Nutrient changes due to processing of some species of *Vernonia* in Cameroon

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ABSTRACT

Vernonia, commonly known as "bitterleaf" or "ndole" in most Central and West African countries, is a widely consumed leafy vegetable in Cameroon. Two main types exist, the non bitter types and the bitter type. 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. Processing methods used are thought to influence the nutrient content of these vegetables. The present study is aimed at determining the effect of some species and traditional processing techniques on the nutrient levels of the bitter (*V. amygdalina, V. calvoana* var. bitter) and non bitter *(V. colorata* , *V. calvoana* var. non bitter) species. The processing techniques used were simple squeeze-washing and rinsing, squeeze-washing and rinsing after boiling with clean water or with different concentrations of natron. Results show that the different *Vernonia* species were good sources of proteins (18.16 ± 2.3 to 24.12g/100g DW), ash (between 7.69±0.11 and 11.96±0.1g/100gDW), vitamin C (137.5±3.3 to 197.5 ± 3.5mg/100gDW), carotenoids (between 30.0 ± 1.0 and 41.5± 0.9mg/100gDW and dietary fibre (24.88 ± 0.95 and 30.12 ± 0.44g /100g DW). Whereas the protein levels reduced slightly after processing ($p<0.05$), fibre levels increased slightly (between 5.79 and 11.58.6%). The levels of reducing sugars and total lipids decreased by 58 and 59% respectively. Losses of vitamin C were more prominent in the bitter species (*V. amygdalina*-77%, *V. calvoana* var bitter- 55%) that required intense squeeze-washing and rinsing. Comparatively, squeeze-washing (W) proved to be the best processing method that ensured minimum loss of vitamins.

Key words: Vernonia, processing, Proteins, Dietary fibre, Total reducing sugars, lipids, vitamin A and C

RÉSUMÉ

Vernonia, plus connu sous le non "bitterleaf" ou "ndole" en Afrique de l'Ouest et Centrale est consommé par la majorité de population du Cameroun. Il existe deux types de *Vernonia*; espèces amère et non amer. Les traitement traditionnels utilisées sont le lavage, le lavage après blanchement, et utilisation de natron. Le présent travail a pour but d'évaluer les effets des espèces et des traitements traditionnels sur les teneur en nutriments dans les espèces de *Vernonia* amer (*V. amygdalina, V. calvoana* var. amère) et non amer (V. *colorata* et *V. calvoana* var. non amère) rencontrées au Cameroun. Les traitements utilisés sont le lavage, le lavage après blanchiment sans natron, le lavage après blanchiment avec une concentration de natron (1N) ou avec une double concentration de natron (2N). Les résultat ont montré que les ces légumes feuilles sont de bonnes sources de protéines, (18,16 ± 2,3 to 24,12±0,98g/100g MS), cendre (entre 7,69±0,11 et 11,96±0,1g/100g MS), vitamine C (137,5 ± 3,3 to 197,5 ± 3,5mg/100gMS), caroténoïdes (entre 30.0 ± 1.0 et 41.5± 0.9mg/100gMS) et fibre alimentaire (24.88 ± 0.95 et 30.12 ± 0.44g /100g MS). Les traitements entraînent une baisse de la teneur en protéines tandis que la teneur en fibres augment (entre 11.9 et 24.6%). Les taux de sucres réducteurs et lipides réduisaient de 58 et 59% respectivement. Les pertes de vitamine C sont plus claires dans les espèces amères (*V. amygdalina*-77%, *V. calvoana* var amère- 55%) qui nécessitent le lavage intense. Le lavage est le traitement qui s'assure le minimum de perte en vitamines.

Mots cles: Vernonia, transformation, Protéines, Fibres Alimentaire, sucres réducteurs, lipides, vitamines A et C

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INTRODUCTION

Malnutrition persists in developing countries in spite of the increase in production of basic foods. Leafy vegetables offer the most rapid and cheapest method of providing substantial supplies of proteins, minerals, fibre and vitamins to the most vulnerable groups (1CN, 1992). However, in Tropical Africa, millions of people still suffer from nutrient insufficiency despite the increased consumption of leafy vegetables.

