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MINERAL AND ANTINUTRIENTS OF FRESH AND SQUEEZED-WASHED BITTER LEAF (*VERNONIA AMYGDALINA*) AS AFFECTED BY TRADITIONAL DE-BITTERING METHODS

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ABSTRACT: *The effect of traditional de-bittering methods on the mineral and anti-nutrient components of fresh and squeezed-washed bitter leaf was studied. Palm oil, Potash, Salt and boiling process was used in the squeeze-washing at 3 pre-processing methods of squeeze-wash and periods of 3 to 8 minutes. The percentage retention and losses of mineral and anti-nutrients increased simultaneously during squeeze-washing. Copper, magnesium, calcium and anti-nutrient had retention of 55 to 100% for samples squeezed-washed with palm oil than the other squeeze- washed samples. This could be due to rigidity of the cells which did not allow much nutrients to leach into the squeezed leaf-water. Loss of minerals and anti-nutrients was observed to be influenced directly by the cause-and-effect of disintegration changes which usually leads to softening due to the severity of the squeeze-washing on the bitter leaf instead of cellular composition or level of minerals and anti-nutrients initially present. Palm oil should be used in the squeeze-washing of bitter leaf for better nutrient retention.*

KEYWORD: Anti-Nutrients, Bitter Leaf, De-Bittering, Mineral, Squeeze-Washing

INTRODUCTION

Green leafy vegetable are important as food both from economic and nutritional standpoint (Bolaji *et al*., 2008). Leafy vegetables contain nutrients which can be absorbed by the body to be used as regulatory and protective material (Saidu and Jideobi, 2009). They occupy an important place among the food crops as they provide adequate amount of many vitamins and minerals for humans (Fasuyi, 2006). Leafy vegetables represent inexpensive but high quality nutritional sources for the poor segment of the population, especially where malnutrition is widespread (Nnamani *et al.*, 2007). They contain phytochemicals which have both positive and detrimental effects. Some of these phytochemicals are anti-nutrients that reduce bioavailability of vitamins and minerals (Chinma and Igyor, 2007). Some forms of these phytochemicals have been used in folk medicine to improve some human health conditions. The potassium content of leafy vegetables is good in the control of diuretic and hypertensive complications, because it lowers arterial blood pressure.

Leafy vegetables contain low calories and negligible quantities of utilize energy. Hence, they are ideal for obsessed people who can satisfy their appetite without consuming much carbohydrate. Some leafy vegetables are known to lower blood pressure (Fugile, 2000).

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These leaves can do much to preserve mother's health and pass on strength to the foetus in pregnant and breast feeding women (Price, 1995).

Vernonia amygdalina, commonly known as bitter leaf mostly grow wild, though it could be cultivated/ domesticated. It is widely consumed in mostly Central African countries both by humans and animals. It is consumed by a large proportion of the Cameroonian population, though its cultivation is limited to the southern parts of the country (Singh *et al.,* 2001). In Nigeria, its consumption is mainly by some Igbo speaking areas in the South-Eastern States (Morah and Obiegbuna, 2002). It is known as Oriwo in Edo, ewuro in Yoruba, Shuwaka in Hausa and Onugbo in Igbo (Igile *et al.,* 1995; Ijeh *et al.*, 1996).

Studies on the nutritional composition of *Vernonia amygdalina* found its nutritional content to conform to the results obtained for green leafy vegetables. It contains phosphorous, ascorbic acid, iron, β –carotene, calcium, water, fibre as well as other nutrients, though it is low in fat (Oshodi, 1992). *V. amygdalina* contains anti-nutritional factors much as alkaloids, saponins, tannins, steroid glucosides (vernoniosides), flavonoids, glycosides (vernomin) and sterols which cause its bitterness (Ologunde *et al.*, 1992; Buttle and Bailey, 1973). Bitter leaf contains appreciable high contents of a-acids, iso-a acid and essential oils which can be used as hop substitutes in beer brewing (Okafor and Anichie, 1983). Processing most times if not always, modifies the nutrient composition especially vegetables which easily lose their water soluble or heat labile nutrient (Agomuo *et al*., 2015).

This work therefore evaluated the minerals and anti-nutrients in fresh and squeeze- washed bitter leaf as affected by traditional methods of de-bittering.

MATERIALS AND METHODS

The fresh bitter leaf vegetables were bought randomly from three different sellers' at a local market in Umuahia, Abia State. This market was chosen because of its peculiarity with the processing and selling of bitter leaf.

