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Determination of Nitrate and Nitrite Contents of Some Edible Vegetables in Guyuk Local Government Area of Adamawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author PA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PH and UTC managed the analyses of the study. Authors PA and PH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Based on research founding's nitrite and nitrate has been discovered as pro-drugs of the vasorelaxant nitric oxide, however many studies have reported that intake of these ions either intravenously or as a food supplements enhanced athletic performance. One of the factors and major causes of gastrointestinal cancer in adults and Methemoglobinemia (blue baby syndrome) in children is nitrosamine derived from nitrate. Approximately 80 to 90 percent of nitrate which enters the human body is through vegetables and fruits, in this present study nitrate and nitrite concentrations in fresh leafy and fruits vegetables in Guyuk was determined and compared with WHO standard limit. The levels of nitrate and nitrite were determined in six edible vegetable samples consumed and cultivated in Guyuk Local Government area of Adamawa State, Nigeria. The vegetables studied were *Spinacea oleracea* (spinach), *Amaranthus caudatus* (Amaranthus), *Lycopersicum esculentum* (Tomato), *Allium cepa* (onion), *Abelmoschus esculent* (Okra), and *Brassica oleracea* (Cabbage). The nitrate and nitrite concentrations were analyzed by ultraviolet

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spectrophotometer method. The results of the present investigation showed no any significant difference in nitrate levels found in most vegetables cultivated during the dry and rainy season harvests. Statistically, the mean nitrates level was higher in spinach (52.50±4.00 mg/kg to 53.50±4.50 mg/kg), Amaranthus (43.50±3.50 mg/kg to 47.50±2.50 mg/kg), Okra (39.00±2.10 mg/kg to 40.50±0.90 mg/kg) and onion (37.50±0.40 mg/kg to 39.00±1.60 mg/kg), intermediate in tomato (34.50±2.40 mg/kg to 36.50±2.40 mg/ kg), and lower in cabbage (15.00±1.60 mg/kg to 20.50±1.50 mg /kg), as compared with those in other vegetables. However, it was discovered that the level of nitrate in all the edible studied vegetable samples was lower than the WHO standard limit. The concentration of nitrite in the different studied vegetables exceeded 1.0 mg/ kg, but is within the permissible limit of the WHO Standard limit. It was observed that the values have no significant difference among most vegetables. It was also noted that nitrate contents in the studied vegetables differs, depending on the kinds of vegetables and were comparable and similar to those vegetables grown and cultivated in other countries.

Keywords: Nitrate; nitrite; edible vegetables; Guyuk Local Government area; Adamawa; Nigeria.

1. INTRODUCTION

Vegetables are highly valued crops; vegetables constitute a major source of human exposure to nitrate and nitrite in human diet. Farmers tend to apply large amount of fertilizer, especially nitrogen fertilizer, this is a reasonable insurance against yield losses from nutrient deficiencies. Today, the use of nitrogen fertilizers plays a key role on plant growth, performance and quality of agricultural products and this is increased in order to boost crop yields and achieve more productivity, thereby, leading to overusing or abuse of nitrogen fertilizers [1-4]. The excessive use of such fertilizers leads to nitrate accumulation in the plants, which causes toxicity and carcinogenic effect, in addition to surface and groundwater pollution these creates environmental hazards [1-4]. Nitrates accumulation in plants, causes different kinds of diseases such as methemoglobinemia in children and gastrointestinal cancer in adults [1-4].

It was estimated that vegetables contributes approximately 80–95% and 16–34% of the average daily dietary intake of nitrate and nitrite, respectively [5,6]. Nitrate concentrations vary significantly, ranging from 1 to 10,000 mg/kg fresh weight, while nitrite level in fresh vegetables are extremely low (< 2 mg/kg), as published literatures [1,7]. Furthermore, nitrate and nitrite have been routinely added during the curing process of certain meat products, serving as a preservative against microorganisms, such as *clostridium botulinum,* that can cause food poisoning. Both nitrate and nitrite are monitored regularly because of their toxicity. There is an increased awareness of the relationship between nitrate and nitrite content in food and water supplies and methemoglobinemia found in infants and the formation of carcinogenic nitrosamines [8,9].

