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EFFECT OF PROCESSING AND PRESERVATION METHODS ON VITAMIN C AND TOTAL CAROTENOID LEVELS OF SOME *VERNONIA* (BITTER LEAF) SPECIES

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ABSTRACT

***Vernonia* is one of the leafy vegetables that can be used in an attempt to alleviate the problem of micronutrient malnutrition, prominent in tropical Africa. Commonly known as Ndole or Bitterleaf in most Central and West African countries, it is one of the most widely consumed leafy vegetable in Cameroon. Though eaten by a large proportion of the population, cultivation is limited to the southern parts of the country and mostly in the rainy season. In order to ensure availability in non-growing areas or seasons, the vegetable is usually processed and preserved. Processing and preservation methods used are thought to influence the nutrient content of these vegetables. The study was aimed at determining the effects of processing and preservation methods on vitamin C and total carotenoid levels of some species of *Vernonia* (*V. amygdalina, V. calvoana* var. bitter*, V. colorata and V. calvoana* var. non bitter*)* consumed in Cameroon. The processing methods were squeeze–washing, boiling and squeeze–washing with 0N, 1N and 2N concentrations of natron. The methods of preservation were sun drying, oven drying at different temperatures (45, 60 and 75°C) and freezing for 0, 10, 30 60 and 120 days. Results show that these leafy vegetables were good sources of vitamin C and total carotenoid. Vitamin C values vary from 137.5±3.3 in *V. calvoana* non bitter to 197.5 ± 3.5mg/100g in *V. colorata,* while total carotenoid levels range from 30.0 ± 1.0 in *V. amygdalina* to 41.5±0.9mg/100g in *V. colorata* for the raw samples. Greater losses of both vitamins were observed for the bitter species. Drying caused significant losses, especially in Vitamin C. The best temperature for drying the vegetables to preserve carotenoids and vitamin C was at 45°C, whereas sun drying and oven drying at 75°C caused the highest losses. Freezing for up to ten days had no significant influence on total carotenoids and vitamin C levels. Comparatively, squeeze-washing proved to be the best processing method that ensured minimum loss of both vitamins.**

Key words: Processing, preservation, *Vernonia*, total carotenoids, Vitamin C

FRENCH

RÉSUMÉ

Vernonia est l'un des légumes à feuilles qui peuvent être utilisés pour essayer d'alléger le problème de malnutrition liée aux micronutriments, qui prédomine en Afrique tropicale. Communément appelé Ndole ou feuille amère dans la plupart des pays de l'Afrique centrale et de l'Ouest, c'est l'un des légumes à feuilles les plus largement consommés au Cameroun. Bien que mangé par une grande proportion de la population, il n'est cultivé que dans les régions du Sud du pays, surtout pendant la saison des pluies. En vue d'en assurer la disponibilité dans des régions où ce légume ne pousse pas ou pendant les saisons non favorables, ce légume est généralement traité et mis en conserve. D'aucuns pensent que les méthodes de traitement et de

conservation utilisées influencent la teneur en nutriments dans ce légume. La présente étude a pour but de déterminer les effets des méthodes de traitement et de conservation sur la vitamine C et sur les niveaux totaux de la caroténoïde de certaines espèces de Vernonia (V. amygdalina, V. calvoana var. amère, V. colorata et V. calvoana var. non amère) consommées au Cameroun. Les méthodes de traitement étaient le lavage/rinçage, la cuisson et le lavage/rinçage avec des concentrations de natron 0N, 1N wr 2N. Les méthodes de conservation étaient le séchage au soleil, le séchage au four à des températures différentes (45, 60 et 75° C) et la congélation pendant 0, 10, 30, 60 et 120 jours. Les résultats montrent que ces légumes à feuilles sont de bonnes sources de vitamine C et de caroténoïde totale. Les valeurs en vitamine C variaient entre 137,5±3,3 en V. calvoana non amer et 197,5 ± 3,5mg/100g en V. colorata, tandis que les niveaux totaux de caroténoïde se situaient entre 30.0 ± 1.0 en V. amygdalina et 41,5±0,9mg/100g en V. colorata pour les échantillons bruts. Des pertes plus importantes des deux sortes de vitamines ont été observées dans les deux espèces amères. Le séchage a causé des pertes considérables, surtout en vitamine C. La meilleure température pour sécher les légumes de façon à ce qu'ils préservent les caroténoïdes et la vitamine C était 45°C, tandis que le séchage au soleil et le séchage au four à 75°C ont causé le plus de pertes. La congélation jusqu'à dix jours n'avait pas d'importante influence sur les niveaux totaux de caroténoïdes et de vitamine C. D'une manière comparative, le lavage/rinçage s'est avéré être la meilleure méthode de traitement qui a assuré le minimum de pertes des deux espèces de vitamines.