The leaves of *Vernonia* (commonly known in most west and central African countries as Ndole or bitterleaf) is widely known in most African and Asian countries as a leafy vegetable used in human nutrition and for the treatment of some major diseases like stomach ache and fever (Gasquet *et al* 1985). It is one of the most widely consumed leafy vegetables in Cameroon. Though consumed by a large proportion of the population, cultivation is limited to the southern parts of the country and in the rainy season. There is therefore a need for storage during the six months of dry season. Post harvest losses are therefore a serious problem, which requires much attention. Two main types exist in Cameroon, the non-bitter type (*V. colorata,* and *V. calvoana* var. non bitter) and the bitter type (*V. amygdalina* and *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 by drying. These processes generally lead to losses of some nutrients and non nutrients (antinutritional factors). Processing and preparation of foods bring about losses in nutrients and the extent of these losses depends on the type of technique used (Bender, 1966).

Studies on the nutritional composition of *Vernonia* are numerous and limited to one specie; *V. amygdalina.* Faboya (1990) demonstrated that ascorbic acid decreases with increase in storage time. Oshodi (1992) found that the dried leaves of *V. amygdalina* were rich in minerals, especially in phosphorus, and that the contents in ascorbic acid were temperature dependent. Little is known about the effect of processing on the different species of *Vernonia (V. amygdalina, V. calvoana* var. bitter, *V. colorata and V. calvoana* var. non bitter*)*. This study is aimed at determining the effect of different processing methods on the nutrient levels of some species of *Vernonia* eaten in Cameroon. This information will be useful in determining the best conditions of processing capable of minimising losses in nutrients and prolonging the shelf life of the different species of V*ernonia.*

EXPERIMENTAL

The young shoots and fresh leaves of *V. amygdalina, V. calvoana* var. bitter, *V. colorata, and V. calvoana var.* non bitter) harvested from an experimental farm in Ngaoundéré, were rinsed in water to remove dust. They were then sorted, sliced and separated into 5 different lots of 450g each that were subjected to different processing conditions:

1. Raw (R): Unprocessed Sample
2. Squeeze-washing (W): the traditional method of squeeze-washing which involves crushing and rinsing to remove the green colour and bad odour was used on 450 gram portions of the sliced samples. The process was intense in the bitter varieties where 40 minutes were used in squeeze washing than the non bitter varieties that used 15 minutes.
3. Blanching and squeeze-washing (Wb): 450g of the fresh sliced samples were blanched in 400 ml of boiling water for 5 minutes, followed by the traditional squeeze-washing.
4. Combination of blanching in natron (a naturally occurring alkaline salt) and squeeze-washing: it involves squeeze-washing after boiling for 5 minutes in 1N (W1) and 2N (W2) concentrations. 450g portions of the fresh sliced samples were blanched in 400 ml of boiling water containing 10g (IN) of natron for 5 minutes before squeeze–washed. These concentrations were determined following a survey of the amount used traditionally.

The raw and processed samples were dried at 45°C, milled to pass through a 1-mm screen and stored in air tight containers for laboratory analysis.

Dietary fibre was determined by enzymatic and gravimetric method (AOAC 1997), crude protein analysed using the method of Devani *et al.* (1989), total lipids were estimated using the method proposed by Bourely (1982), and total reducing sugars analysed by colorimetric method (Dubois et al 1956). The vitamin C levels were determined using

N-Bromo-succinimide (Evered, 1960) recommended for the pigmented solutions while carotenoid was first extracted using a mixture of hexane - acetone 30/70 (v/v) then separated by column chromatography and quantified using a spectrophotometer (AOAC, 1965). All the analyses were done using triplicate samples. Data are reported as mean and standard deviation. 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

The levels of proteins of the different species of *Vernonia* as influenced by the different processing techniques are presented in table 1. The protein values for the unprocessed samples varied between 18.16 ± 2.3 in *V. colorata* and 24.12g/100g DW in *V. calvoana* var. non bitter. The levels of protein in the unprocessed leafy vegetables of all the species studied were slightly different at P>0.05 from values obtained when the leafy vegetables were subjected to different processing conditions.