The amount of, nitrate naturally produced in human body is approximately 62 mg daily. Nitrates are naturally present, in most fruits and vegetables and in food and also added as an additive or preservative to food [1,4,10-13]. Nitrate usually enters in to drinking water through contaminated ground water caused by fertilizer, animal, or human waste.

Eighty percent of nitrate in human diet is present mainly in beetroots and mostly in leafy green vegetables such as spinach and lettuce [5,6,14]. Many reports from different studies have revealed the effects of nitrates on human body by its direct consumption in the form of nitrate – rich dietary supplements or inorganic nitrates [15,16]. In this study nitrate and nitrite concentrations in available vegetables grown and cultivated in Guyuk Local Government area of Adamawa State were investigated.

2. MATERIALS AND METHODS

2.1 Area of Study

Guyuk Local Government area of Adamawa State lies between latitude 11°26 ' and 11°34 'N and Longitude 14°12 ' and 14°34 'E.

A total of six fresh edible vegetables samples were collected from three different farms along the bank of river Katanbara in Guyuk Local Government area of Adamawa State, Nigeria. These vegetables include *Spinacia oleracea* (spinach), *Amaranthus caudatus (*Amaranthus), *Solanm lycopersicum* (Tomato), *Allium cepa* (Onion), *Abelmoschus esculent* (Okra) and

Brassica oleracea (Cabbage). The edible vegetables were randomly collected in three (3) reference point, sample site A: Kutugam, sample site B: Purokayo and sample site C: Lakumna, the samples were collected in the rainy and dry seasons between the months of May to December 2011 and January 2012.

Vegetable samples were washed under tap water for about 30 seconds to remove sort dirt or wind borne particles that may have been present on any of the vegetables and then rinsed with distilled water for several times. Each sample was then cut and homogenized with a cutter and a homogenizer, and immediately stored at $-20^{o}C$ before it is subject to analysis. Fifty (50) ML of distilled water was added to the well homogenized sample weighed 1 g in a 100 ml volumetric flask. The flask was then moved in a boiling water bath for 20 min. at 80℃, shaken up and laid on the table until cooled down, and then diluted to a final volume of 100 ml with distilled water. It was followed by filtering through a 0.45 µm syringe filter. The first filtrate of 3 mL was discarded and following filtrate of 1 mL was collected for the determination of the nitrate and nitrite. All samples were immediately analysed within 1hr after sample preparation.

2.2 Statistical Analysis

Data were presented as mean ± standard deviation of three replicates analysis and ranges (minimum and maximum).

2.3 Reagent and Standards

All reagents used in this study were of analytical – reagent grade. All the glassware used was firstly soaked in 10% HCl for 24 hr, and then rinsed with distilled water for several times. The standard nitrate solutions were prepared according to [5] by dissolving 0.25 g of KNO_3 in 250 ml of water, which gave rise to a 0.004 gmL^{-1} of stock solution. Serial dilutions were made to obtain the standard concentrations; 1.0, 4.0, 8.0, 12.0, 16.0 and 20.0 mgL^{-1} nitrate –N

2.4 Nitrate and Nitrite Analysis

Nitrate and Nitrite contents were determined according to AOAC 2000 [17] and Diazo method [18]. Ultraviolet detections were at 410 nm and 254 nm respectively.

3. RESULTS AND DISCUSSION

According to comparison, nitrate concentration in all the samples was lower than standard limit of World Health Organisation (Table 1) and are consistent to Sayed and Rezvan studies 2014 [1].