Mots-clés: ***Traitement, conservation, Vernonia, total des caroténoïdes, vitamine C***

INTRODUCTION

Malnutrition persists in developing countries in spite of the increase in production of basic foods. One of the most common forms of malnutrition is Vitamin A deficiency which affects about 250 million preschool children world wide [1]. In Africa, vitamin A deficiency affects close to 18 million preschool children, of which 1.3 million are affected by xerophthalmia. Problems related to vitamin C deficiency may not be directly emphasized but its role in enhancing absorption of iron is to be mentioned, as iron deficiency is prominent in most developing countries and could very much be linked to its absorption.

Leafy vegetables are the most available and cheapest source of substantial amounts of vitamins A and C to the most vulnerable groups [2]. However, in Tropical Africa, millions of people still suffer from vitamin A and C deficiency despite the increased consumption of leafy vegetables. These leafy vegetables are relatively inexpensive, easily and quickly cooked and rich in several nutrients especially β-carotene and vitamin C, which are essential for human health. These vegetables also provide some minerals such as iron. *Vernonia*, commonly known as Bitterleaf, is widely used in most West and Central African countries both for human and animal consumption. Though consumed by a large proportion of the Cameroonian population, cultivation is limited to the southern parts of the country and in the rainy season. Post-harvest losses are evident during the six months of Vernonia glut. Therefore there is need for storage use during the six months of dry season. Two main types exist in Cameroon; the non-bitter types (*V. colorata, V. calvoana* var. non bitter) and the bitter types (*V. amygdalina, V. calvoana* var. bitter). Local processing involves squeeze-washing the raw or boiled leafy vegetable to remove the bitter taste and foam. The leaves are tenderised and the greenish colour preserved by boiling with natron. The washed bitterleaf can be preserved by freezing or drying. These processes generally lead to losses of some nutrients and anti- nutrients (anti nutritional factors). Processing and preparation of foods brings about losses in nutrients and the extent of these losses depends on the type of technique used [3].

Studies on the nutritional composition of *Vernonia* are numerous and limited to one species: *V. amygdalina*. In 1990, Faboya demonstrated that ascorbic acid decreases with storage time [4]. Oshodi found that the dried leaves of *V. amygdalina* were rich in minerals, especially in phosphorus, and that the content of ascorbic acid was temperature dependent [5]. Little is known about the effect of processing and preservation on the different species of *Vernonia (V. amygdalina, V. calvoana* var. bitter*, V. colorata and V. calvoana* var. non bitter*)*. This study was aimed at determining the effect of different processing and preservation methods on the vitamin A and C levels of four species of *Vernonia* available in Cameroon. This information will be useful in determining the best methods of preservation and processing which cause minimum losses of total carotenoids and vitamin C in different species of V*ernonia*.

Materials and Methods

Samples were collected in an experimental farmland in Ngaoundéré, Cameroon in the months of October and November. The leaves and growing shoots were sorted, rinsed under running tap water, left to drain, and subsequently sliced using a stainless steel kitchen knife before submitting them to the different treatments.

