55	232.65^a±34.27
Onion	27.78^a±2.36	29.93^a±2.14
Tomato	41.15^a±7.49	39.99^a±8.87
Groundnut	900.78^a±6.87	904.60^a±16.36
Bean	720.00^a±10.92	713.43^a±20.56
Garlic	114.93^a±5.04	108.14^a±8.16

The values given above are mean of replicate values (n = 8) Within row mean values with different letters are significantly different p<0.05

Table 2: Concentration (μg g^{-1}) of nitrite in some fresh vegetables obtained from Alau Dam and Gongula, Maiduguri, Nigeria between the periods of October-November 2005

Vegetables	Alau-Dam	Gongula
Spinach	22.53^a±6.61	20.96^a±3.46
Lettuce	8.19^a±1.56	8.18^a±0.37
Cabbage	10.40^a±1.14	10.36^a±1.94
Carrot	21.27^a±2.93	18.69^a±2.71
Onion	13.94^a±1.75	14.69^a±0.50
Tomato	15.44^a±2.68	16.90^a±0.68
Groundnut	195.44^a±11.15	199.42^a±15.40
Beans	94.65^a±4.54	95.37^a±9.32
Garlic	17.26^a±1.57	16.28^a±1.96

The values given above are mean of replicate values (n = 8) Within row mean values with different letters are significantly different p<0.05

The results obtained from the determination of nitrate in the vegetable samples indicated that the values generally ranged from low to high values. The trend of the variation of nitrate levels in these vegetables is in the order: groundnut>spinach>beans>carrot>lettuce>cabbage>garlic>tomato>onion. The results from the determination of nitrite also indicated that groundnut, spinach and beans recorded high concentrations of nitrites. The trend of the variation showed that groundnut>beans>spinach>carrot>garlic>tomato>onion>cabbage>lettuce. These results are in line with the reports that leafy vegetables contain high amounts of nitrate and nitrite (Kenneth, 1990). The high concentrations of nitrate and nitrite recorded in the groundnut and beans samples can be attributed to the fact that these groups of vegetables belong to the nitrogen-fixing family (*Leguminosae*), which contain the nitrogen-fixing bacteria in their root nodules.

High concentrations of nitrate in vegetables can reflect the over applications of nitrate containing fertilizers to the soils on which these vegetables are grown (Hardison *et al.*, 1996). The levels of nitrate and nitrite vary from one country to the other; this is due to the different farming practices and the uncontrolled applications of fertilizers and manures (especially nitrogen fertilizers) to the soils to improve crop yields. Based on the Acceptable Daily Intake (ADI) (Ketiku *et al.*, 2000) said that the critical values of nitrate content in the same kind of vegetable may vary broadly from country to country owing to differences in vegetable consumption and in vegetable production practices (Radojevic and Bashkin, 1999) gave the maximum permissible nitrate level (in mg kg^{-1}) of spinach as: USA, 3600; Russia, 2100; the Netherlands, 400; the Czech Republic, 730. For other leafy vegetables, the Netherlands and Austral employed a maximum allowance value of 3000 mg kg^{-1}. Russia employed a maximum allowance value of 150 mg kg^{-1} for tomato, 200 mg kg^{-1} for carrot and 2000 mg kg^{-1} for lettuce. The concentrations of nitrate and nitrite observed in these vegetables were however below the daily dietary exposure to the intake of nitrate and nitrite (50-150 mg day^{-1} for Western diet and >300 mg day^{-1} among Vegetarians) as observed by Corre and Breimer (1979). The concentrations of nitrate and nitrite in each of the vegetable samples were not significant ($p>0.05$) between the 2 sample areas.

Figure 1 show a plot of concentration of sulphate and phosphate against vegetable in the vegetable sample.

