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Spectroscopic Evaluation of Nitrate and Nitrite Concentrations in Selected Fruits and Vegetables

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Abstract— *This study evaluates the concentration of nitrate and nitrite in selected fruits and vegetables sold in Lafia, Nasarawa state. A total of 15 samples comprising two different fruits (orange and watermelon) and three vegetables (spinach, onion and pepper) were used for this purpose. Nitrate content was obtained by derivative spectrophotometric method and nitrite by colorimetric method. The results showed that all fruits and vegetables analyzed contained detectable levels of nitrate and nitrite except for the orange and watermelon purchased from seller B which had nitrate and nitrite content below the detection limit respectively. The mean nitrate concentrations of the samples were 1348.91 mg/kg for orange, 3539.98 mg/kg for watermelon, 3540.00 mg/kg for spinach, 1434.55 mg/kg for onion and 2669.27 mg/kg for pepper. In addition, the mean nitrate concentrations of watermelon, spinach and pepper were above the maximum level of 2500 mg/kg stipulated by EC, WHO and FAO as opposed to those of orange and onion which were below the specified maximum level. Mean nitrite concentrations which ranged from 0.00025 mg/kg to 0.00039 mg/kg for the fruits and 0.00037 mg/Kg to 0.00056 mg/kg for the vegetables were all below the 100 mg/kg maximum level specified by WHO.*

Keywords— *Nitrate, Nitrite, Fruits, Vegetables, Lafia.*

I. INTRODUCTION

A good nutrition is a fundamental requirement for the wellbeing of adults and the healthy development of children [1]. However, a number of diseases bewildering man today have their origin in diet either directly or indirectly [2, 3]. Methaemoglobinaemia or blue baby syndrome is one of such diseases caused by the accumulation of nitrate which is converted to nitrite in the body. Nitrates (NO_3^-) and nitrites (NO_2^-) are natural chemical compounds found in the soil, water, plants and even the human body. Exposure to nitrates and nitrites also stems from occupational factors mainly due to inhalation and dermal routes especially workers in the explosive and fertilizer industry while farmers experience periodic exposures while handling fertilizers [4]. Due to the undeniable nutritional value of fruits and vegetables, the World Health Organization (WHO) recommends an intake of a minimum of 400 g (five servings) of fruits and vegetables per day for the prevention of chronic diseases [10]. However, fruits and vegetables are known to accumulate nitrates and nitrites owing to the excessive use of nitrogen based fertilizers, organic manure and unfavourable post-harvest conditions (storage and processing methods). Other factors are light intensity, type of soil, temperature, humidity, frequency of plants in the field, plant maturity, vegetation period or season and species variation [11, 12, 13, 14]. It is important to determine the nitrate and nitrite content in fruits and vegetables due to the potential health threat to humans when present in high concentration although of major concern is the level of nitrite which plays an essential role in curing meats, react with secondary or tertiary amines to produce nitrosamines, which may sometimes be powerfully carcinogenic [15, 16].

Several authors have determined the concentration of nitrate and nitrite in fruits and vegetables. Sayed and Rezvan, 2014 measured nitrate and nitrite concentrations in vegetables and fruits and discovered the average of nitrate concentration to be higher in the former while Abu-dayeh, 2006; Susin and Gregorcic, 2006; Razaei *et al.*, 2014; Sayeed and Rezvan, 2014; Alexander, Handawa and Charles, 2016 had nitrate concentrations within the constraints of very low (< 200 mg/kg), low (200-500 mg/kg), medium (500-1000 mg/kg), high (1000-2500 mg/kg) and very high (>2500 mg/kg). The afore-mentioned studies also discussed the associated risks inherent in high uptake of nitrates and nitrites [17, 18, 19, 20, 21]. This research seeks to quantify the nitrate and nitrite contents from the selected fruits and vegetables sold in Lafia.

II. MATERIALS AND METHODS

Sampling and Sample Preparation

Lafia is a town in the central part of Nigeria and the capital city of Nasarawa state. It's on coordinate's 8°29'30"N 8°31'0"E/8.49167°N 8.51667°E [22] and characterised with a tropical savannah climate with an average annual temperature of 36 °C [23]. A total of five different fruits and vegetables (two fruits and three vegetables) were collected from three distinct sellers at Makurdi road and the Lafia modern market all in Lafia town. The fruit samples include orange and watermelon while the vegetable samples were spinach, onion and pepper. The samples were washed in distilled water to remove dust and dried in a forced-air oven at 65 °C for 24 hours, ground and mixed thoroughly [24].