To determine the effect of squeeze-washing and boiling with or without natron, a 5x4 factorial design was used. The species *V. amygdalina, V. calvoana* var. bitter*, V. colorata and V. calvoana* var. non bitter were subjected to five different treatments: Raw (R), squeeze-washing (W), squeeze-washing and boiling in 0N (Wb), 1N (W1) and 2N (W2). Concentrations of natron (0N) indicates boiling without natron, whereas 1N is the average quantity of natron used by housewives – 10 g/ 450 g fresh leafy vegetables boiled to tenderise the leaves and preserve the colour for ten minutes.

To determine the influence of drying, a 5x2x4 factorial design was used. Five treatments as above, two species *(V. amygdalina and V. calvoana* var bitter) and four conditions of drying- sun drying and drying in a moisture oven at 45 °C, 60 °C and 75 °C.
The effect of duration of freezing was determined on only one species (*V. calvoana*), stored at –18°C in a freezer for 10, 30, 75, and 120 days (5x5 factorial design).
All samples were later dried, ground to fine powder and stored in a refrigerator inside air tight containers awaiting analysis

The vitamin C levels were determined using N-Bromo-succinimide recommended for the pigmented solutions, while total carotenoid was first extracted using a mixture of hexane - acetone 30/70 (v/v) then separated by column chromatography and quantified using a spectrophotometer [6, 7]. All the analyses were done using triplicate samples. Experimental results were subjected to Analysis of Variance (ANOVA) and differences between means were assessed by Duncan's new multiple range test using the statistical package statsgraphics 2000.

RESULTS

Changes in vitamin C levels

Table 1 shows the levels of vitamin C in the four species of *Vernonia* as affected by different processing techniques. Vitamin C values for the raw leaves of all four species of *Vernonia* varied from 137.5 ± 3.3 in *V. calvoana* var. non bitter to 197.5 ± 3.5 mg/100 g in *V. colorata.* These values were generally high for all the raw species of *Vernonia* as compared to the processed samples. However, losses were noticed between 55% and 77% in *V. calvoana* var. bitter and *V. amygdalina* respectively, with the greatest losses in the bitter species (*V. amygdalina* and *V. calvoana* var. bitter*)*. For processing conditions, simple squeeze-washing had the least loss of vitamin C while the highest destruction was observed when natron was used ($P<0.05$).

The effect of temperature on the vitamin C levels of *V. calvoana* var. bitter and *V. amygdalina* is presented in Tables 2 and 3, respectively. Vitamin C levels dropped with increase in temperature. The highest losses were found in the sun-dried samples for either the raw or processed samples. Raw samples of *V. amygdalina* had values that dropped from 166.5 ± 2.1 mg/100 g to 73.0 ± 9.9 mg /100 g, and the values for *V. calvoana* var bitter also dropped from 178.5 ± 16.2 to 61.0 ± 12.7 mg /100 g, ie 56% loss for the two species when sun dried. The losses due to drying in the processed samples were lower for both species than for the raw samples.

Table 4 presents the effect of freezing duration on the vitamin C levels of *V. calvoana* var. bitter. For both the raw and processed leafy vegetables, freezing for between zero and ten days showed no significant difference ($P>0.05$) in the values of vitamin C as was in the case when these samples were frozen for 30, 75 and 120 days.

Changes in total carotenoids

The levels of total carotenoids in the different species subjected to different processing techniques are shown in Table 5. Total carotenoids levels range from 30.0 ± 1.0 in *V. amygdalina* to 41.5 ± 0.9 mg /100 g in *V. colorata* for the raw samples. These raw samples were considerably reduced when subjected to different processing methods ($P<0.05$). More losses of carotenoids were observed for the bitter species (*V. amygdalina* had 50% and *V. calvoana* var. bitter had 67%). Meanwhile *V. calvoana* var. non bitter had 43% and *V. colorata* had 49% loss. The greatest losses were noticed when 2N natron was used in processing. Table 6 and 7 show the effect of different drying conditions on the carotenoid levels of *V. calvoana* and *amygdalina* respectively. Losses due to drying were significant for the raw samples with maximum losses of up to 43% when samples of *V. amygdalina* were dried at 75 °C, while carotenoid levels dropped from 38.5 ± 0.3 to 26.1 ± 0.2 mg /100 g, ie 32% when solar radiation was used for drying *V. calvoana* var. bitter. Table 8 shows the effect of duration of freezing at –18°C on the carotenoid levels of *Vernonia calvoana.* Significant losses were observed after ten days of freezing the raw samples at $P< 0.05$. Freezing for ten days resulted to significant variation for both the raw and processed samples of *V. calvoana* var. bitter ($P>0.05$)

