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Effect of Different Processing Methods on Nutritional Composition of Bitter Leaf (vernonia *amygdalina)*

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ABSTRACT: *Effect of different processing methods on proximate, vitamins and minerals composition of vernonia amygdalina were evaluated using standard procedure and method. Leaves of vernonia amygdalina were subjected to boiling, sun drying squeeze washing+ salt and squeeze washing+boiling. Proximate values of the fresh vegetables were generally higher than it processed counterparts. The least moisture and Fiber content were recorded for sundried leave, while the least lipid, proteim and ash contents were recorded for Squeeze+boiled leaf. Squeeze+salt processing methods had the least effect on the proximate content when compared with other processing methods. The vitamin C and A contents of the fresh leave were 195.5±0.14 mg/100g and 0.38±0.02mg/100g respectively. The vitamin C and A contents were more lowered in Squeeze+boil sample, while Sundrying had the least effect on vitamin C and vitamin A content when compared with other processing methods. The fresh leaf also had higher mineral contents than the processed vegetable except for Na which was highest in Squeeze+salt sample. The least Fe, Ca and K contents were recorded for Squeeze+boil sample. In conclusion Vernonia amygdalina possesses considerable amounts of nutrients. However, the traditional method of processing this vegetable results in loss of some of the vital nutrients*

KEYWORD: *vernonia amygdalina, proximate, minerals, vitamin, processing methods*

I. INTRODUCTIONS

Nutritional importance of vegetables can not be neglected in our daily meals. Vegetables are edible parts of the plants, which are usually cooked and salted before consumption with other foods. Fresh vegetables are important foods both from an economic and nutritional point of view and vegetable of all types are valuable part of our diet. They play an important part in maintaining general good health owing to the presence of mineral element and vitamin (Adegunwa *et al*., 2011). Leafy vegetables are consumed as cooked complements to the major staples like cassava, cocoyam, guinea corn, maize, millet, rice and plantain (Nwanekezie and Obiakor-Okeke, 2014)

The high biological value of leafy vegetables depends on the pronounced content of the minerals compound especially, calcium, magnesium, phosphorus and iron (Jaworska and Kmiecik, 2009). In general these crops also contain significant amounts of beta-carotene, folic acid and dietary fibre. Of this group of vegetables, leafy vegetable are seasonal crops with a limited value for processing. The protein in leaves is low but what is present is of the high grade. The quantity of vitamin E in leafy vegetables increases with their greenness (Kochhar, 2001).

V. amygdalina Delile, commonly known as bitter leaf, is a shrub or small tree belonging to the family Asteraceae. *V. amygdalina* is a popular African vegetable which grows in several parts of tropical and subtropical Africa (Erasto *et al*., 2006). It is known as 'Chusar-doki' in Hausa, 'Ewuro' in Yoruba, 'Onugbu' in Igbo, 'Ityuna' in Tiv, 'Oriwo' in Edo, 'Etidot' in Ibibio, and 'Grawa' in Amharic. *V. amygdalina* is drought tolerant though it grows better in a humid environment (Ikeh *et al.,* 2014). It is used to a large extent in tropical Africa for its culinary and medicinal purposes, it is also used in the traditional treatment of malaria, diabetes, diarrhea, veneral disease, hepatitis, gastrointestinal problems, skin disorders, cough, constipation and in the treatment of wounds (Ajebesone and Aina, 2004). However, the plant pass through different processing method such as blanching, squeeze-washing, boiling and sun drying before comsumption which may results in nutrient lost and it is difficult to assess the nutritional values of the final product in this regard. It was in the light of this that the present study was designed to evaluate the effect of different processing methods on Nutritional composition of *Vernonia amygdalina* It is hoped that the data generated from this study will help in establishing the best processing method with higher nutrient retaintion for *Vernonia amygdalina.*

II. MATERIAL AND METHOD

Materials

Plant Collection

Fresh leaves of *V. amygdalina* was collected from from Baddegi, Niger State Nigeria. Taxonomic authentication of the plant was conducted by a Botanist in the Department of Biological Science, Federal University of Technology Minna, Niger State

Reagent and chemicals

All chemicals used were of analytical grade and were products of Sigma Chemical Co., USA. Distilled water was used for all the washing, cleaning and preparation of solutions used. All the glassware was thoroughly cleaned with liquid detergents, rinsed with distilled water before being oven dried at 105^0C.

