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Nitrate contents in some vegetable leaves in Sokoto Metropolis, Nigeria

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Popular Nigerian vegetables namely, cabbage, spinach, bitter leaf, water leaf, ewedu, roselle and lettuce obtained from Sokoto metropolis, Nigeria were analysed for nitrate contents by ultra-violet (UV)-spectrophotometric method. The fresh leaves of the samples were chopped and ground using mortar and pestle for the analysis. Absorbance of each sample was obtained in three replicate and the calibration graph of standards nitrate were used in determining the concentration of each sample. Cabbage, spinach, ugwu and lettuce contain the lowest amount of nitrate in this study (0.109 ± 0.035 µgml^{-1}, 1.530 ± 0.130 µgml^{-1}, 1.730 ± 0.328 µgml^{-1} and 2.185 ± 0.157 µgml^{-1}) in comparison with the nitrate contents of samples like roselle, ewedu, water leaf and bitter leaf which contain the highest amount (2.938 ± 0.060 µgml^{-1}, 3.682 ± 0.140 µgml^{-1}, 3.924 ± 0.160 µgml^{-1} and 4.351 ± 0.190 µgml^{-1}). These values fall within those recommended for nutritional purpose.

Key words: Nitrate contents, vegetables, Sokoto, absorbance.

INTRODUCTION

A number of ailments have their origin in our diet, either directly or indirectly. Many modern diseases are as a result of nutritional deficiencies. Fortunately, in many cases, by simply increasing the vegetables intake can solve these problems as long as they have not been ignored for too long (Mason, 2010; Sasathorn et al., 2015). Green vegetables are a major source of dietary nitrate intake. Nitrate may have several beneficial health effects mediated through reactive N intermediates, including antibacterial effects and effects on gastric mucosal integrity (Andra's et al., 2014; Keszei et al., 2013).

Nitrate is a naturally occurring compound that is part of nitrogen cycle, as well as approved food in which they play an important role in the nutrition and function of plants. Nitrates are important components of vegetables, they occur widely in our drinks and food (Okafor and Ogbonna, 2003; EFSA, 2008). High levels of nitrate tend to occur in the leaves whereas lower level occurs in the seeds and tubers. Nitrates are nitrogen-oxygen chemical units which combine with various organic and inorganic compounds (Croitoru, 2012). Once taken into the body, nitrates may be converted in to nitrites. Crop containing high level of nitrates can be identified by laboratory test. Nitrates in vegetables and fruits have no taste or smell (FFTC, 2007). Nitrates occur naturally in fruits and vegetables, but only in small quantities, they can rise to high levels in intensively grown crops (Croitoru, 2012).

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Nitrate concentrations in vegetables depend on the biological properties of the plant culture, light intensity, type of soil, temperature, humidity, frequency of plants in the field, plant maturity, vegetation period, harvesting time, storage time and source of nitrogen (Shohreh et al., 2015; Tamme et al., 2006).

The high concentration of nitrogen in fertilized soil may lead to the high nitrate level in edible vegetables and toxic level of nitrate may be produced by microbial activity in the gastrointestinal tract of the consumer of such vegetables (Tanaka et al., 1982; Thomson et al., 2007). Nitrates and their precursor's nitrites are both naturally occurring substances and are produced by living cells (Shohreh et al., 2015). They are involved in many important chemical reactions in the body. Vegetables and fruits sources of nitrates are considered healthy whereas preserved meat sources are not. Indeed 70 - 80% of our consumption of nitrates is thought to be from plant sources as well as from water (NYR, 2013; Contam, 2008). Nitrates are soluble salts of nitric acid. The solubility of nitrates is important, as they are absorbed in solution by plants through their root system (Tamme et al., 2006). Nitrates occur in the soil through the effect of lightning or atmospheric nitrogen and oxygen, and through the decay of dead plants and animals, as well as by use of fertilizers (Harwood, 2008).

High nitrate content is a potential human threat especially to infants, causing the problem known as methemoglobinemia, also called "blue baby syndrome" (Andra's et al, 2014). When nitrate is taken in by eating food and drinking water, it is converted in the gut to nitrite, which then combines with haemoglobin to form methemoglobin, thus, decreasing the ability of the blood to carry oxygen in the human body (FFTC, 2007).

Some studies have raised a concern about cancer causing-potential of nitrates and nitrites which are used as preservative and colour enhancing agents in meat. Nitrates react with amino acids to form nitrosamine which has been reported to cause cancer in humans (ATDSR, 2007; Pham et al., 2008).