The results of the investigation of the nitrate contents, of the selected vegetables are reported in Table 1. It was shown that the average nitrate concentration in leafy vegetables (Spinach and Amaranthus) is more than fruit vegetables, (okra, tomato and onions). It was observed that spinach, and amaranthus contained a higher level of nitrates, whilst okra, onion and tomato have an intermediate level and cabbage contained a lower level of nitrates compared with those in other vegetables. The average mean nitrate content of the six vegetables ranges as follows (mg/kg): Spinach, 52.50±4.00 to 55.50±1.50; amaranthus, 43.50±3.50 to 47.50±2.50; okra, 39.00±2.10 to 40.50±0.90; onion, 37.50±0.40 to 39.00±1.60; tomato, 34.50±2.40 to 36.50±2.40; and cabbage, 15.00±1.60 to 20.50±1.50. The reports of many studies have shown that the nitrate content in vegetables can vary from 1 to 10,000 mg/kg, the level of nitrate variation depends not only on genetic factors such as kinds or strains of the vegetables, but also on environmental factors such as nitrogen content of the soil, temperature, amount of sunlight and conditions of cultivation and storage [19-21].

Meanwhile, many studies have reported that the concentration of accumulated nitrates needed for subsequent survival and growth in different parts of vegetables fluctuate. A higher concentration of nitrate was observed in greenish-yellow leafy vegetables [19-21]. According to the report of Scharpf [22] a lower concentration of nitrate was recorded in the vegetable groups of cucumbers and tomatoes (below 500 mg/kg), while higher concentrations were recorded in the groups of lettuce, Spinach, radish and Chinese cabbage [19,22].

Tables 1 and 2 showed the average contents of nitrate and nitrite in six edible vegetables determined in three different locations A, B and C along river Katanbare collected in rainy and dry season respectively. The average nitrate content in spinach ranged from 52.50±4.00 to 55.50±1.50 mg/kg with the highest concentration in site B (55.50±1.50 mg/kg) and the lowest in site C (52.50±4.00 mg/kg). The nitrite average content in spinach ranged from 10.00±1.80 mg/kg in C to 14.00± 2.25 mg/kg in B, the nitrate and the nitrite contents is higher in site B, in the concentration trend order of B>A>C. The average nitrate concentration in amaranthus ranged from 43.50±3.50 to 47.50±2.50 mg/kg. The highest level was recorded in site B and the lowest level in site C. The result of the investigation indicated that site B contained higher concentrations of nitrate and nitrite in almost all the vegetables analyzed, this may be due to the high application of fertilizers by farmers, which might partly explain the higher concentration of nitrate and nitrite in site B soil.

According to comparison, of nitrate and nitrite concentrations (Figs. 1 and 2) the vegetable samples are from highest to lowest including spinach >amarantus> okra > onion> tomato> cabbage, while the nitrite level in all samples is in the trend order amarantus> spinach> onion> tomato> okra> cabbage.

Nitrate levels of vegetables harvested in dry and rainy seasons Table 1 showed no significant difference. Some studies conducted in Europe and other places have shown that nitrate levels of vegetables in winter harvests were higher than those in the summer. However, this difference was not observed in our study, which showed no significant difference in nitrate contents. This disparity in data may be due to different environmental conditions (i.e. length of exposure to sunlight, cultivation methods and seeding time). Guyuk farmers use fertilizers in both dry and rainy season farming, which might partly explain the higher nitrite levels in vegetables.

As seen in Table 2, the nitrite contents in most vegetables were above 1 mg/kg, but within the WHO permissible standard limit of 100 mg/kg. There was no significant difference in nitrite levels of vegetables harvested between the dry and rainy seasons. The comparison of nitrite in different vegetables in Fig. 2, showed that

Table 1. Average concentration of nitrate in the samples and standard limit of sample according to World Health Organization (mg/kg)

Sample	Rainy season May- July (A)	Rainy season August –Oct (B)	Dry season Nov. – Jan. (C)	Range	Standard Limit
Spinach	53.00 ± 2.20	55.50±1.50	52.50 ± 4.00	52.50±4.00-55.50±1.50	(5) 3000–2000
Amaranthus	45.00±3.20	47.50±2.50	43.50±3.50	43.50±3.50-47.50±2.5	-
Tomato	35.50±1.42	36.50±2.40	34.50±2.40	34.50±2.40-36.50±2.40	5(300)
Onion	38.50±3.20	39.00±1.60	37.50±0.40	37.50±0.40-39.00±1.60	(5) 80
Okra	39.50 ±1.25	40.50±0.90	39.00±2.10	39.00±2.10-40.50±0.90	-
Cabbage	17.50± 2.10	20.50±1.50	15.00±1.60	15.00±1.60-20.50±1.50	(5) 500–900