The total reducing sugar of the raw samples ranged from 13.08 ± 1.44 to 15.78 ± 0.14g/100g DW (table 2). No significant difference (P>0.05) in the level of reducing sugar was observed in all species. However, processing conditions considerably affected the total reducing sugar levels. Samples boiled with natron (W2) had as much as 58% loss of total reducing sugar for *V. calvoana* while washed *V. amygdalina* (W) had only 12% loss.

The effect of processing on the dietary fibre levels of the species of *Vernonia* is shown in table 3. Dietary fibre values ranged from 24.88 ± 0.95 in *V. colorata* to 30.12 ± 0.44g /100g DW in *V. calvoana* var. non bitter for the raw samples. This value increased by as much as 11.58% when *V. amygdalina* was squeeze washed without being boiled. The non

Table 1: Effect of the different processing techniques on the crude protein levels of the different *Vernonia* species (g/100g DW)

	V. amygdalina	*V. calvoana* var. bitter	*V. colorata*	*V. calvoana* var. non bitter
R	19.23±0.20[a]	21.34±0.32[a]	18.16±2.30[a]	24.12±0.98[a]
W	19.46±0.49[a]	20.67±0.41[a]	17.01±0.32[a]	24.26±0.87[a]
Wb	18.89±0.34[a]	19.24±0.13[a]	18.93±0.20[a]	23.26±0.94[a]
W1	18.74±0.24[a]	19.39±0.16[a]	18.82±0.88[a]	21.60±1.90[a]
W2	18.06±0.78[a]	18.02±0.17[a]	17.98±0.79[a]	22.10±0.71[a]

Means not sharing a common superscript letter in a column are significantly different at p< 0.05; R =Raw, W= Squeeze-washing, WB = Blanching and squeeze-washing. W1= blanching in 1N natron and squeeze-washing; W2= Blanching in 2N natron and squeeze-washing.

Table 2: Effect of the different processing techniques on the levels of total reducing sugars of the different *Vernonia* species (g/100g DW).

	V. amygdalina	*V. calvoana* var. bitter	*V. colorata*	*V. calvoana* var. non bitter
R	14.31±0.40[a]	15.79±0.14[a]	14.81±1.53[a]	13.08±1.44[a]
W	12.58±0.39[b]	13.75±0.27[b]	12.56±0.72[b]	10.57±1.06[b]
Wb	11.87±0.20[c]	11.18±0.56[c]	12.20±0.19[b]	11.03±0.81[b]
W1	10.98±1.11[c]	7.67±0.68[d]	8.21±0.31[c]	7.25±0.58[c]
W2	10.60±0.84[c]	6.66±1.13[d]	8.48±0.12[c]	7.48±0.26[c]

Means not sharing a common superscript letter in a column are significantly different at p< 0.05; R =Raw, W= Squeeze-washing, WB = Blanching and squeeze-washing. W1= blanching in1N natron and squeeze-washing; W2= Blanching in 2N natron and squeeze-washing.

Table 3: Effect of the different processing techniques on the dietary fibres levels of the different *Vernonia* species (g/100g DW).

	V. amygdalina	*V. calvoana* var. bitter	*V. colorata*	*V. calvoana* var. non bitter
R	25.47±0.29 [a]	27.58±0.47 [b]	24.88±0.25 [a]	30.12±0.46 [a]
W	27.91±0.74 [b]	29.91±0.39 [a]	26.32±0.50 [b]	32.48±0.46 [b]
Wb	27.76±0.37 [b]	29.07±0.35 [a]	26.15±0.08 [b]	32.12±0.33 [b]
W1	26.98±0.45 [b]	29.38±0.27 [a]	25.71±0.02 [b]	31.28±0.61 [b]
W2	27.73±0.65 [b]	29.32±0.46 [a]	25.90±0.86 [b]	32.40±0.83 [b]

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

Table 4: Effect of the different processing techniques on the levels of total lipids of the different *Vernonia* species (g/100g DW)

	V. amygdalina	*V. calvoana* var. bitter	*V. colorata*	*V. calvoana* var. non bitter
R	4.70±0.40 [a]	4.00±0.40 [a]	7.19±0.17 [a]	2.57±0.45 [a]
W	2.26±0.01 [b]	3.13±0.30 [b]	4.30±0.49 [b]	2.25±0.21 [a]
Wb	2.13±0.57 [b]	2.14±0.10 [c]	4.73±0.52 [b]	1.22±0.09 [b]
W1	1.94±0.28 [b]	2.47±0.07 [c]	4.52±0.38 [b]	1.03±0.25 [b]
W2	2.83±0.21 [c]	2.43±0.12 [c]	4.73±0.14 [b]	1.11±0.07 [b]