Determination of Nitrate Concentration

Sample extraction

Portions (0.1000 g) of sample tissues were suspended in 10 mL distilled water, kept at 45 °C for 1 hour and then filtered through Whatman filter paper. Samples were extracted and analyzed within 24 hours after extraction when stored at 4 °C. The derivative spectrophotometric determination of nitrate was done according to Lastra [24].

Calibration

Aliquots (0.1 mL) of working standard solutions (10–100 mg/L NO_3^-–N) in a 30 mL tube were mixed thoroughly with 0.4 mL salicylic acid solution. After 20 minutes at room temperature, 9.5 mL 2 N NaOH solution was slowly added to obtain 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, and 1.0 mg/L NO_3^- –N solutions.

Determination of Nitrate Concentration Calculation

A 0.1 mL volume of the preceding extract was thoroughly mixed in a 30 mL tube with 0.4 mL salicylic acid solution. After 20 minutes at room temperature, 9.5 mL 2 N NaOH solution was slowly added [24].

Nitrate–N in plant tissue was expressed as mg NO_3–N/kg dry weight:

$$Cx = \frac{(1D_{388-440-a}) \times 1000}{b \times w}$$

where $1D_{388–440}$ corresponds to the spectrophotometric measurement of the sample, *b* and *a* correspond to the slope and the intercept of the calibration curve of $1D_{388–440}$ versus NO_3^-–N concentration (mg/L), respectively; *w* = tissue weight (g). The value was converted to NO_3^- by multiplication with a factor of 4.43.

Quantitative determination of nitrite

The colorimetric method for the determination of nitrite was done according to Flemer [25].

Statistical Analysis

A descriptive analysis encompassing means, standard deviation and range (minimum and maximum values) were computed.

III. Results and Discussion

The results from this study show that all the samples analyzed contained detectable levels of nitrate except for the orange purchased from seller B with nitrate level below the detection limit. The mean nitrate concentrations of the fruits are 1348.91 mg/kg for orange and 3539.98 mg/kg for watermelon (Table 1) while the vegetables had mean nitrate concentrations of 3540.00 mg/kg, 1434.55 mg/kg and 2669.27 mg/kg for spinach, onion and pepper respectively (Table 2). Furthermore, the mean concentrations of nitrate in different fruits and vegetables in descending order were spinach (3540.00 mg/kg) > watermelon (3539.98 mg/kg) > pepper (2669.27 mg/kg) > onion (1434.55 mg/kg) > orange (1348.91 mg/kg) as shown in Fig. 1a. Also, mean nitrate concentrations and range of spinach obtained in this study were similar to that reported in Hong-Kong with a mean nitrate concentration of 3100 mg/kg and a range of 1100 - 5823.86 mg/kg [26]. However, for the other fruits and vegetables analyzed, the mean nitrate concentrations and range reported in other literatures were considerably lower than those in the present work with mean nitrate concentrations and ranges of 49.88 mg/kg and 2.76-105.97 mg/kg, 38.50 mg/kg and 52.50-55.0 mg/kg for onions [18, 21], 26.61 mg/kg and 13.95-37.7 mg/kg for watermelon [20] and 42.60 mg/kg for pepper [17] respectively. While those in the other literatures had nitrate concentrations: very low (< 200 mg/kg), low (200-500 mg/kg), medium (500-1000 mg/kg), high (1000-2500 mg/kg) and very high (>2500 mg/kg) [17, 18, 19, 20]. However, the present study reports nitrate concentrations ranging from high (1000-2500 mg/kg) to very high (>2500 mg/kg). Maximum levels (ML) of nitrate by the European commission are defined only for spinach and lettuce (EC, 2002). ML prescribed for lettuce (2000–4000 mg/kg for fresh samples) depends on the time of yield, harvest and the type of growing (in the open or under protected conditions) and in spinach (2000–3000 mg/kg for fresh samples) based on the time of yield, harvest and the type of processing (canned or frozen spinach) [19]. The spinach sample used for this study was harvested in June, 2017. The mean concentration of nitrate obtained was 3540.00 mg/kg which was above the maximum level of 2500 mg/kg stipulated by the European commission on the basis of the time of [26]. Also, mean concentrations of watermelon, spinach and pepper from this investigation showed that the content of nitrate was more than the maximum level of 2500 mg/kg as recommended by the WHO and FAO [27]. Nevertheless, the mean nitrate concentrations of oranges and onions were below the maximum level set by WHO and FAO. Even at that, the high levels of nitrates in all the fruit and vegetable samples are alarming as continuous consumption of these fruits and vegetables increases the exposure to nitrate and without a reduction in the intake of these food substances (especially those with nitrate concentration exceeding WHO, FAO and EC limits) it may lead to undesirable effects. In addition, Swallow [28] explained that an important factor that affects nitrate accumulation in plants is the environmental conditions. Nitrate level was found to be affected by high sunlight intensity because it increases the nitrate levels in plant tissues by increasing the activity of the nitrate reductase. This nitrate reductase converts the nitrogen in plant to nitrate. This is a very imperative factor as the intensity of sunlight in Lafia is high. Also, Chung *et al.*, [29] and Tremblay *et al.* [30] showed that the type of crop is a primary factor that affects the nitrate level such that certain foods tend to accumulate large amounts of nitrate. These food substances include: leafy vegetables (such as spinach, lettuce, and cabbage) and root vegetables (such as carrots, beet, and broccoli) though at a much lower level than the leafy vegetables. This may explain why the spinach in this study had a nitrate concentration that exceeded the maximum level of WHO, FAO and EC.