Methods

Processing Techniques:

Fresh leaves sample of *V. amygdalina* was subjected to some conventional food processing techniques as reported by (Babalola, *et al*., 2010). The Various processing methods are described below:

Boiling: This involved placing Fresh leaves sample of *V. amygdalina* in boiling water for some minutes (5 min)

Sun drying: This required a thorough drying until crisp with solar energy after cutting the sample with a sharp knife

Squeeze: Washing with salt - This process involved the tearing apart of the tissues with hand and subsequent rinsing in water with addition of 10% table salt (w/w of sample)

Squeeze: Washing with boiling - This process involved the tearing apart of the tissues with hand and subsequent boiling in water (Babalola, *et al*., 2010).

Proximate Analysis

Determination of Moisture content:

Two grammes each of *V. amygdalina* samples was placed in the crucible and heated at 105˚ C until a constant weight was attained. The moisture content was calculated as loss in weight of the original sample and expressed as percentage moisture content.

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - w_1} \times 100$$

Where:

W1 = initial weight of empty crucible

W2 = weight of crucible +sample before drying

W3= final weight of crucible + sample after drying

Determination of crude protein:

A (0.5g) each of *V. amygdalina* samples was digested with 5 ml of concentrated sulphuric acid in the presence of Kjeldahl catalyst. The nitrogen from the protein in the sample was converted to ammonium sulphate that reacted with 2.5 ml of 2.5 % Brucine reagent, 5 ml of 98 % sulphuric acid to give a coloured derivative and the absorbance read at 470 nm. The percentage nitrogen was calculated and multiplied by 6.25 to obtain the value of the crude protein (A.O.A.C., 1990).

$$\% \text{ Nitrogen} = \frac{Vs - Vb \times Nacid \times 0.01401}{W} \times 100$$

Where: Vs =titer value of the sample

Vb= of acid required to titrate

Nacid= normality of acid

W= is the weight of sample in grams

Estimation of crude lipid:

This estimation was performed using the Soxhlet extraction method. Ten grammes of each *V. amygdalina* samples were weighed and wrapped with a filter paper and placed in a thimble. The thimble was covered with cotton wool and placed in the extraction column that was connected to a condenser. 200 ml of n – Hexane was used to extract the lipid (A.O.A.C., 1990).

$$\%Fat = \frac{W_2 - W_3}{\text{Weight of sample}} \times 100$$

Where W_2=wt of filter paper and sample before extraction

W_3=wt of filter paper and sample after extraction

Determination of crude fibre:

Each of *V. amygdalina* samples (5g) and 200 ml of 1.25 % H_2SO4 were heated for 30 min and filtered with a Buchner funnel. The residue was washed with distilled water until it was acid free. 200 ml of 1.25% NaOH was used to boil the residue 30 min, it was filtered and washed several times with distilled water until it was alkaline free. It was then rinsed once with 10% HCl and twice with ethanol. Finally it was rinsed with petroleum ether three times. The residue was put in a crucible and dried at 105^{o}C in an oven overnight. After cooling in a desiccator, it was ignited in a muffle furnace at 550^{o}C for 90 minutes to obtain the weight of the ash.

% fiber content = The loss in weight after incineration × 100.