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

Table 5: The effect of processing on ash levels of the four species of *Vernonia* in g/100g DW

	V. amygdalina	*V. calvoana* var. bitter	*V. colorata*	*V. calvoana* var. non bitter
R	7.72±0.11 [a]	10.52±0.30 [a]	11.84±O.27 [a]	11.96±0.15 [a]
W	5.65±0.8 [b]	9.08±O.18 [b]	10.10±0.21 [b]	10.78±0.19 [b]
Wb	5.37±0.59 [b]	6.46±0.27 [c]	8.42±0.08 [c]	8.85±0.14 [d]
W1	6.19±0.46 [b]	6.15±0.26 [c]	8.86±0.6 [c]	9.491±0.21 [c]
W2	5.40±O.37 [b]	6.61±0.18 [c]	10.09±0.19 [b]	10.85±0.12 [b]

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

bitter varieties had lower percentage increases (5.79 and 7.84% increases for *V. calvoana* var. non bitter and *V. colorata*).

Total lipid levels for the raw samples varied with species and ranged from 2.5 ± 0.45g / 100g DW for *V. calvoana* var. non bitter to 7.19 ± 0.17g/ 100g DW for *V. colorata* (Table 4). From these results, it was observed that *V. amygdalina* had 4.7 ± 0.4 g/100g DW total lipids. The levels of total lipids also decreased when these samples were subjected to the different treatments. Loss of total lipids increased with squeeze-washing and blanching with or without natron. This explains why *V. calvoana* non bitter had 12% while *V amygdalina* had 59% loss.

Results of the ash levels of both processed and unprocessed *Vernonia* are presented in table 5. These levels are high between 7.72 ±0.01 and 11.96±0.15g/ 100g DW for the unprocessed samples. In all the species there was a significant decrease in ash levels due to processing of these leafy vegetables. These losses were however minimal for the samples processed using kanwa.

Table 6: Effect of different treatments conditions on the Vitamin C levels of the different species of *Vernonia* (mg/100g DW).

	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}

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

Table 7: Effect of different treatments on the levels of carotenoid of the different species of *Vernonia*. (mg/100g).

	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^{cd}	12.8 0.4^{c}	21.2 2.1^{b}	20.2 0.3^{c}

Means not sharing a common superscript in a column are significantly different at $p< 0.05$; R =Raw, W= Squeeze-washing, WB = Blanching and squeeze-washing. W1 =blanching in1N natron and squeeze-washing; W2= Blanching in 2N natron and squeeze-washing

Table 6 shows the levels of vitamin C in the species of *Vernonia* as affected by different processing techniques. Vitamin C values for the raw leaves of all species of *Vernonia* varied from 137.5 3.3mg/100g DW in *V. calvoana* var. non bitter to 197.5 ± 3.5mg/100g DW in *V. colorata.* These values were generally high for all the species of *Vernonia.* 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 levels of carotenoids in the different species subjected to different processing techniques are shown in Table 7. Carotenoid levels range from 30.0 ± 1.0 in *V. amygdalina* to 41.5±1.0mg/100g DW 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.

DISCUSSION

High values of proteins were obtained for the different species of untreated *Vernonia* with *V. calvoana* var. non bitter having the highest value of proteins. In addition, these values reduced slightly when different processing techniques were applied. Values for both raw and processed samples are close to values obtained by Igile *et al.* (1995), for raw *V. amygdalina* and Favier *et al.*, (1988) for other leafy vegetables. Animal proteins are generally known to have a high biological value than plant proteins but plant foods, when rightly combined with other foods can satisfactorily meet the protein needs of adults (Favier and Virobin, 1996).