The nitrite concentration in the fruits and vegetables were relatively low with nitrite concentrations not exceeding 1.0 mg/kg and the average nitrite concentration of the fruits ranging from 0.00025 mg/kg for watermelon to 0.00039 mg/kg for oranges. Nitrite concentrations of orange were consistent for all the sellers with a value of 0.00039 mg/kg but that of watermelon ranged from below the detection limit

(seller B) to 0.00039 mg/kg (seller C) as presented in Table 3. The nitrite content of spinach ranged from 0.00036-0.00094 mg/kg with that of seller D having the highest value of 0.00094 mg/kg and sellers E and F having the same values (0.00036 mg/kg). Onions from sellers D, E and F all had nitrite concentrations of 0.00039 mg/kg. The values obtained from pepper were within the range of 0.00036 - 0.00039 mg/kg as depicted in Fig. 1b. Average nitrite concentrations of the vegetables were 0.00056 mg/kg, 0.00039 mg/kg and 0.00037 mg/kg for spinach, onion and pepper respectively as shown in Table 4.

Table 1: Nitrate concentration (mg/kg) in selected fruits sold in Lafia

S/N	Samples	Seller A (mg/kg)	Seller B (mg/kg)	Seller C (mg/kg)	Mean (mg/kg)± SD	Range (mg/kg)
1	Orange	2183.95	BDL	1862.77	1348.91±0.05	BDL-2183.95
2	Watermelon	4110.95	2398.05	4110.95	3539.98±0.08	2398.05-4110.95
	Maximum level = 2500 mg/kg					

Key: SD= Standard deviation, BDL= Below detection limit

Table 2: Nitrate concentration (mg/kg) in selected vegetables sold in Lafia

S/N	Samples	Seller D (mg/kg)	Seller E (mg/kg)	Seller F (mg/kg)	Mean (mg/kg)±SD	Range (mg/kg)
1	Spinach	4003.92	5823.86	792.22	3540.00±0.2	792.22-5823.86
2	Onion	1434.57	2291.02	578.07	1434.55±0.1	578.07-2291.02
3	Pepper	1541.59	4282.26	2183.95	2669.27±0.1	1541.59-4282.26
	Maximum level=2500 mg/kg					

Key: SD= Standard deviation

Table 3: Nitrite concentration (mg/kg) in selected fruits sold in Lafia

S/N	Sample	Seller A	Seller B	Seller C	Mean±SD	Range
1	Orange	0.00039	0.00039	0.00039	0.00039±0.00	
2	Watermelon	0.00036	BDL	0.00039	0.00025±0.01	BDL-0.00039
	Maximum level=100 mg/kg					