Tanaka et al. (1982) reported a sensitive and direct spectrophotometric method for the determination of nitrates in vegetables using 2-sec-butylphenol. The basis for this method is that 2-sec-butylphenol reacts quantitatively with nitrate in acidic solution. Gaya and Alimis (2006) also reported a spectrophotometric determination of nitrate in vegetables using phenol. The method is based on the measurement of the absorbance of yellow sodium nitrophenoxide formed via the reaction of phenol with the vegetable-based nitrate in the presence of sulphuric acid.

This current work demonstrated the effectiveness of a standard calibration plot for the determination of the concentration of nitrate in an unknown sample. The investigation also aimed at determining the contents of nitrate in the vegetable leafs consumed in Sokoto, Nigeria using spectrophotometric method and also to see if the level of nitrate found in the leafs is in line with the approved daily in take.

MATERIALS AND METHODS

Materials

Sodium hydroxide (MF: NaOH, MW: 40.00 g/mol, CAS: 1310-73-2, Assay: 97%), silver sulphate (MF: Ag_2SO_4, MW: 311.80 g/mol, CAS: 10294-26-5, Assay: 99.99%), sodium carbonate (MF: Na_2CO_3, MW: 105.99 g/mol, CAS: 497-19-8 Assay: 99.99%), sulphuric acid (MF: H_2SO_4, MW: 98.08 g/mol, CAS: 7664-93-9, Assay: 99.99%), toluene (MF: $C_5H_5CH_3$, MW: 92.14 g/mol, CAS: 108-88-3, Assay: 99.80%) and phenol (MF: C_6H_5OH, MW: 94.11 g/mol, CAS: 108-95-2, Assay: 99.50%), were purchased from Sigma-Aldrich (Dorset, UK). Vegetable samples, namely: spinach, lettuce, water leaf, bitter leaf, ugwu, ewedu, roselle and cabbage were purchased from Sokoto fish, meat and vegetable market. The materials were used as received.

Methods

Wavelength determination (λ_{max})

A stock solution of 100 μgml^{-1} of nitrate was prepared in deionised water to determine the lambda max. Subsequently, a standard solution of 25 μgml^{-1} was prepared in a 25 ml volumetric flask, and a Cary 60 UV/Vis spectrophotometer was used to determine the wavelength of maximum absorption in the range 200 - 800 nm. Figure 1 shows the spectrum of nitrate.

Calibration graph

A Cary 60 UV/Vis spectrophotometer (Agilent technologies) was used to determine the concentration of nitrate. A stock solution of 100 μgml^{-1} of nitrate was prepared in deionised water. Absorbance of 8 standards solutions (0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0, 25.0 μgml^{-1}) each with three replicates were determined at 282 nm. Beer's Law calibration plots of absorbance versus concentration of nitrate showed no deviation from linearity with regression coefficients ≥ 0.9999 and an intercept of 0.0039.

Sample preparation

Fresh vegetable samples were chopped and ground using mortar and pestle till homogenous slurry was formed, 10 g of the slurry was taken in to a 250 ml beaker. 70 ml of deionised water and 2.5 ml of 4% NaOH solution were added. The content of the beaker was warmed at 80°C for 25 min with occasional shaking. The resulting solution was filtered through a fluted filter paper into a 100 ml volumetric flask and made up to the mark with deionised water. An aliquot of 4 ml of the diluted solution was taken into a test tube cooled in an ice. 1 ml of 5% Ag_2SO_4 solution was added followed by subsequent addition of 7 ml of concentrated H_2SO_4 solution and 0.1 ml of 5% phenol solution. The solution was allowed to stand for 20 min with occasional shaking and the resulting mixture was extracted with toluene after shaking for another 10 min in a 50 ml separating funnel. The lower aqueous layer was discarded, the organic phase was washed twice with 10 ml of deionised water by shaking for 2 min and each time discarding the aqueous phase. The organic phase was extracted again by shaking for 1 min with 10 ml of 10% Na_2CO_3 solution and then the resultant product was collected in a test tube. The procedure was carried out for all the vegetable samples as described above.

