JASEM ISSN 1119-8362
Full-text Available Online at www.bioline.org.br/ja
J. Appl. Sci. Environ. Mgt. *March*, 2006
Vol. 10 (1) 79 - 82

Spectrophotometric Determination of Nitrate in Vegetables Using Phenol

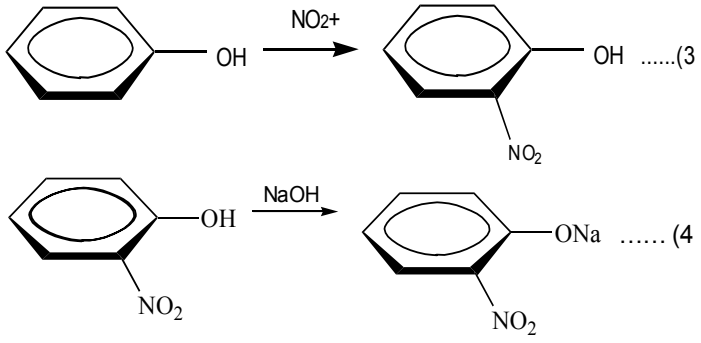
***GAYA, U I; ALIMI, S**
Department of Chemistry, Bayero University, P.M.B 3011, Kano, Nigeria
**Corresponding Author. E-mail: umargaya2000@yahoo.com*

ABSTRACT: A rapid and sensitive spectrophotometric method for the determination of nitrate in vegetables is described. 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 presence of sulphuric acid. The analytical concentration of the acid has marked effect on the nitrate determined. The colour development was rapid and remained stable overnight. Analysis of six vegetable samples containing nitrate gave satisfactory mean recoveries of 76 to 123% in 18 determinations. The proposed method is reproducible and sensitive to lower level concentrations. @JASEM.

The high concentration of nitrogen in soil may lead to the high nitrate levels in edible vegetables and toxic levels of nitrite may be produced by microbial activity in the gastrointestinal tract of the consumer of such vegetables (Tanaka *et al*., 1982). Nitrate containing vegetables are potential causes of methemoglobinemia, a disease which may occur in children older than 6 month (Sanchez-Echaniz *et al.*, 2001). Since nitrate-nitrogen is lost during digestion (Rowell, 1994), the determination of nitrate as nitrate-nitrogen via digestion becomes difficult. Pfeiffer and Smith (1975) determined nitrate in baby food using ion selective electrode which showed a good correlation with the AOAC xylenol method. Some of the methods employed are based on the nitration of bi-substituted phenol such as 2,4-xylenol, 3,4-xylenol and 2,6-xylenol (Tanaka *et al*., 1982). Due to the involvement of distillation stage the 2,4-xylenol method is time consuming (Tanaka *et al*., 1982) and therefore unsuitable for routine food analysis. The 2,4-xylenol method was modified by Toyoda *et al*. (1978) and a gas-liquid chromatographic method was developed. Basset *et al.* (1978) reported a titrimetric method for the nitrate determination which could be erroneous if applied to vegetables. Tanaka *et al*. (1982) reported a sensitive and direct spectrophotometric method for the determination of nitrate in vegetables using 2-*sec*-butyl phenol. The basis for the method is that 2-*sec*-butylphenol reacts quantitatively with nitrate in acidic solution. Beijaars *et al.* (1994) reported a determination of nitrate in vegetables by continuous flow procedure and subsequent reaction of the nitrate extracted with sulfanilamide and N-1-naphthylethylenediamine to form a reddish-purple azo dye which was measured colorimetrically at 530nm. This research aimed at developing a method for the determination of nitrate in vegetable products by using phenol as the active reagent. The principle is based on the nitration of phenol and the formation of the corresponding sodium salt. The elementary processes involved are

$$NO_3^- + H_2SO_4 \rightarrow HSO_4^- + HNO_3 \quad \ldots\ldots\ldots\ldots (1$$

$$HNO_3 + H_2SO_4 \rightarrow NO_2^+ + {}^-OSO_3H + H_2O \quad \ldots\ldots\ldots (2$$



Ultimately, 1 mol of sodium nitrophenoxide is produced from 1 mol of nitrate-nitrogen. Since sodium nitrophenoxide absorbs ultraviolet light it provides a basis for the determination of nitrate as nitrate-nitrogen. The reaction is quantitative and analogous to that reported by Tanaka *et al*. (1982).

