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NITRATE-NITRITE, VITAMIN C AND IN-VITRO METHEMOGLOBIN FORMATION FROM SOME VEGETABLES

by

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ABSTRACT

The content of nitrate, nitrite and Vitamin C in eleven samples of fresh vegetables were determined. The levels of nitrate were beyond 300 ppm recommended in six of the vegetables, namely: Celosia sp, Lactuca sativa, Amaranthus hybridus, Brassica oleracea variety acephala, Talinum sp, Spinacia oleracea. Minute quantities of nitrite were detected in Celosia sp and Daucus carota. With accompanying high concentration of Vitamin C (a reducing agent) present in Celosia sp, this vegetable may be a potential source of nitrite in foods. Effect of cooking caused less than 25% reduction in nitrate-nitrite content of the vegetables.

When an in vitro test of methemoglobin formation was carried out with extract from the samples, modified hemoglobin formed paralleled content of nitrate in the vegetables. It is suggested that caution must be exercised in the use of some of these vegetables in infant foods as they may contribute to induction of methemoglobin.

INTRODUCTION

The hazards associated with nitrate-nitrite in the environment have been reviewed (1 - 4). Although nitrate as such does not constitute a hazard, there exists the possibility of conversion of nitrates to nitrites (5) which can in turn serve as precursors of carcinogenic nitrosamines (6). The main hazard associated with nitrate in foods is methemoglobinemia in infants (7).

Nitrates and nitrites may occur naturally in high concentrations in vegetables consumed by humans or in forages fed to livestock. Intake of nitrate also comes from water supplies that are high in nitrate content (4). Most vegetables contain a large proportion of indigestible fibrous materials but they are also good sources of minerals, vitamins and proteins. Leafy vegetables are generally used by Nigerians in the preparation of soup, therefore, the leaf protein content of most of them has been determined (8). Some leaf proteins have also been used in the nutritional rehabilitation of malnourished children (9).

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Recently, the nitrate-nitrite in some cereals and baby foods used in Nigeria was reported (10). However, such studies have not been done on vegetables used during weaning and rehabilitation of malnourished children. The objective of this study is to determine the nitrate-nitrite and accompanying vitamin C, which may serve in conjunction with other reducing substances to convert nitrate to nitrite, in some of these vegetables. The work is extended to the determination of the potential capacity of the vegetables in the formation of methemoglobin.

METHODS

(a) Preparation of samples.

All samples were obtained fresh from the University of Benin Farm Project (a project established to grow local vegetables with modern techniques of farming and application of fertilizers) in the morning and stored in cellophane bags prior to determination on the same day.

The bulk sample in each case was divided into three parts. Each sub-sample of 10g was homogenized with 20ml of distilled water and later made up to 200ml in a standard flask. The flask was quickly immersed in a water bath (80^oC) and held for 10 minutes. The resulting extract was passed through a column of aluminium oxide to remove the pigments and then filtered through No. 41 whatman paper for analyses.

(b) Estimation of Nitrate-nitrite and Vitamin C.

The method based on reduction of nitrate to nitrite with cadmium followed by the formation of a diazo compound with sulfanilic acid and N-1-napthyl-ethylenediamine introduced by Elliot and Porter (11) for nitrate in bacon and later modified for determination of nitrate in vegetables by Kenny and Walshe (12) was used. The nitrite due to the reduction of nitrate was calculated by substracting the determination done without reduction on some of the same sample.

The procedure for the determination of ascorbic acid (Vitamin C) was by Wahba et al (13).

For the study of the effect of cooking on nitrate-nitrite, fresh samples of some vegetables were sub-sampled and parts were boiled in distilled water for thirty minutes, extracted and analysed for nitrate-nitrite.

(c) In vitro methemoglobin formation.