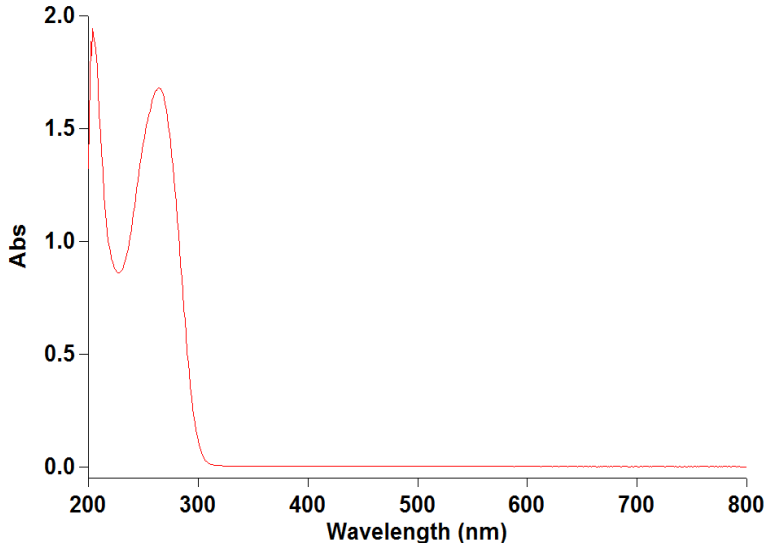

Figure 1. UV spectrum of nitrate.

Table 1. Absorbance (A.U) and concentration (µgml^{-1}) of nitrate + STDEV.

Concentration (µgml^{-1})	Absorbance (A.U)			Mean (A.U)	STDEV
	1st	2nd	3rd		
0	0	0	0	0	0
2.5	0.0857	0.0897	0.0911	0.0889	0.0028
5.0	0.1832	0.1756	0.1727	0.1772	0.0054
7.5	0.2732	0.2820	0.2676	0.2743	0.0073
10.0	0.3627	0.3672	0.3575	0.3625	0.0049
12.5	0.4559	0.4586	0.4522	0.4556	0.0032
15.0	0.5439	0.5425	0.5399	0.5421	0.0020
20.0	0.7349	0.7335	0.7283	0.7322	0.0035
25.0	0.9262	0.9198	0.9165	0.9208	0.0049

UV/visible spectroscopy

Absorbance eight of the standards and the samples were measured with Cary 60 UV/Visible spectrophotometer at 282 nm. The samples containing total nitrate content were placed into a cell equipped with a quartz window. Measurements were made in triplicates for both the standards and the samples. Concentrations in the liquid samples were analysed using the equation of the graph and the mean of the absorbance of nitrate and their corresponding standard deviations were calculated.

RESULTS AND DISCUSSION

Calibration graph

Table 1 shows the absorbance of standard concentration of nitrate in three replicate with their mean and standard deviation. These results were utilised in building a calibration graph of the known standards. The data presented in Table 1 can also be seen in Figure 2.

The data point represents the mean of 3 results with SD error bars. The graph shows the line equation and R^2 value is included to aid further calculations. The error bars on the graph are very small which shows the significance of the observed data to the linear relationship.

From Table 1, the mean of three replicates of the absorbances were plotted against the concentration of the standards and standard deviation where each replicate was used to calculate error bars on each data point. Looking at the graph (Figure 2), the plot generated exhibited an excellent linearity, the equation of the graph $y = 0.0368 x – 0.0039$ shows the gradient of 0.0368, intercept – 0.0039 and $R^2 = 0.9999$ which is very close to +1. The observed results are due to careful handling during solution preparation, making the calibration plot less susceptible to random errors which can affect the