**Corresponding Author. E-mail: umargaya2000@yahoo.com*

MATERIALS AND METHODS

Unicam UV 1 uv-visible spectrophotometer v1.30 was used for analysis. Fresh vegetable samples were obtained from Dorayi ward, forming part of Kumbotso Local Government, Kano, Nigeria. All reagents used were of analytical-reagent grade. The vegetable sample was chopped and ground in a porcelain mortar till homogeneous slurry was formed. 10g of the slurry was taken into a 250cm^3 beaker by washing with 70 cm^3 distilled water and 2.5ml of 4% NaOH was added. The content of the beaker was warmed at 80^{0}C for 25min with occasional shaking. The resulting solution was filtered through a fluted filter paper into 100 cm^3 volumetric flask and made up to the mark. An aliquot of 4 cm^3 was taken into a test tube cooled in ice. 1 cm^3 of 5% Ag_2SO_4 solution was added followed by subsequent addition of 7 cm^3 of 98% H_2SO_4 and 0.1 cm^3 of 5% phenol solution. The solution was allowed to stand for 20min while shaking occasionally. The resulting mixture was extracted in 50 cm^3 separating funnel by adding toluene and shaking for 5 to 10min. The lower aqueous layer was discarded. The organic phase was washed twice with 10ml of distilled water by shaking for 2min and each time discarding the aqueous phase. The organic phase was extracted again by shaking for 1 min with 10 cm^3 of 10% Na_2CO_3 solution and collected in a test tube. Absorbance was read at 407nm. Since 4cm^3 of the 100cm^3 filtrate was used for analysis.The amount of nitrate (µg/g) in the vegetable was calculated by the formula

$$\text{Nitrate} = \frac{C \times 100}{W_s \times 4}$$

where C = Concentration of nitrate in the sample as from calibration graph (µg cm^{-3}) (Fig 1a); W_s = Weight of the slurry used (g).

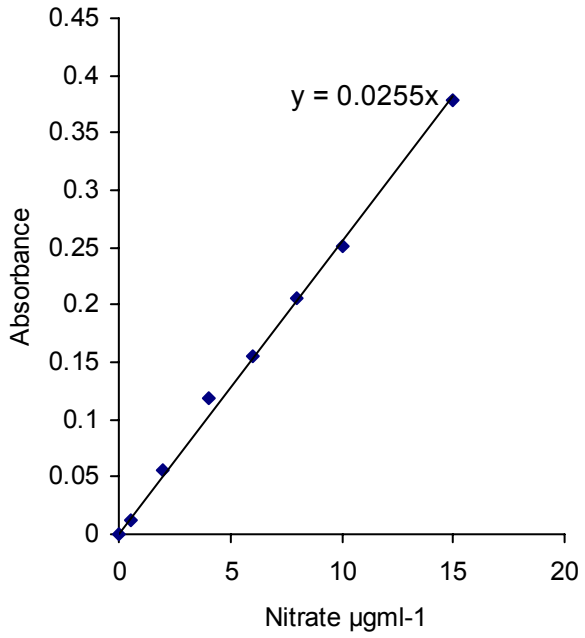


Fig 1a. Nitrate calibration curve

Aliquots of standard nitrate-nitrogen solutions were taken through the procedure at the same time as the samples and calibration curve was prepared. A recovery test was attempted intraday to ensure validity of the method. In the filtration step above, 5 cm^3 of 50µgcm^{-3} solution was added to the filtrate and made up to 100 cm^3 with distilled water. The procedure was then applied. To investigate the effect of reaction time 4cm^3 of standard nitrate solution at level of 5µgcm^{-3} was used for the nitrate determination and absorbance of the coloured product was read after time intervals of 2, 5, 10, 15, 20 and 25 minutes. The concentration of H_2SO_4 was varied to determine the suitable amounts required for effective nitration in the procedure. 4cm^3 aliquots of 10µgcm^{-3} standard nitrate-nitrogen solutions were added to five different test tubes and carried through the procedure with addition of 0, 40, 60, 80 and 98 % v/v sulphuric acid solutions respectively. A graph of absorbance against % acid was plotted. The effect of increasing amounts of phenol and Na_2CO_3 were studied at levels of 10µgcm^{-3}. For each of the reagents concentrations of 3, 5, 8, 10 and 15% were used in the procedure and a graph of absorbance against concentration was plotted in each case. The effect of the use of a different solvent for extraction was investigated by carrying samples of standard nitrate solution at level of 10µgcm^{-3} through the procedure and adding 10ml of benzene instead of toluene. The process was repeated with xylene, ethanol and CCl_4 and observations were made.