Blood was obtained from the blood bank of the University of Benin Teaching Hospital, Benin City. Preparation of Oxyhemoglobin and Methemoglobin with sodium nitrite was as reported by Russo and Sorstokke (14). To determine the potential capacity of the vegetable in forming methemoglobin, five leafy vegetables (four on basis of high nitrate content and one based on low nitrate content) were extracted, prepared and the nitrate content was reduced to nitrite with Cadmium. 3ml of oxyhemoglobin solution with absorbance of 0.8 at 500nm was pipetted into a cuvette followed by 3ml of 0.2M Potassium phosphate buffer (pH 7.0). 1ml of the decolorized extract was added to the cuvette and the content was thoroughly mixed. The formation of methemoglobin was determined from a decrease in absorbance reading at 560nm at 5 minutes interval for 80 minutes and up to 160 minutes in one case of the vegetable (Telfaris, spp) with low nitrate level. A control experiment was run simultaneously with equivalent concentration of sodium nitrite as per nitrite determined for the vegetables to ensure that the nitrite alone was responsible for the methemoglobin formed.

RESULTS AND DISCUSSION

Table 1 shows the level of nitrate-nitrite in eleven vegetables commonly consumed in Nigeria. Celosia sp contains about twice the recommended nitrate levels (300ppm) in vegetables (15), and above the 500ppm recommended by FAO (16). Other vegetables of high nitrate content which are beyond the recommended limit of 300ppm are Lactuca sativa, Amaranthus hybridus, Brassica oleracea variety acephala, Talinum triangulare, Spinacea oleracea and Veronica sp. Other vegetables analysed have safe levels of nitrate, whilst Telfaris sp has only trace

Table 1: Nitrate-nitrite content in vegetables

Sample	Number of sample	Range (ppm)	Mean (ppm)	c.v.%
Celosia sp	3	500 - 673	568	31
Lactuca sativa	3	467 - 553	500	17
Amaranthus hybridus	3	450 - 555	495	21
Brassica oleracea variety acephala	3	460 - 534	492	16
Talinum triangulare	3	370 - 440	400	18
Spinacia oleracea	3	320 - 355	342	10
Daucus carota	4	190 - 300	256	43
Veronica sp	3	190 - 237	211	23
Brassica oleracea	3	117 - 124	120	6
Corchorus olitorus	3	90 - 107	95	11
Telefaris sp	3	18 - 22	20	20

amount. Insignificant quantities of nitrite were detected in Celosia sp (16.7ppm) and Daucus carota (12.5ppm).

Table 2 shows the vitamin C content of the vegetables examined. High level of vitamin C was detected in Celosia sp, whilst relatively low levels were estimated in other vegetables with high nitrate content.

Table 2: Vitamin C content in vegetables

Sample	Number of sample	Range (mg/100g)	Mean (mg/100g)	C.V.%
Celosia sp	3	130 - 176	158	30
Lactuca sativa	3	16 - 22	14	32
Amaranthus hybridus	3	30 - 48	38	47
Brassica oleracea variety acephala	3	23 - 32	27	33
Talinum triangulare	3	47 - 51	49	8
Spinacia oleracea	3	61 - 67	65	9
Daucus carota	3	21 - 29	24	34
Veronica sp	3	80 - 83	82	4
Brassica oleracea	3	41 - 59	48	38
Corchorus olitorus	3	69 - 74	71	7
Telefaris sp	3	48 - 56	52	15

It is as well that, except for Celosia sp, all other vegetables with high nitrate content have low vitamin C. Storage of vegetables with high reducing substances (vitamin C is a naturally occuring reducing agent) and high level of nitrate may bring about a quantitative conversion of nitrate to nitrite. Such vegetables may then become unsafe for use in infant foods. Reductive conversion for Spinach has been reported by Phillips (17). Perhaps the trace amounts of nitrite in Celosia sp and Daucus carota represent the product of such initial reduction.

Table 3 shows that less than a 25% of the nitrate is lost during cooking, although cooking may serve to destroy nitrate reductase present in fresh vegetables, thereby eliminating a pathway of nitrate reduction

(1B), the enzyme may become significant in nitrate conversion when vegetables are stored improperly.