DISCUSSION

Vitamin C values were high for all four species of *Vernonia* with as much as 77% losses after processing. Singh *et al*. obtained closely similar values for vitamin C in some Indian leafy vegetables [8]. Bender also observed similar trends, and proved that in leafy vegetables, losses in vitamin C were a function of the method of processing [3]. In this same light Machlin, found 50% *loss* after cooking *V. amygdalina* [9]. These losses are justified since vitamin C is thermo sensitive and hydrosoluble. Losses were also found in samples that were dried at different conditions. The maximum losses observed after drying samples at 75 °C and using solar radiation, was confirmed by the works of Oshodi who proved that vitamin C levels in vegetables are temperature dependent [5]. Generally vitamin C levels in *V. calvoana* var. bitter were stable when these vegetables, processed or raw were stored at –18°C for ten days. After this period, significant losses were observed. Faboya observed that vitamin C could be conserved by storing leafy vegetables in a refrigerator [3]. The losses observed after ten days of freezing were more likely to be during the thawing process when some water is lost together with some Vitamin C [9].

Vitamin A deficiency remains a major problem in Cameroon, affecting mostly the people in the Northern provinces [10, 11]. Its role in vision and growth regulation has made the public health officials to look for urgent and rapid methods of combating the problem. Leafy vegetables remained one of the most important and cheapest sources of Vitamin A. Processing generally leads to losses [12]. High levels of carotenoids, found in the raw samples of the different species of *Vernonia* were destroyed during processing. Higher values of carotenoids were found in fresh bengal gram leaves [8] but values fall within the range of values obtained for spinach and amaranth [13, 14]. Processing caused losses and these losses were more in the bitter species: *V. amygdalina* and *V. calvoana* var. bitte*r*. This could be explained by the fact that processing was more intense in the bitter species. High losses, also found when 2N natron was used in processing proved that though carotenoid is not hydrosoluble, it could be destroyed by the use of alkaline during cooking. The losses observed as a result of processing in the present study are higher than those in the literature [12, 15], and this can be attributed to variations in processing methods. Losses in total carotenoids were observed when the different species of Vernonia were dried under different conditions. Yamini, however, found varying trends due to drying of different leafy vegetables in India at different conditions [16].

CONCLUSION

The different species of *Vernonia* are good sources of Vitamin A and C. However, processing generally leads to significant losses. Squeeze-washing and rinsing seem to be the best treatment to preserve both vitamins A and C. Losses were more prominent in the bitter species that require intense squeeze-washing and rinsing. Drying at 45°C is best recommended while freezing for less than ten days is most appropriate for the conservation of vitamin A and C. Nevertheless, because of the increased demand for these nutrients, and limited possibility to avoid processing and conservation, it is pertinent that supplementation of Vitamin A and exploitation of other sources of Vitamin C - such as from fruits - be considered imperative.

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Table 1.

Vitamin C levels in raw and processed forms of different species of *Vernonia* (mg/100 g)

	V. amygdalina	**V. calvoana** var. bitter	**V. colorata**	**V. calvoana** var. non bitter
R	166.5±2.1 [a]	178.5±16.2 [a]	197.5±3.5 [a]	137.5±3.3 [a]
W	75.5±6.4 [b]	117.0±12.7 [b]	95.0±4.2 [b]	98.5±16.3 [b]
Wb	57.5±6.4 [c]	79.5±2.1 [c]	90.0±4.2 [b]	95.0±7.1 [b]
W1	66.0±1.4 [bc]	97.0±18.4 [b]	77.0±1.4 [c]	53.0±1.8 [c]
W2	38.0±1.4 [d]	63.0±2.8 [c]	75.0±2.1 [c]	51.0±4.2 [c]

Values are means±SD
Means not sharing a common superscript letter in a column are significantly different at $p < 0.05$
R =Raw, W= Squeeze-washing, Wb = Boiling and squeeze-washing. W1 boiling in1N natron
and squeeze-washing; W2= Boiling in 2N natron and squeeze-washing

Table 2.