Determination of ash content:

The total ash content of a substance is the percentage of inorganic residue remaining after the organic matter has been ignited. 2 g each of *V. amygdalina* samples was placed in a crucible and ignited in a muffle furnace at 550oC for 6 hours. It was then cooled in a desiccator and weighed at room temperature to get the weight of the ash. (A.O.A.C., 1990).

$$\textbf{\%Ash content} = \frac{\text{Weight of ash}}{\text{Weight of original food}} \times 100$$

Carbohydrate determination:

The carbohydrate content was determined by subtracting the summed up percentage compositions of moisture, protein, lipid, fibre, and ash contents from 100 (Otitoju, 2009).

%Carbohydrate:= 100 – (%Protein + %Moisture + %Ash + %Fibre)

III. VITAMIN ANALYSIS

Determination of Vitamin C

This was determined using the AOAC.967.21 (1996) method. Five grams each of *V. amygdalina* samples was diluted with 10% trichloroacetic acid (TCA) to 100.0ml mark of 100ml volumetric flask. 2, 6-dichlophenolindophenol was titrated to 10.0ml of the vegetable filtrate. Ascorbic acid was calculated as:

Ascorbic acid, (mg/100g) = (A-B) X C X 100/s X (100/10)

Where A = Volume in ml of indophenol solution used in the sample.

B = Volume in ml of indophenol solution used for the blank

C = Mass in mg of ascorbic acid equivalent to 1 ml of standard indophenol solution.

S = weight of the sample taken (g)

100/10 = total extraction volume / volume of titrated sample

Determination of Vitamin A

1g each of *V. amygdalina* samples weighed using the weighing balance. 10ml of distilled water was added into the sample and hemonized using ceramic mortar. The solution was sieved with filter paper. 2mls of the filtrate was pipette and discharged into a test-tube. 2mls of 1M KOH was added to the filtrate in the test-tube. The solution was shaken thoroughly for 1 minute. The solution was heated in a water bath at 60 degrees Celsius for 20minutes. After cooling, 20mls of xylene was added to the solution. The solution was mixed thoroughly using the cyclo-mixer or vortex mixer. After mixing, it was centrifuged for 10minutes. The absorbance was taken at 335A. The solution was radiated and absorbance was taken again. It was calculated as thus;

% Vitamin = absorbance before radiation – absorbance after radiation

Mineral Analysis

The method of A.O.A.C (1990) was employed for the determination of mineral content. One gramme each of *V. amygdalina* samples was placed in a crucible and ignited in a muffle furnace at 550OC for 6 hours. The resulting ash was dissolved in 10 ml of 10 % HNO3 and heated slowly for 20 minutes. After heating, it was filtered and the filtrate was used for the determination of mineral content. Atomic absorption spectrophotometer (AAS) was used to determine Ca, and Fe, while flame photometer was used for the determination of Na and K in the filtrate

Statistical analysis

The data obtained were subjected to Analysis of Variance (ANOVA) using SAS statistical package. Means were separated using Duncan's Multiple Range Test (DMRT). Significance was accepted at $P < 0.05$. The data is given as mean $\pm$SEM.

RESULTS

PROXIMATE COMPOSITIONS

The proximate contents of vernonia *amygdalina* as affected by different processing methods are shown on Table 1. proximate values for the raw 57.00$\pm$0.45, 4.17$\pm$0.20, 18.10$\pm$0.40, 9.23$\pm$0.71, 8.02$\pm$0.20 and 348$\pm$0.5 for Moisture, lipid, protein, ash, fiber and Carbohydrate respectively. These values were generally high for the raw vegetables when compared with their processed counterparts. The least moisture content (12.43$\pm$0.43) and Fiber (6.72$\pm$0.03) content was recorded for sundried leave, while the least lipid content (1.13$\pm$0.13), proteim (9.77$\pm$0.99), and ash (3.32$\pm$0.99) contents was recorded for Squeeze+boiled leaf, Squeeze+salt processing methods had the least effect on the proximate content when compared with other processing methods.