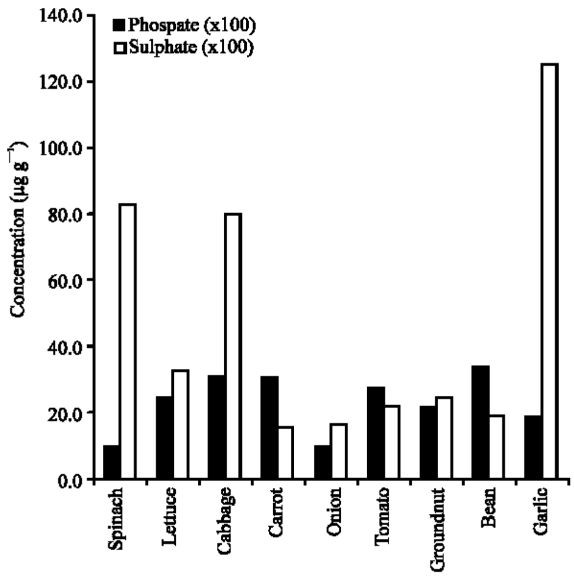

Fig. 1: Phospate and sulphate concentrations (µg g^{-1}) in vegetables

From the figure, it was observed that the phosphate concentrations gave values in the range of 967.69±29.08 to 3390.50±26.26 µg g^{-1}.The lowest and highest values were obtained in onion and beans, respectively. The concentrations of Sulphate in these vegetables varied from 1520.88±24.95 to 12500.47±99.49 µg g^{-1}. The lowest and highest values were observed in carrot and garlic, respectively. The results from the determination of phosphate indicate that each of the vegetable samples recorded high concentrations. The trend of the variation showed that, beans>cabbage>carrot> tomato>lettuce>groundnut>garlic>spinach>onion. The high concentrations of phosphate in these vegetable samples can be attributed to the uncontrolled applications of phosphate fertilizers and manures to the soils on which these vegetables are grown. The concentrations of phosphate in each of the vegetable samples were significantly different, ($p<0.05$) between the two sample areas. However, the concentrations of phosphate in these vegetables were lower than the maximum tolerance daily intake of dietary phosphate of 70.0 mg kg^{-1} body weight (WHO\FAO, 1985).

The results of the analysis of the vegetable samples for sulphate indicated high concentrations of sulphate in all of the samples. The trend of the variation showed that, garlic>spinach>cabbage>lettuce>groundnut>tomato> beans>onion>carrot. This can also be attributed to the uncontrolled application of sulphate fertilizers and manures to the soils on which these vegetables are grown.

The levels were however lower than the maximum permissible limits prescribed by world health organization (Walker, 1990).

CONCLUSION

Results of levels of nitrates, nitrite, phosphate and sulphate in some fresh vegetable grown and consumed as food in maiduguri, Borno state, Nigeria revealed the anions concentration to be below the maximum permissible limits for vegetable as prescribe by World health organization. The consumption of these vegetables therefore does not pose health hazards to human and animals. However periodic evaluations of the vegetables should be carried out to monitor possible accumulations of the anions in future.

REFERENCES

Anjana, S. Umar, M. Iqbal and Y.P. Abrol, 2006. Are nitrate concentrations in leafy vegetables within safe limits? Proceedings of the Workshop on Nitrogen in Environment, Industry and Agriculture, New Delhi, India, pp: 81-84.

Cantliffe, D.J., 1973. Nitrate accumulation in table beets and spinach as affected by nitrogen, phosphorus and potassium nutrition and light intensity, Agron. J., 65: 563-565.

Corre, W.J. and T. Breimer, 1979. Nitrate and nitrite in vegetables. Literature survey N39 Centre for Agricultural Publishing and Documentation Wageningen, pp: 30-37.

Dapoigny, L., S.D. Tourdonnet, J. Roger-Estrade, M.H. Jeuffroy and A. Fluery, 2000. Effect of nitrogen nutrition on growth and nitrate accumulation in lettuce (*Lactuca sativa* L.) under various conditions of radiation and temperature. Agronomie, 20: 843-855.