RESULTS AND DISCUSSION

The formation of sodium salt of nitrophenol through the reaction of vegetable- based nitrate with phenol was quantitative and provided a fundamental line for the

determination of nitrate. The reaction was confirmed by the procedures of Boxer (1997) where upon addition of drops of concentrated HCl the yellow colour of the sodium phenoxide was discharged. The resulting alkaline extract was found to absorb at wavelength maximum of 407nm when measured with spectrophotometer. There was rapid colour development and no measurable change in absorbance for 25minutes of standing after full colour development (Fig 1).

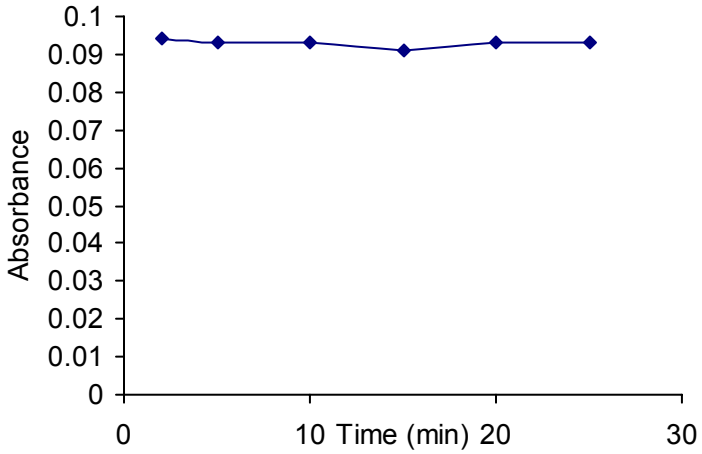


Fig 1 Effect of reaction time for nitration

The colour remained overnight. This is an indication of the stability of the sodium nitrophenoxide. Evidently, the time of reaction does not have modifying effect in the analysis. Prior to the choice of toluene, other solvents were tested as extractants but were found to be unsuitable. Benzene, xylene, ethanol and CCl_4 formed emulsion during the extraction process. Further, benzene extract appeared to be a dirty brown coagulation. Tanaka *et al*. (1982) found toluene suitable for extraction of phenoxide in the nitrate determination with *2*-sec-butyl phenol. Toyoda et al. (1978) used hexane to extract 6-nitro-2,4-xylenol in a modified 2,4-xylenol method for the determination of nitrate.

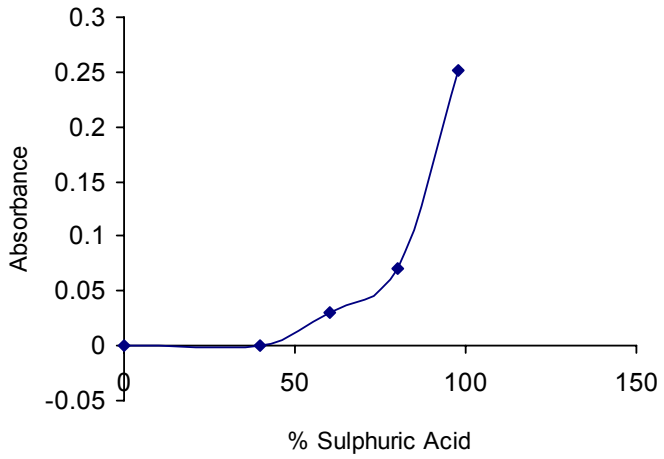


Fig 2 Effect of sulphuric acid concentration on the nitrate determination

Satisfactory recoveries of 76 to 123% were obtained when the sample of spinach, lettuce, water leaf, potato, tomato juice and onion were spiked with nitrate at level of 50 μgcm^{-3} (Table 1). Pfeiffer and Smith (1975) used the similar nitrate addition method to ensure correct electrode response during nitrate determination. BCS (1983) had recoveries of up to 80 to 114% of the nitrate added to six different vegetables, cheeses and meat products at levels of 10 to 30 μgcm^{-3}. Toyoda *et al.*(1978) reported recoveries of 83 to 100% from several foods. Sulphuric acid has a marked effect on the nitration of phenol and hence the determination of nitrate. Fig 2 indicated that with dilute sulphuric acid solution the nitration was not effective.