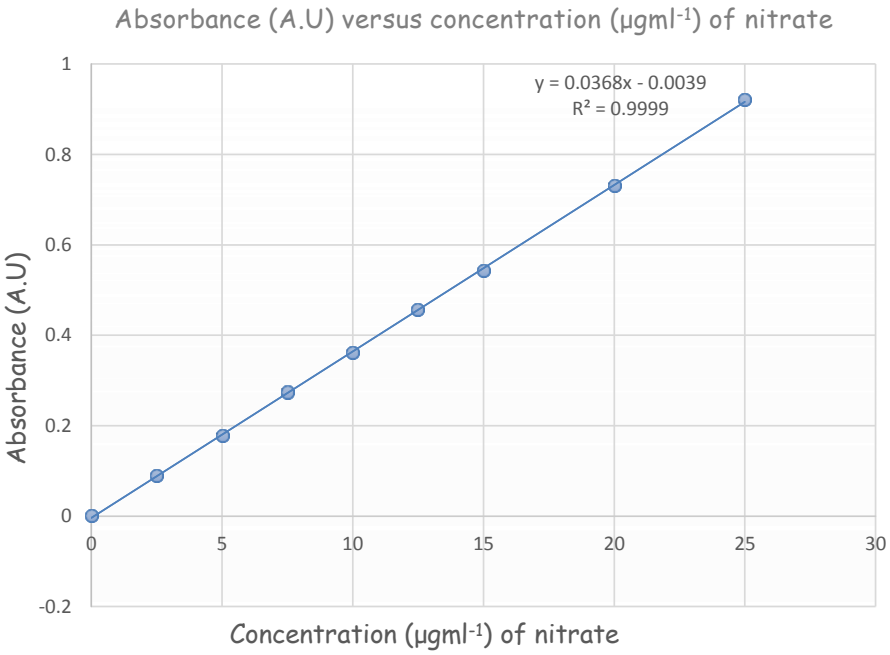

Figure 2. Graph of absorbance (A.U) versus concentration (µgml^{-1}) of nitrate.

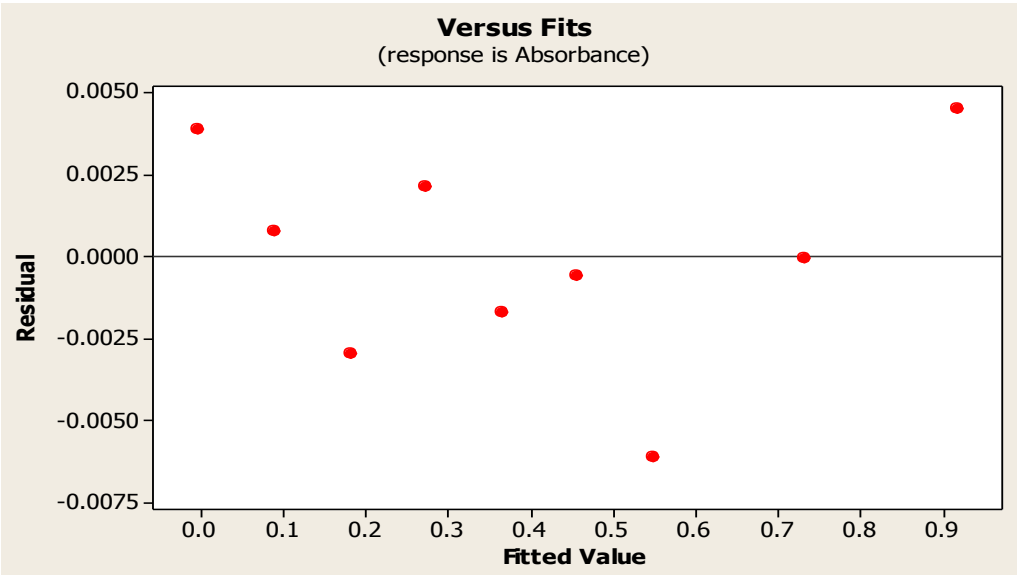

Figure 3. Residual versus fitted value of the calibration graph.

results.

The graph shows the residual on the vertical axis and fitted value (independent variable) on the horizontal axis. The linear regression model for the data is appropriate because the points in the graph are randomly distributed on the horizontal axis indicating a good fit of the graph in Figure 2.

In order to examine whether the set of data in Figure 2 is a good fit, residuals of the line of the graph above was investigated, this graph revealed a fairly random distribution relationship between the residual and concentration, thus signifying that a decent fits to the data (Figure 2) is provided by a straight line model.

Linearity and the best-fit line were obtained by linear regression (Figure 3) which operates by obtaining the line that gives a minimum value for the sum of the squares of the distances of all the points from that line. The best-fit line occurs at the standard concentration of 20 µgml^{-1} and