Dich, J., R. Jivinen, P. Knekt and P.L. Pentill, 1996 Dietary intakes of nitrate, nitrite and NDMA in the Finish Mobile Clinic Health Examination Survey, Food Add. Contam., 13: 541-552.

Durner, J., D.F. Klessig, 1999. Nitric oxide as a signal in plants, Curr. Opin. Plant Biol., 2: 369-374.

Hardison, A., P.A. Gonzalez, I. Frias and J. Raguera, 1996. The Evaluation of the contents of nitrates and nitrites in food products for infants. J. Food. Comp. Anal., 9: 13-17.

Hill, M.J., 1999. Nitrate toxicity: Myth or reality. Brit. J. Nutr., 81: 343-344.

Ikemoto, Y., M. Teraguchi and Y. Kogayashi, 2002. Plasma level of nitrate in congenital heart disease: Comparison with healthy children, Pediatr. Cardiol., 23: 132-136.

Ishiwata, H., T. Yamada, N. Yoshiike, M. Nishijima, A. Kawamoto and Y. Uyama, 2002. Daily intake of food additives in Japan in five age groups estimated by the market basket method. Eur. Food Res. Tech., 215: 367-374.

Kenneth Helrich, 1990. Official Method of Analysis of AOAC (5th Edn.), AOAC Inc. Arlington USA., pp: 56-58.

Ketiku, A.O., M.K.C. Sirdhar and T. Akinruli, 2000. Nitrate and nitrite content of selected Nigeria foods. Afr. J. Biomed., 3: 65-70.

Lamattina, L., C. Garcia-Mata, M. Graziano and G. Pagnussat, 2003. Nitric oxide: The versatility of an extensive signal molecule, Ann. Rev. Plant Biol., 554: 109-136.

Luo, J., Z. Lion and X. Yan, 1993. Urea transformation and the adaptability of three leafy vegetables to urea as a source of nitrogen in hydroponic culture. J. Plant Nutr., 16: 797-812.

Maynard, D.N., A.V. Barker, P.L. Minotti and N.H. Peck, 1976. Nitrate accumulation in vegetables, Adv. Agron., 28: 71-118.

McCall, D. and J. Willumsen, 1998. Effects of nitrate, ammonium and chloride application on the yield and nitrate content of soil-grown lettuce. J. Hortic. Sci. Biotech., 73: 698-703.

NAAS, 2005. Policy options for efficient nitrogen use, Policy paper Nat. Acad. Agric. Sci. New Delhi, pp: 1-4.

Nosengo, N., 2003. Fertilized to death. Nature, 425: 894-895.

Radojevic, M. and V.N. Bashkin, 1999. Practical Environmental Analysis, Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge, UK., pp: 225-403.

Reddy, K.S. and R.C. Menary, 1990. Nitrate reductase and nitrate accumulation in relation to nitrate toxicity in *Boronia megastigma*. Physiol. Plantarum, 78: 430-434.

Santamaria, P., 2006. Nitrate in vegetables: Toxicity, content, intake and EC regulation. J. Sci. Food Agic., 86: 10-17.

Santamaria, P., 2006. Nitrate in vegetables: Toxicity, content, intake and EC regulation. J. Sci. Food Agric., 86: 10-17.

Vieira, R., E.P. Vasconselos and A.A. Montereiro, 1998. Nitrate accumulation, yield and leaf quality of turnip greens in response to nitrogen fertilization, Nutr, Cycl. Agroecosys., 51: 249-258.

Walker, R., 1990. Nitrates, nitrites and N-nitroso compounds. A Review of the Occurrence in Food and Diet and the Toxicological Implications. Food Add. Contam., 7: 717-768.

WHO/FAO, 1985. WHO/FAO Food Additives Data Systems. Evaluation by the Joint WHO/FAO Expert Committee on Food Additives 1956- 1984. FAO Food and Nutrition Paper 30/Rev Rome.