Levels of Vitamin C of *V. calvoana* var. bitter (mg/100 g) after dying

	R	W	Wb	W1	W2
"30"	61.0±12.7 [d]	51.0±12.7 [b]	47.0±1.4 [c]	54.0±1.4 [bc]	28.5±5.0 [b]
45°C	178.5±16.2 [a]	117.0±1.4 [a]	79.5±2.1 [a]	97.0±18.4 [a]	63.0±2.8 [a]
60°C	105.5±6.4 [b]	65.0±14.1 [b]	64.0±8.5 [b]	62.5±3.5 [b]	60.0±4.2 [a]
75°C	78.5±2.1 [c]	65.0±1.4 [b]	62.0±5.7 [b]	57.5±3.5 [b]	57.5±2.1 [a]

Values are means±SD *"30"* indicates sun drying
Means not sharing a common superscript letter in a column are significantly different at $p < 0.05$
R =Raw, W= Squeeze-washing, Wb = Boiling and squeeze-washing. W1 boiling in1N natron
and squeeze-washing; W2= Boiling in 2N natron and squeeze-washing

Table 3.

Vitamin C levels of V. *amygdalina* (mg/100 g) after drying

	R	W	Wb	W1	W2
"30"	73.0±9.90	60.0±1.41	28.0±1.41	39.0±1.41	23.5±2.1
45°C	166.5±2.12	75.5±6.36	57.5±24.75	66.0±1.41	38.0±1.4
60°C	74.0±0.71	66.0±14.14	43.0±4.24	43.0±1.41	29.0±1.4
75°C	77.0±0.00	57.5±0.71	24.0±2.83	41.5±2.12	25.0±0.7

Values are means±SD *"30"* indicates sun drying
Means not sharing a common superscript letter in a column are significantly different at $p < 0.05$
R =Raw, W= Squeeze-washing , Wb= Boiling and squeeze-washing. W1 boiling in1N natron
and squeeze-washing; W2= Boiling in 2N natron and squeeze-washing

Table 4.

Vitamin C levels of *V. calvoana* var. bitter (mg/100 g) after freezing

	R	W	Wb	W1	W2
0 day	178.5±16.26^{a}	117.0±12.7^{a}	79.5±2.12^{a}	97. 0±18.4^{a}	63.0±2.8^{a}
10days	185.0±21.2^{a}	110.0±10.6^{a}	70.5±10.6^{a}	80.0±14.1^{a}	60.0±9.9^{a}
30days	131.5±7.8^{b}	71.0±12.7^{b}	67.5±10.61^{a}	62.5±10.6^{a}	60.0±14.1^{a}
75days	129.0±19.8^{b}	67.0±9.9^{b}	51.5±4.95^{b}	45.5±7. 8^{b}	46.5±0.7^{ab}
120days	115.0±21.2^{b}	71.0±2.8^{b}	51.5±2.12^{b}	53.5±7.8^{b}	48.0±4.2^{ab}

Values are means±SD
Means not sharing a common superscript letter in a column are significantly different at $p < 0.05$
R =Raw, W= Squeeze-washing, WB = Boiling and squeeze-washing. W1 boiling in1N natron
and squeeze-washing; W2= Boiling in 2N natron and squeeze-washing

Table 5.