Key: SD= Standard deviation, BDL= Below detection limit

Table 4: Nitrite concentration (mg/kg) in selected vegetables sold in Lafia

S/N	Samples	Seller D	Seller E	Seller F	Mean ±SD	Range
1	Spinach	0.00094	0.00036	0.00036	0.00056±0.01	0.00058
2	Onion	0.00039	0.00039	0.00039	0.00039±0.00	0
3	Pepper	0.00036	0.00036	0.00039	0.00037±0.01	0.00003
	Maximum level=100 mg/kg					

Key: SD= Standard deviation

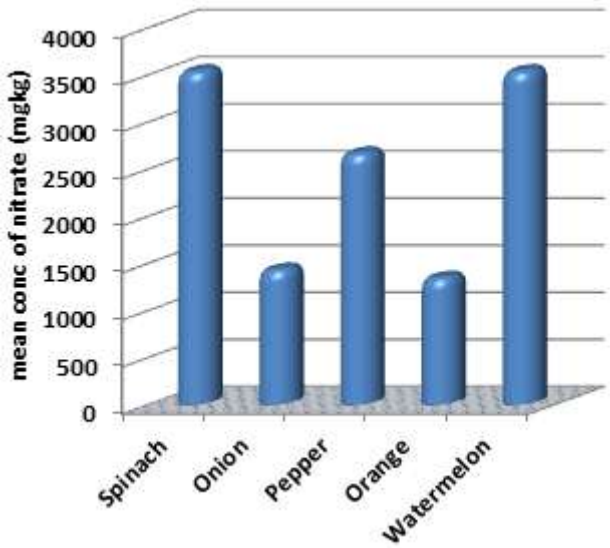

Fig. 1a: Mean concentration of nitrate in the fruits and vegetables

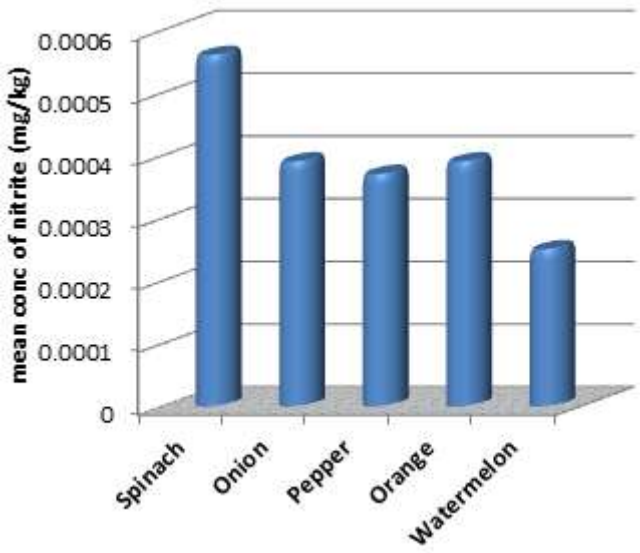

Fig. 1b: Mean concentration of nitrite in the fruits and vegetables

The mean concentrations of nitrite in different fruits and vegetables in descending order were spinach (0.00056 mg/kg) > orange and onion (0.00039 mg/kg) > pepper (0.00037 mg/kg) > watermelon (0.00025 mg/kg). In addition, mean nitrite concentrations and range of the fruits and vegetables obtained in this study were lower than those reported in other literatures [17, 20, 21, 26]. However, continuous intake of low nitrite containing food substances can bioaccumulate in the

body system which can lead to cancer and methaemoglobinaemia [30]. The nitrite concentrations in all the selected fruits and vegetables were below the maximum level of 100 mg/kg specified by WHO [21].

IV. CONCLUSION

The study showed that nitrate and nitrite were present in all the selected fruits and vegetables except for the orange and watermelon purchased from seller B. Although the nitrate concentration of orange and onion were below the WHO and FAO maximum level, the high values of nitrate in other fruits and vegetables studied may likely pose a health challenge as nitrate is reduced to nitrite which is a toxic matter. On the other hand, the nitrite content in all the samples were low and are therefore unlikely to pose any health threat. However, in order to maximize the health benefits of consuming fruits and vegetables, measures have to be taken to reduce the nitrate and nitrite exposure while maintaining the recommended intake of fruits and vegetables of the general populace.

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