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EFFECT OF WILTING AND PROCESSING ON THE NITRATE AND NITRITE CONTENTS OF SOME NIGERIAN LEAF VEGETABLES

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ABSTRACT

Nitrate and nitrite contents of seven varieties of commonly consumed leaf vegetables were determined. Effect of cooking and wilting on the contents of these factors were investigated. Nitrate ranged from 48.10 in *ewuro* to 270.0 ppm *ogunmo* (mean 116.43 ± 78.31) while nitrite ranged from 0.024 ppm in *tete* to 0.064 in *ogunmo* (mean 0.044 ± 0.018).

Cooking reduced the nitrate levels in all the samples but nitrite levels inexplicably increased in all sample. On wilting nitrate levels decrease while nitrite levels increased up to 83% in *tete*. The nitrate and nitrite levels were not considered hazardous but toxicological implications of high consumption of these factors is briefly highlighted.

INTRODUCTION

Many studies have demonstrated consistently that the leaf, stem and flower vegetables are generally rich in nitrate (Wilson, 1949; Jackson *et al.*, 1967; Teotia *et al.*, 1988). There is a great deal of concern over the nitrate and nitrite content of both human and animal food and water supplies. This is due to several reports relating these factors to infant methemoglobinemia and to the possible formation of carcinogenic nitrosamines (Mirvish, 1977; Walker, 1990). Gilbert *et al.* (1946) analysed many plants for their nitrate content, and observed that the ingestion of foods containing as much nitrate as was found by them could be hazardous. Instances of poisoning in animals on cultivated pastures also abound (Webb, 1952; Lichtenwalne *et al.*, 1971). Nitrite is

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shown to arise from microbiological reduction of nitrate in foods or water when such foods are stored at room temperature (Jones and Griffith, 1965; Siciliano *et al.*, 1975). Nitrite concentration in fresh, undamaged plant tissues are usually very low but post-harvest storage and wilting process favours high nitrite accumulation and a decrease in nitrate content (Phillips, 1968).

In view of the potential hazard due to the presence of nitrate and nitrite prior to ingestion, the International Agency for Research on Cancer and WHO (1977; 1978) have recommended the reduction of nitrate and nitrite concentrations in foods and vegetables, especially in infants food for weaning. The problem is particularly acute in infants since children under three months of age are more susceptible to methemoglobinemia (Betke, 1953; Philips, 1971). From stand-point of the critical health significance of nitrate and nitrite in food, this paper investigates the possible consumer hazard that may arise from consumption of some fresh, processed and wilted local leafy vegetables.

List of Vegetables:

Local Name	Botanical Name
Tete	*Amaranthus hybridus*
Ewedu	*Corchorus olithorus*
Ilasa	*Hibiscus esculentus*
Ewuro	*Vernonia amygdalina*
Soko	*Celocia argentea*
Soko pupa	*Celocia spp.*
Ogunmo	*Solanium nodiflorum*

MATERIALS AND METHODS

Seven varieties of fresh leafy vegetables were collected from the University Teaching and Research Farm. The effect of cooking on the nitrate and nitrite contents were determined after boiling 20 g of each fresh sample in 80 ml of water under practical conditions for 10 minutes and separating from the cooking water. To investigate the effect of wilting the vegetables were spread out under tree shade and sampled at 2 hours intervals over a period of 8 hours. By the end of 8 hours the vegetables lost their fresh appearance and wilting was quite obvious.

Pretreatment of samples

20 g of each of the fresh, cooked and wilted vegetable samples were macerated with 80 ml of double distilled water until a fine slurry was formed. The slurry was then centrifuged. About 5 g of HgCI was added to the supernatant (as a deproteinizer) and allowed to stand for 15 minutes. 10 g of

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activated charcoal was added to remove plant pigments and, after shaking vigorously, filtered using watman No. 32 filter paper. A clear solution was obtained.

Estimation of nitrite

The colorimetric method of Montgomery and Dymock (1961) was adopted. 1 ml of the clarified sample supernatant was used as a blank and sulfanilic acid replaced with an equal amount of double-distilled water. Absorbance measurements of the resultant pink solutions were made at 550nm. Nitrite-N values were extrapolated from a standard curve of nitrite.

Estimation of nitrate

Nitrate was determined essentially by the method of Harper (1924) as modified by Bassir and Maduagwu (1978). To 25 ml of the clarified sample solution, 1 ml of nitrate-free silver sulphate (4g/l) were added to remove any interfering chloride ions. Precipitated chloride was removed by filtration. Loss of nitrate was prevented by the addition of 0.2 g of magnesium oxide to 1 ml of filtrate. The optical density of yellow nitrophenolic colour developed was measured in an EEL photoelectric colourimeter using a blue filter. Double distilled water was used as blank and nitrate concentrations were read off a standard curve of nitrate as nitrate-N.

All estimations were carried out in duplicate.