Levels of carotenoid of the different species of Vernonia (mg/100 g) after processing

	V. amygdalina	*V. calvoana* var .bitter	**V. colorata**	**V. calvoana** var. non bitters
R	30.0±1.0 [a]	38.5±0.3 [a]	41.5±0.9 [a]	35.8±0.2 [a]
W	21.6±0.4 [b]	16.9±1.0 [b]	26.0±0.5 [b]	28.5±0.2 [b]
Wb	21.9±0.4 [b]	16.5±1.4 [b]	21.2±3.3 [b]	27.8±1.8 [b]
W1	16.4±3.4 [c]	14.9±1.8 [b]	22.5±1.0 [b]	21.8 ± 3.9 [c]
W2	14.9±0.4 [c d]	12.8±0.4 [c]	21.2±2.1 [b]	20.2±0.3 [c]

Values are means±SD
Means not sharing a common superscript letter in a column are significantly different at $p< 0.05$
R =Raw, W= Squeeze-washing, Wb = Boiling and squeeze-washing. W1 boiling in1N natron
and squeeze-washing; W2= Boiling in 2N natron and squeeze-washing

Table 6.

Carotenoid levels of *V. calvoana* var bitter (mg/100 g) after drying

	R	W	Wb	W1	W2
45°C	38.5±0.3[a]	16.9±1.0 [a]	16.4±1.5 [a]	14.9±1.8 [a]	12.8±0.4 [b]
60°C	34.2±0.8 [b]	17.0±2.3 [a]	14.6±3.8 [a]	15.4±2.6 [a]	17.0±0.2 [a]
75°C	36.2±0.5 [c]	13.4±0.8 [ab]	13.9±4.6 [a]	12.4±0.8 [b]	12.2±2.2 [b]
"30"	26.1±0.2 [d]	14.2±3.3 [ab]	12.8±1.4 [a]	12.7±1.7 [b]	17.5±0.7 [a]

Values are means±SD *"30"* indicates sun drying
Means not sharing a common superscript letter in a column are significantly different at $p< 0.05$
R =Raw, W= Squeeze-washing , WB = Boiling and squeeze-washing. W1 boiling in1N natron
and squeeze-washing; W2= Boiling in 2N natron and squeeze-washing

Table 7.

Carotenoid levels of *V. amygdalina* (mg/100g) after drying

	R	W	Wb	W1	W2
45°C	30.0±1.0 [a]	21.6±0.4 [a]	21.9±0.3 [a]	16.4±3.4 [a]	14.9±0.4 [a]
60°C	19.5±0.1 [b]	12.3±0.8 [b]	12.1±0.6 [b]	12.1±2.6 [a]	11.6±0.3 [b]
75°C	17.1±1.3 [b]	13.1±1.3 [b]	11.2±0.0 [c]	8.4±0.2 [b]	9.8±0.8 [c]
"30»	17.7±0.5 [b]	13.4±1.2 [b]	14.3±1.2 [b]	7.0±0.2 [c]	8.7±0.6 [c]

Values are means±SD *"30"* indicates sun drying
Means not sharing a common superscript letter in a column are significantly different at $p< 0.05$
R =Raw , W= Squeeze-washing , Wb = Boiling and squeeze-washing. W1 boiling in1N natron
and squeeze-washing; W2= Boiling in 2N natron and squeeze-washing

Table 8.

Carotenoid levels of *V. calvoana* var bitter (mg/100 g) after drying

	R	W	Wb	W1	W2
0day	38.5±1.3 [a]	16.9±1.0 [a]	16.5±1.5 [a]	14.9±1.8 [a]	12.8±0.4 [a]
10days	37.0±.2 [a]	16.8±0.4 [a]	12.0±0.1 [b]	11.10±1945 [a]	11.0±0.5 [b]
30days	23.9±0.5 [b]	14.9±0.6 [b]	10.4±0.2 [c]	10.3±0.1 [ab]	6.7±0.6 [c]
75days	13.9±0.4 [c]	10.5±1.8 [c]	8.3±1.4 [d]	9.1±0.8 [b]	8.7±1.8 [b]
120days	14.1±158 [c]	10.4±0.7 [c]	11.0±0.6 [c]	6.1±0.2 [c]	6.7±O.6 [c]

Values are means±SD
Means not sharing a common superscript letter in a column are significantly different at $p< 0.05$
R =Raw, W= Squeeze-washing, WB = Boiling and squeeze-washing. W1 boiling in1N natron
and squeeze-washing; W2= Boiling in 2N natron and squeeze-washing

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