The graph indicated sharp rise in the amount of nitrate detected as the concentration of the acid is increased. Nitrate was not detected when sulphuric acid concentration was below 40%. The use of 98% H_2SO_4 was therefore recommended for the procedure. Nearly equal amounts of nitrate were determined successfully in aliquots of standard nitrate-nitrogen solutions at a level of 10μgcm^{-3} by using different phenol concentrations of 3, 5, 8, 10 and 15% (Fig 3). In the proposed procedure 5% phenol was recommended. There was no significant difference in the amount of nitrate determined with Na_2CO_3 concentration of 3, 5, 8, 10 and 15%. Therefore, the variation of the concentration of Na_2CO_3 solution in the procedure does not affect the nitration reaction (Fig 4). However, observation showed that at concentrations higher than 15% the solution became saturated at room temperature. For this reason, 10% Na_2CO_3 was recommended. Interfering chlorides were precipitated by the addition of $1cm^3$ of 5% Ag_2SO_4 solution.

Table 1 Recoveries of nitrate added as KNO_3 to certain vegetable products at level of 50µgcm^{-3}

Sample	Nitrate Found(µg)	No of Determinations	Mean Recovery (%)
Spinach	308 ± 14	3	123
Lettuce	296 ± 9	3	118
Water Leaf	267 ± 11	3	101
Potato	236 ± 8	3	94
Tomato Juice	185 ± 6	3	74
Onion	189 ± 6	3	76

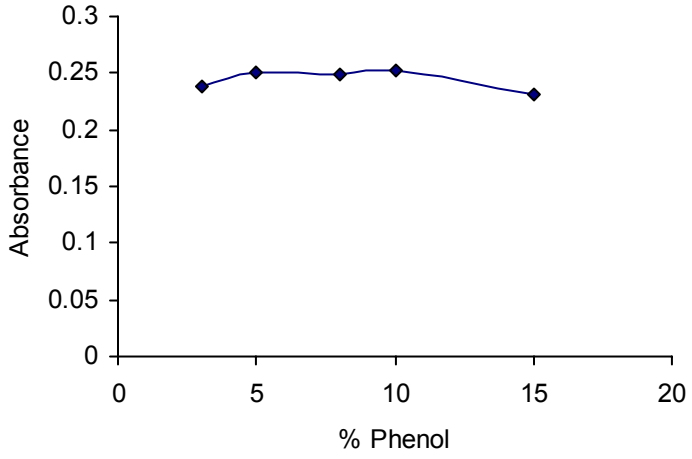


Fig 3. Influence of increasing phenol concentration on the absorbance of nitrate

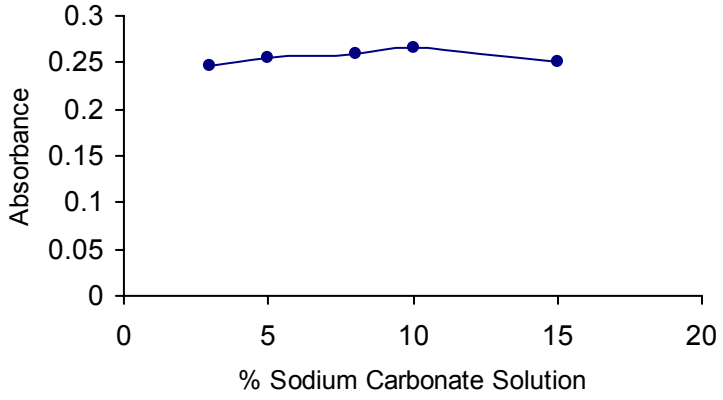


Fig 4. Influence of Na_2CO_3 concentration on the nitrate determined

Conclusion: The determination of nitrate using the developed method is rapid and precise. The nitrate recovery test in Table 1 indicated that the method is specific for nitrate. The remarkable constancy of the absorbance of the coloured solution with time (Fig 1) indicated suitability for analytical use. The reagents involved are common, cheap and suitable for routine analysis.

Acknowledgement: The authors acknowledge the contribution of Musa Beli of Central Laboratory Complex, Bayero University Kano.

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