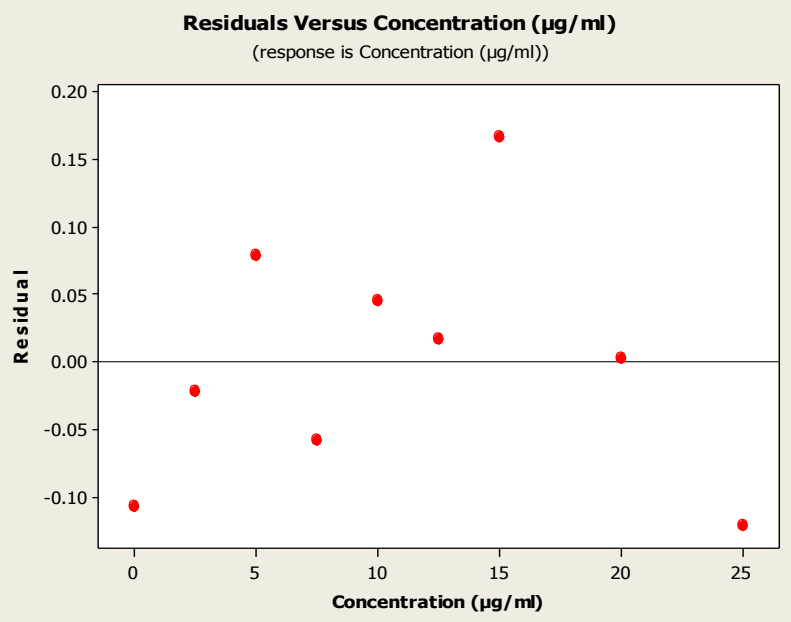

Figure 4. Residual versus concentration (µgml^{-1}) of the calibration graph.

Table 2. Total nitrate content in vegetables.

Sample	Botanical name	Amount of nitrate (µgml^{-1})
Cabbage	*Brassica Sativa*	0.109 ± 0.035
Ugwu	*Theifricia Occidentalis*	1.730 ± 0.328
Ewedu	*Chochorus Sativa*	3.682 ± 0.140
Water leaf	*Talinun Triangulare*	3.924 ± 0.160
Bitter leaf	*Vernonia Amygdalinan*	4.351 ± 0.190
Spinach	*Spinacia Oleracea*	1.530 ± 0.130
Lettuce	*Lactiva Sativa*	2.185 ± 0.157
Roselle	*Herbiscus Sabdariffa*	2.938 ± 0.060

the least fit-line occur at concentration of 25 µgml^{-1}. The accuracy of the plot was checked by plotting a graph of residual versus fitted values and also a plot of residual as a function of concentration (Figures 3 and 4). Data generated from the two plots is randomly distributed which signifies the certainty of the graph. As discussed earlier, looking at the trend pattern in Figures 3 and 4, data is randomly distributed and the instrument response increases as the concentration of the standards increased. Problems of non-linear range and matrix effect that normally occur due to an instrument problem were not witnessed which also signifies that the results obtained relied on interpolation (certainty of the results).

In summary, the resultant calibration graph proved suitable for use in the nitrate analysis.

LOD and LOQ

Detection limits and quantification limits. The limits of detection (LOD) of the proposed method were determined at a signal-to-signal ratio of 3, whereas the limits of quantification were obtained at a signal-to-signal ratio of 10. The results showed LOD of 0.522 µgml^{-1} and LOQ of 17.391 µgml^{-1} for the graph analysed.

Nitrate contents in the vegetable samples

Eight leafy vegetables samples were collected from local market during the period of June 2010. Three replicates of each sample were analysed and nitrate contents were evaluated as the mean of three measurements. The contents obtained are detailed in Table 2.

Spectrophotometric analysis was carried out in order to determine the amount of nitrate in the vegetable samples. The amount of nitrate from within all the vegetable was studied using Cary 60 spectrophotometer. From the Table 2, it shows that nitrate content was found in detectable amount in all the vegetables investigated. The

table illustrates the amount of nitrate in the vegetable samples. Vegetables such as cabbage, spinach, ugwu and lettuce contain the lowest amount of nitrate in this study (0.109 ± 0.035 μgml^{-1}, 1.530 ± 0.130 μgml^{-1}, 1.730 ± 0.328 μgml^{-1} and 2.185 ± 0.157 μgml^{-1}) in comparison with the nitrate contents of samples like Roselle, ewedu, water leaf and bitter leaf which contain the highest amount (2.938 ± 0.060 μgg^{-1}, 3.682 ± 0.140 μgg^{-1}, 3.924 ± 0.160 μgml^{-1} and 4.351 ± 0.190 μgml^{-1}). These results are in comparison with the study carried out by Ann et al. (2014) on how high-nitrate vegetable diet increases plasma nitrate and nitrite concentrations and also reduces blood pressure in healthy women.

Conclusion

The locally available vegetables are valuable and natural sources of nitrate. The results show that these vegetable leaves are a very good source of nitrate. This can be testified from the fact that nutritive recommendation for nitrate is 3.70 $mgkg^{-1}$ body weight. This paper presents a spectrophotometric method usable for simultaneously determining nitrate in vegetables with high sensitivity, accuracy and precision. Such method is extremely important in the biomedical research regarding the formation of nitric oxide and in the toxicological research regarding the presence of nitrate as toxins in vegetables or biological material. Other techniques such as HPLC and GC-MS could also be adopted in improving this research.

Conflict of interests

The authors did not declare any conflict of interest.

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