Values are means ± SD and ranges (minimum and maximum). Each type of vegetable was analyzed in triplicate. The numbers in the bracket are used resource number. (A, B, and C reference points of sample collection)

Table 2. Average concentration of nitrite in the samples (mg/kg)

Sample	Rainy season May- July (A)	Rainy Season August- October (B)	Dry season Nov.- Jan. (C)	Range
Spinach	13.00±1.30	14.00±2.25	10.00±1.80	10.00±1.80-14.00±2.25
Amaranthus	14.00±1.12	15.00±2.88	12.00±2.42	12.00±2.42-15.00±2.88
Tomato	12.00±3.50	14.00±1.66	11.00±0.88	11.00±0.88-14.00±1.66
Onion	13.00±2.10	14.00±1.20	12.00±2.40	12.00±2.40-14.00±1.20
Okra	10.00±1.60	11.00±1.70	9.00±1.66	9.00±1.66-11.00±1.70
Cabbage	3.00±1.26	4.00±1.50	2.00±0.50	2.00±0.50 - 4.00±1.50

Values are means ± SD and ranges (minimum and maximum). Each type of vegetable was analyzed in triplicate. (A, B, and C reference points of sample collection) amaranthus has the highest nitrite content within the studied vegetables followed by onion, tomato, spinach and lower in okra and cabbage, also site B is higher in its nitrite content than the other sites.

Standard limit values which are considered healthy to use according to average of nitrate concentration in each vegetable in (mg/kg) is estimated in Table 3. The level of nitrate accumulation value in different organs of vegetables is from highest to lowest including leaflets > leaves > stems > roots > inflorescent > bulb > onion > fruits > seeds [1,15,23]. The leaves contain higher level of nitrate than the seeds and bulbs [1, 23].

Table 3. Standard limit which is healthy to use according to average of nitrate concentration

Kind of vegetable	Dairy consumption value (g/kg)
Spinach	103
Amaranthus	-
Tomato	356.496
Onion	12.2058
Okra	-
Cabbage	36.66

Average of nitrate concentration (mg/kg)

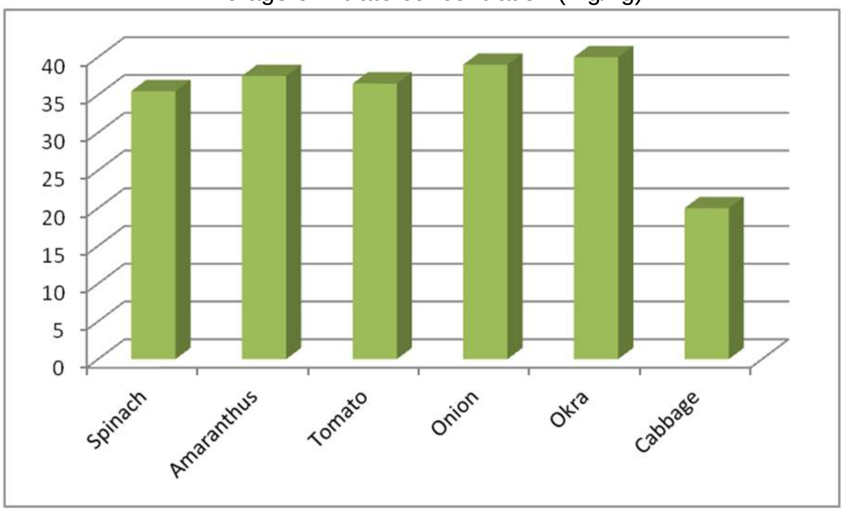

Fig. 1. Comparison of nitrate concentration in vegetables

Average of nitrite concentration (mg/kg)