Vitamins

The vitamin C and A contents of vernonia *amygdalina* as affected by different processing methods are shown on Table 2. The vitamin C and A contents of the fresh leave are195.5$\pm$0.14 mg/100g and 0.38$\pm$0.02mg/100g respectively. These values were also high for the raw vegetables when compared with their processed counterparts. The vitamin C (87.9$\pm$0.11mg/100g) and A (0.09$\pm$0.02) contents are more lowered in Squeeze+boil sample, while Sundrying had the least effect on vitamin C and A content when compared with other processing methods

Minerals.

The Minerals contents of vernonia *amygdalina* as affected by different processing methods are shown on Table 2.the fresh leaf contain Sodium (30.12± 0.12mg/100g), Potassium (9.50 ± 0.12mg/100g), Iron (3.77±0.10mg/100g) and Calcium (83.19$\pm$0.22mg/100g). These values were generally high for the raw vegetables when compared with their processed counterparts except for Sodium (43.11± 0.70mg/100g) which is highest in Squeeze+salt sample. The least iron content (2.99±0.01mg/100g) and Calcium (71.09$\pm$0.03mg/100g), and Potassium (8.20 ± 0.11mg/100g) was recorded for Squeeze+boil sample.

Table 1: The proximate composition of vernonia *amygdalina* at different processing methods

Sample	Proximate Composition (%)					
	Moisture	**Lipid**	**Protein**	**Ash**	**Fiber**	**Carbohydrate**
fresh	57.00±0.45	4.17±0.20	18.10±0.40	9.23±0.71	8.02±0.20	3.48±0.11
Boiled	62.35±0.22	2.31±0.11	12.70±1.32	8.72±1.01	7.93±0.11	5.99±0.40
Sundried	12.43±0.43	3.19±0.03	14.10±2.13	7.21±0.42	6.72±0.03	56.35±0.15
Squeeze+salt	47.45±1.09	3.25±0.30	16.20±1.21	7.99±1.21	7.11±0.30	18.01±0.32
Squeeze+boil	45.54±0.43	1.13±0.13	9.77±0.99	3.32±0.99	7.09±0.13	33.15±0.23

Data are Mean ± SEM of triplicate determination

Table 2: The Minerals composition of vernonia *amygdalina* at different processing methods

Sample	**Minerals (mg/100g)**			
	Sodium	**Potassium**	**Iron**	**Calcium**
fresh	30.12± 0.12	9.50 ± 0.12	3.77±0.10	83.19±0.22
Boiled	28.01± 0.10	8.20 ± 0.22	3.29±0.22	76.21±0.51
Sundried	28.25± 0.11	9.50 ± 0.16	3.11±0.01	79.96±0.11
Squeeze+salt	43.11± 0.70	8.50 ± 0.32	3.00±0.13	80.61±0.23
Squeeze+boil	26.01± 0.11	8.20 ± 0.11	2.99±0.01	71.09±0.03

Data are Mean ± SEM of triplicate determination

Table 3: The vitamins composition of vernonia *amygdalina* at different processing methods

Sample	vitamins (mg/100g)	
	Vitamin C	Vitamin A
fresh	195.5±0.14	0.38±0.02
Boiled	112±0.26	0.13±0.00
Sundried	175±2.46	0.23±0.11
Squeeze+salt	125±4.16	0.16±0.01
Squeeze+boil	87.9±0.11	0.09±0.02

. Data are Mean ± SEM of triplicate determination

IV. DISCUSSION

Vegetables plays an important role in human diet, they are important source of both digestible and indigestible carbohydrate. They are also good sources of Vitamin C, Beta carotene and other nutrients and are responsible for more subtle feelings of daily well-being and for protection from long-term degenerative disease (Oboh, 2005).

Analysis of proximate composition give information on the basic chemical composition of food, the compositions are moisture, ash, crude fat, protein and carbohydrate. Moisture content is an index of water activity. The increase in the moisture contents of the boiled leaves as compared to the fresh leaves could be as a result of water absorption by the fibres and other natural chemical component of the vegetables (Ajala, 2009). high moisture content will increase susceptibility of the vegetables to microbial attacked, in this study sundried leaves contain the least moisture contents this will favour their preventive properties against microbial attacked and thus the storage life of the sundried vernonia *amygdalina* will be high (Adeyeye and Ayejugo 1994).