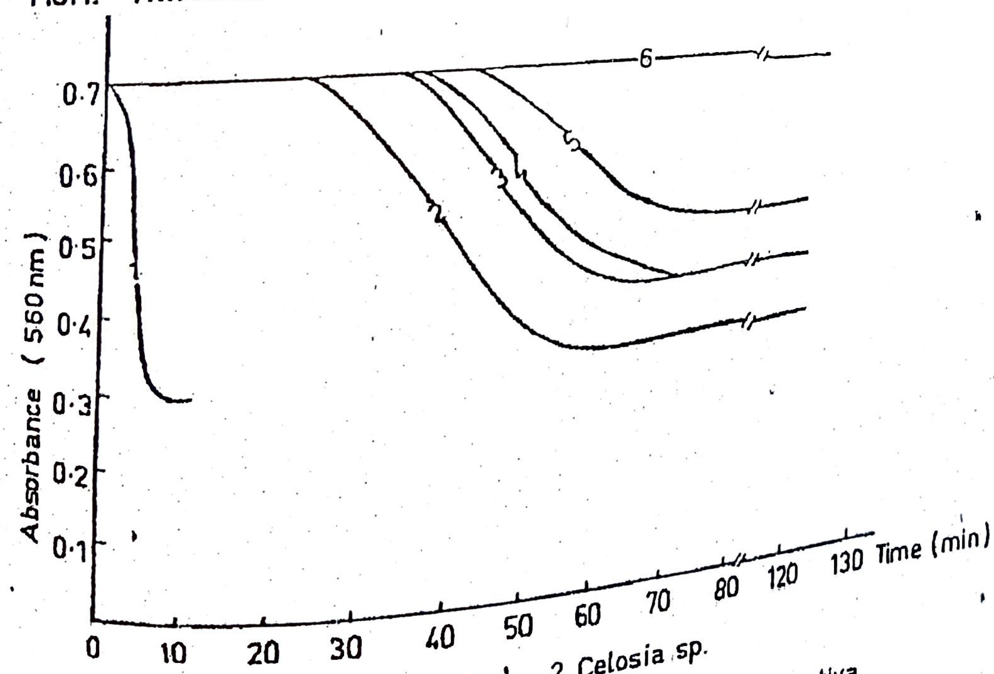
Table 3: Effect of cooking* on Nitrate-nitrite in vegetables

Sample	Nitrate-nitrite (ppm)		% loss of Nitrate-nitrite
	Cooked	Fresh	
Celosia sp	413	530	21
Amaranthus hybridus	466	555	16
Veronica sp	190	237	20

*Boiled at 100°C for 30 minutes.

Fig. 1 and Table 4 show the kinetics of methemoglobin formation with vegetable extracts. In Fig. 1 control experiments run with 0.025M $NaNO_2$ had a change of 0.4 absorbance unit in 5 minutes, while the extract with *Celosia sp* had a similar change (0.37A) in thirty minutes after about an initial thirty minutes lag period. The kinetics of the other vegetables,

FIG.1. Kinetics of Methemoglobin Formation With Vegetable Extracts.



1. Control (0·025M $NaNO_2$) 2. Celosia sp.

3. *Brassica olearacea* var acephala 4. *Lactuca sativa*

5. *Spinacia olearacea* 6. Telfaris sp.

had similar activity curves which were all consistent with an initial step that involved a single electron transfer from nitrite to the bound dioxygen of oxyhemoglobin (19).

Table 4: Methemoglobin formation with vegetable extract

Sample	Absorbance	Time (min)	A/min x 100
Control-0.025M $NaNO_2$	0.40	5	8.00
Celosia sp	0.37	30	1.23
Brassica oleracea variety acephala	0.26	30	0.87
Lactuca sativa	0.25	30	0.83
Spinacia oleracea	0.20	30	0.67
Telfaris sp	0.02	30	0.07

The steepness of the slope, the initial lag period (figure 1) or rate of methemoglobin formation per minute (Table 4) would therefore provide a quantitative assessment of the in vitro capacity of the vegetables in forming methemoglobin when run and compared with the appropriate level regarded as unsafe. Celosia sp approached such an unsafe level, while methemoglobin was virtually unformed with extract from Telfaris sp.

This study serves to indicate that nitrate levels in some Nigerian vegetables, especially in Celosia sp, Brassica oleracea variety acephala and Lactuca sativa may warrant periodic monitoring, especially with increasing use of fertilizers in the country. Nitrite consumed from all sources in Nigeria has not been published, although a recent investigation was reported on the critical nitrate-nitrite-nitrosamine levels of some fermented Nigerian beverages (20). What is currently being sought is the reduction in the rate of nitrates in vegetables and from all other sources through various contracts which provide for the limited use of fertilizers, the choice of varieties, preventive health measures and application of relevant technology to enable the accepted norm to be fed to infants, thus reducing the risk of methemoglobinemia (7).

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