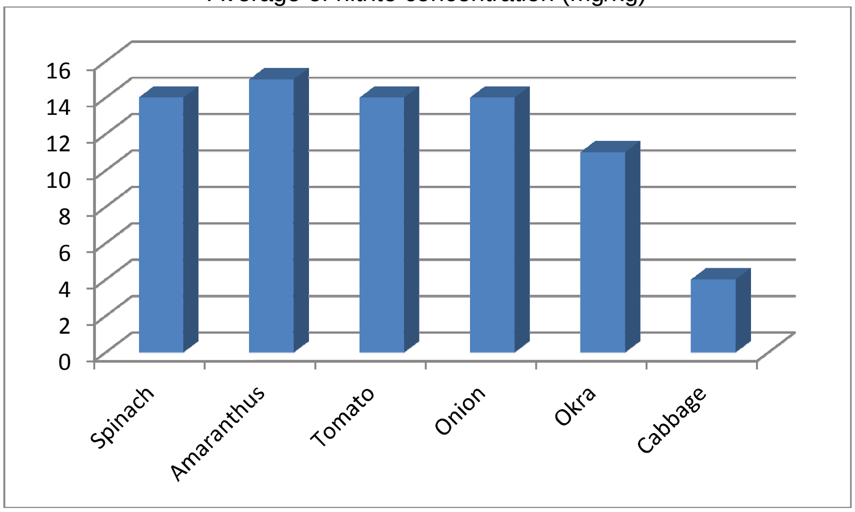

Fig. 2. Comparison of nitrite concentration in vegetables

3.1 International Efforts to Evaluate the Safety of Vegetable Nitrates

Various publications have asserted that such antioxidant components such as ascorbate, a-tocopherol, b-carotene, phenol compounds and indole that occur in plants have strong suppression effects on the formation of nitrosamines, thereby reducing the risk of cancer [24,25]. Furthermore, the panel of the IFT (Institute of Food Technologist Expert Panel on Food Safety and Nutrition) has endorsed the principle that nitrate-containing foods are safe and it also ruled out any scientific basis that would necessitate the establishment of a regulatory regime for such foods. Additionally, the EERO (European Environmental Research Organization) of the EU composed of toxicology professionals from England, Germany, the Netherlands, Belgium and the MIT, USA, have arrived at the final conclusion as follows: Vegetables are a major source of nitrates but they also supply the minimum requirements of vital nutrients and antioxidant agents including vitamins C and E, carotenoid, which in turn suppress the formation of nitrosamine and cyanosis, toxic form of nitrites detected in the human body. Furthermore, there is a great body of evidence that indicates that vegetables have preventive effects against cancer and no scientifically appealing evidence of the toxicity of vegetable nitrates is available [25]. The USA has no regulations in place about the level of nitrates in vegetables, while the EU has established guidelines, but neither the USA nor the EU has found any clear evidence that current nitrate levels in vegetables are harmful to human health. Note that the EU nations were forced to set guidelines as a measure to prevent trade disputes [26].

4. CONCLUSION

From this investigation it was observed that the nitrate concentration recorded in cabbage was very low as compared to other studies done in other places. This is because the concentration of aggregated nitrates needed for subsequent survival and growth in different vegetables fluctuate, also the nitrate content in vegetables can vary from 1 to 10,000 mg/kg, this is because the level of nitrate variation depends not only on genetic factors such as kinds or strains of the vegetables, but also on environmental factors such as nitrogen content of the soil, temperature, amount of sunlight and conditions of cultivation and storage. The result of this investigation also showed that the nitrate concentration in all the samples was lower than the standard limit of World Health Organisation, therefore, the levels of nitrates and nitrites in vegetables grown in Guyuk were lower than the values obtained in other places. In conclusion, it is believed that antioxidant agents ubiquitous in vegetables play a beneficial role as an inhibitor of the nitrosamine formation from nitrites. Therefore, nitrates consumed from vegetables are said to be harmless to human health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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