RESULTS AND DISCUSSION

The concentration of nitrate and nitrite in both fresh and cooked vegetables are shown in Tables 1 and 2 respectively. Nitrate content varied from 48.0 in *ewuro* to 270.0 ppm in *ogunmo*, while the nitrite ranged between 0.024 in *tete* to 0.064 ppm in *ogunmo*. While the nitrite contents were lower, the nitrate values compare well with reported values for several leaf vegetables (Siciliano *et al.*, 1975). Spinach, beets, radishes, egg plant, celery, lettuce, collards and turnip greens are among the vegetables that generally contain high concentrations of nitrate (Jackson *et al.*, 1967; Splittstoesser *et al.*, 1974; Teotia, 1988). Spinach for example is widely reported to contain as much as 2,000–3,000 ppm in nitrate concentration (Wilson, 1949). However, the absolute values reported for leaf vegetables vary widely because of genetic, environmental, sampling and maturity factors which thus limits literature comparisons of data from this study (Regan *et al.*, 1968; Cantiliffe, 1973).

Cooking reduced the total nitrate content by between 24% in *soko pupa* to 61% in *ewedu* of their initial levels (Table 1). In contrast nitrite contents increased on cooking by a range of 9% in *soko pupa* to 78% in *tete*

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TABLE 1

Nitrate Content Of Fresh and Cooked Vegetables (PPM).

	Tete	Ewedu	Ilasa	Ewuro	Soko	Sokopupa	Ogunmo
Fresh	62	71	101	48	94	169	270
Cooked	39	28	62	33	54	129	115
% Decrease	37	61	39	31	43	24	57

TABLE 2

Nitrite Content Of Fresh and Cooked Vegetables (PPM).

	Tete	Ewedu	Ilasa	Ewuro	Soko	Sokopupa	Ogunmo
Fresh	0.024	0.054	0.052	0.026	0.025	0.062	0.064
Cooked	0.109	0.107	0.105	0.062	0.028	0.105	0.104
% Increase	78	50	51	58	11	9	39

(Table 2). It is possible some intrinsic factors may have influenced rise in nitrite levels on cooking (Beevers and Hageman 1969), which is contrary to the observations of Phillips (1968). The influence of wilting on the nitrate and nitrite levels are shown respectively in Tables 3 and 4. While the nitrate levels decreased (except in *ogunmo*), the nitrite contents increased on wilting. The percentage decomposition of nitrate varied from 20% in *soko pupa* to 65% in *ewedu*. Accumulation of nitrite was highest in *tete* (83%) and lowest in *soko* (11%) after 8 hours wilting period. Variation of the nitrate and nitrite levels with wilting time does not have any correlation with the initial levels and probably reflects the magnitudes of endogenous nitrate and nitrite reductase and microbial activities in the samples (Sander 1968).

The nitrate and nitrite contents in both the fresh, cooked and wilted leaf vegetables in this study are somewhat low compared to reports on other widely consumed vegetables and thus present little or no health hazard. However, since vegetables intake is the major contributor of nitrate in the diet (White, 1975), consumption of large quantities of vegetables containing high levels of nitrate is highly inadvisable especially in infant feeding (Bisazz *et al.*, 1978). High levels of nitrate suggest concomitantly high levels of nitrite and thus increased potential for toxicity (Ayanaba *et al.*, 1973). The nitrate and nitrite contents in the vegetables would certainly augment that which may be present in drinking water and other ingested foods. Their relevance in the

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TABLE 3

Effect Of Wilting On The Nitrate Contents (PPM).

Time (hrs.)	Tete	Ewedu	Ilasa	Ewuro	Soko	Sokopupa	Ogunmo
0	62	71	101	48	94	169	270
2	53	53	91	42	69	149	200
4	50	44	85	37	65	142	175
6	46	35	73	33	58	136	150
8	37	25	69	27	58	135	125
% Decrease	40	65	32	44	38	20	54

TABLE 4

Effect Of Wilting On The Nitrite Contents (PPM).

Time (hrs.)	Tete	Ewedu	Ilasa	Ewuro	Soko	Sokopupa	Ogunmo*
0	0.024	0.054	0.052	0.026	0.025	0.062	0.064
2	0.026	0.062	0.063	0.055	0.027	0.104	6.061
4	0.033	0.104	0.024	0.060	0.027	0.105	0.033
6	0.104	0.105	0.104	0.060	0.028	0.106	0.027
8	0.044	0.081	0.076	0.041	0.028	0.088	0.027
% Increase	83	49	51	58	11	42	(57.8)*

*Decrease in Nitrite content.

possibility of endogenous formation of carcinogenic nitrosamines from ingested precursors and the attendant health risk cannot be over emphasized (Marletta, 1989). Bacteria which reduce nitrate to nitrite also catalyse the formation of nitosamines (Sander, 1968; Leaf e*t al.*, 1989) and induction of tumors in animals by simultaneous feeding of nitrate and nitrosatable amines have been reported (Sen *et al.*, 1969).

Nitrite is usually secreted in the saliva when food is chewed. Salivary nitrate appears to be the product of microbial reduction of ingested nitrate (Tannenbaum *et al.*, 1978, Walter and Smith, 1981) and higher levels occur in the saliva of smokers (Soboleva, 1969).

Ingestion of high amounts of nitrite may also lead to lowering of blood pressure (Asbury and Rhodes, 1964), with adverse effects on the thyroid gland and vitamine A and E metabolism (Becher, 1967). High doses of nitrate is shown to cause retardation in growth and fertility and other pathological consequences in animals (Johannsen and Kuehnert, 1969; Soboleva, 1971; Soboleva and Starkov, 1971). Meanwhile as far as possible vegetables should

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be used when fresh, particularly tete in which an 83% rise in nitrite content occurred on wilting.

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