The levels of reducing sugars in all species were similar in the unprocessed leaves but processing conditions resulted to considerable losses. The loss was more pronounced in samples treated with natron. This is an indication of the fact that natron being a base, hydrolysis the sugars present in the samples into soluble forms (Cheftel *et al,* 1977). Igile *et al.* (1995), found slightly higher values of reducing sugars in *V. amygdalina.* The difference may be due to the physiological state of the plant before harvesting (Singhal and Kulkarni, 1988). These sugars are the molecules responsible for Millard reaction during blanching of leafy vegetables and therefore responsible for the brown colour if proper precautions are not taken (Cheftel *et al* 1977).

Vernonia have high levels of dietary fibre which varied significantly in the different species. Though slight increases were observed due to different processing techniques ($P<0.05$), these increases were more in the bitter species. This can be attributed to the fact that though, there were losses of other soluble nutrients in the vegetable which could cause an increase in fibre levels; there were also losses of soluble fibre during processing which is more in the bitterspecies where processing was more intense with more losses of the other soluble nutrients. Craplet *et al.* (1979) and Tanya *et al.* (1997) affirmed that leafy vegetables are particularly rich in dietary fibre. Eun – Hee *et al.* (1993) found the average levels of dietary fibre in leafy vegetables of Asian countries to be 33% DW. The present study reports values that are slightly lower. High levels of dietary fibre in leafy vegetables are advantageous for their active role in the gastrointestinal tract (Jenkin *et al.* 1986). It is worthy to recall that dietary fibres are constituents of plant foods that remain undigested by human intestinal enzymes (Lairon, 1990); in effect they are essentially made up of cellulose, hemicelluloses, lignin and pectin. Nyman *et al.* (1987) and Lintas and Cappelloni (1988) found for culinary and industrial treatments slight increases for different leafy vegetables.

Total lipids levels for the raw samples varied with species. Igile *et al* (1995), found similar values for *V. amygdalina.* Singhal and Kulkarni, (1987) reported higher values for *Amaranthus* spp. Generally these low values of total lipids in these samples corroborate the findings of many authors which showed that leafy vegetables are poor sources of lipids. The levels of total lipids also decreased when these samples were subjected to the different treatments. These losses are linked to squeeze washing and to the effect of heat during boiling.

The high values of ash observed in all species of *Vernonia* is a good indicator that these food samples are good sources of minerals when compared to values obtained for cereals and tubers (FAO, 1968). These values were also found to be higher than values obtained for some *Amaranthus* species (Singhal and Kulkarni, 1987). Higher losses in ash due to different processing methods could be explained by the fact that these minerals remain soluble in water in the course of squeeze washing. However, these losses were not so high in samples treated with natron (19.5%) and this could be explained by the fact that natron contains some minerals that could remain after processing

Vitamin C values were high for all species of *Vernonia* with as much as 77% losses after processing. Bender (1966) observed this same trend for other vegetables, and proved that in leafy vegetables, losses in vitamin C were a function of the method of processing. In this same light Machlin, (1984) found 50% *loss* after cooking *V. amygdalina.* These losses are justified since vitamin C is thermo sensitive and hydro soluble.

Vitamin A deficiency remains a major problem in Cameroon, affecting mostly the people in the Northern provinces (Domngang *et al,* 1990). 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 (Nagra and Khan, 1989). High levels of vitamin A, found in the raw samples of the different species of *Vernonia* were destroyed during processing. These losses were more in the bitter species: *V. amygdalina* and *V. calvoana* var. bitter. 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 vitamin A is not hydro soluble, it could be destroyed by the use of alkaline in cooking. The losses observed as a result of processing in the present study are higher than those reported by Nagra and Khan (1989) and Renquist *et al* (1978), and this can be attributed to variations in processing methods.

CONCLUSION

The different species of *Vernonia* (*V. calvoana var. bitter, V. amygdalina,* and *colorata* var. bitter and *V. calvoana* var. non bitter) are good sources of proteins, carotenoids, vitamin C and dietary fibre with levels particularly higher in most cases in the non bitter species (*colorata* and *calvoana* var. non bitter). Unfortunately, some of these nutrients are lost during processing. Boiling with high levels of natron favours more losses in reducing sugars (58%) though these same treatments had a milder effect on the levels of crude proteins. Squeeze-washing and rinsing seems to be the best treatment to preserve both vitamins A and C. Due to the increased demand for these nutrients, and the lack of possibility to avoid processing, it is therefore pertinent that preference be given to the non bitter species.

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