The ash content give a measure of total amount of inorganic compounds like minerals present in a sample. The ash content of the fresh leaves of vernonia *amygdalina* was higher than the processed sample this is an indication that fresh leaves of vernonia *amygdalina* contain more minerals than the processed leaves The decrease in the ash content of processed vegetables could be as a result of processing during which some of the inorganic salt in the vegetables might have leached off (Yaciuk and Sofose 1981).

Lipids are distinct and diverse set of small molecules consisting of eight general compound classes including fatty acyls, glucerolipids, glycerophospholipids sphingophospholipids sterol lipids, phenol lipids, saccharolipids and polyketides (Ezeocha and Ojimelukwe, 2012). All the method use in processing vernonia *amygdalina* cause reduction in lipid with the Squeeze+boil causing the highest reduction. With boiling the fat must have melted into the boiling water thus causing a reduction in the fat content. However, excess fat consumptions has been implicated in the etiology of certain cardiovascular disease such as cancer and aging (Anha *et al*., 2006).

Protein is an essential component of human diet needed for the replacement of tissue and for the supply of energy and adequate amount of required amino acid. Protein deficiency cause growth retardation, muscle wasting, oedema, abnormal swelling of the body and collection of fluid in the body of children (Mounts, 2000). All the processing methods evaluated in this study cause reduction in the protein contents of vernonia *amygdalina*. Boiling +squeeze washing cause the highest reduction , this reductions may be due to the fact that during boiling cellular protein are denatured and the chlorophyll which are bound to protein may be released such free chlorophyll are highly unstable and are readily converted to pheophytin which is olive green to brown in colour (Komolafe and Obayanju, 2003).

Vitamin C has anti-infective properties, promotes wound-healing, may boost the immune system and help to ward off infections, while vitamin A helps to maintain good sight and prevents certain diseases of the eye. Both vitamins also have antioxidant properties and may protect against some forms of cancer (Wright, 2002). Sundrying had the least effect on vitamin C and A content of vernonia *amygdalina* when compared with other processing methods while Squeeze+boil cause the most reduction on vitamin C and A content of vernonia *amygdalina*. The losses observed in this study are very high most especially when the vegetables were subjected to boiling and squeeze-washing with or without salt. Loss as a result of boiling is justified since vitamin C is water-soluble and heat labile (Egerg *et al.*, 1977). Thus vitamin C is easily leached into the boiling medium

The nutritive metals basically calcium, iron Sodium and Potassium were determined in the vegetable. All processing method cause reduction in the minerals analysed except for squeeze washing which cause increase sodium concentration. Sodium is the principal exteacellular cation and is used for acid – base balance and some osmo-regulation in the body fluid (Odoemena and Ekanem, 2006). Potassium is responsible for nerve action and is very important in the regulation of water and electrolyte balance and acid – base balance in the blood and tissues (National Research Council [NRC], 1989). Calcium is necessary for the strong bones and teeth. It is relatively high in cereals, nuts and vegetable (James, 1996). Iron is an important constituent of heamoglobin. vernonia *amygdalina* can contribute these minerals and enhance their availability in daily life. These vegetable can supplement the daily requirements of Ca, Fe and Na, which have been put by (FAO/WHO, 2001) at (260 mg/day), (0.425 mg/g) and Na (0.099 mg/g) respectively (Weigert, 1991).

V. CONCLUSION

The study showed that Vernonia amygdalina possesses considerable amounts of proximates, minerals, vitamins. However, the traditional method of processing this vegetable including the boiling, squeeze washing and salting or squeeze washing and boiling results in loss of some proximates, Ca, Fe, K, Vitamin C and ß-carotene.

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