Sample preparation: The fresh bitter leaves were sorted, de-stalked and rinsed in water to remove dust and dirt, and were left to drain. They were then divided into six parts. The six parts were individually subjected to different local de-bittering methods.

The first de-bittering method was normal squeeze-washing. The bitter leaves were squeeze-washed by breaking, squeezing and rinsing of the sample to remove bitterness. The process of squeezing and rinsing was done in 3 pre-processing rounds; 8mins each, for the first and second intervals while the third interval is for 5mins making a total of 21mins. Rinsing was done when enough foam was formed that prevented further successful squeeze-washing.

The second de-bittering method was squeeze-washing and boiling. The sample was squeeze-washed using the same procedure as described in the first process but not as intense as was done to the sample subjected to only squeeze washing. In this process, squeeze- washing was mild as it was for only 14mins (3 pre-processing rounds of 8mins, 3mins.) And this made it possible. For the bitter leave samples to retain high level of bitterness. The mildly squeeze-washed bitter leaves were then introduced into boiling water in an iron pot to boil for 2mins at $115^{O}C$.

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The third treatment involved the addition of 5g of salt while squeeze-washing the bitter leaf. The process lasted for 11mins after 3 rounds of squeeze-washing.

The fourth treatment was squeeze washing with the addition of 5g of ground potash. Also this process lasted for 11mins at three rounds of squeeze-wash.

The last treatment involved the addition of 5ml of Palm oil in order to retain the fibrous tissue of the leaves.

The unprocessed sample served as the control. Before any of these samples was analyzed, it was reduced in size by pounding in a mortar with pestle.

Determination of mineral contents of bitter leaf: The minerals were determined by atomic absorption photometry technique by James (1995).

Calcium, Potassium and Sodium Determination: The ash of each sample obtained was digested by adding 5ml of 2M HCL to the ash in the crucible and heat to dryness on a heating mantle. 5ml of 2M HCL was added again, heated to boil, and filtered through a Whatman No. 1 filter paper into a 100ml volumetric flask. The filtrate was made up to mark with distilled water and made ready for reading of concentration of Calcium, potassium and sodium on the Jenway Digital Flame Photometer (PFP7 Model) using the filter corresponding to each mineral element.

The concentration of each of the element was calculated using the formula:

$$\%Ca \text{ or } \%K \text{ or } \%Na = \frac{\text{Meter Reading (MR)} \times \text{Slope} \times \text{Dilution factor}}{1000}$$

Determination of Mg, Cu, Mn, Fe, Zn using BUCK 200 AAS: The digest of the ash of each sample above as obtained in calcium and potassium determination was washed into 100ml volumetric flask with deionised or distilled water and made up to mark. These diluents were aspirated into the Buck 200 Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements was read at their respective wavelengths with their respective hollow cathode lamps using appropriate fuel and oxidant combination.

Determination of anti-nutrients present in the vegetables: The anti-nutrients were determined using the method of Association of Official Analytical chemist (A.O.A.C, 2005).

Phytate Determination: Two grams (2g) of each sample was weighed into 250ml conical flask. Each sample was soaked in 100mls of 2% Hydrochloric acid in the conical flask for 3h. This was filtered through a double layer of hardened filter paper. 50ml of each filtrate was placed in 0.50ml conical flask and 107ml distilled water was added in each case to give proper acidity. 10ml of 0.3% Ammonium thiocyanate (NH_4SCM) solution was added into each solution as indicated. This was titrated with standard iron (III) chloride solution which contained 0.000195g iron per ml. The end point was slightly brownish- yellow which persists for 5mins. The % phytate was calculated using the formula:

$$\%\ \text{Phytic acid} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100 \times 3.55}{\text{Wt. of Sample}}$$

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Oxalate Determination: Two grams (2g) of each sample was boiled in 40ml of water for 30mins in a reflux condenser. 10ml of 20% Na_2CO_3 was added and boiled for another 30mins. The liquid extract was filtered and washed with hot water until the water did not show any alkaline reaction and concentrated and filtered into a small volume and cooled. With constant stirring, HCL (1:1 molar ratio) was added in drops until the final acid concentration after neutralization is about 4% at which stage a heavy precipitate appeared (which was allowed to flocculate). The extract was carefully filtered into a 250ml flask and made up to mark. It was kept overnight, and then supernatant liquid was filtered through a dry filter paper in a dry beaker.

An aliquot of this filtration was put in a beaker and diluted with water to 200ml and made to ammonic acid and then acidified with laconic Acid. In the cold media, 10ml of a 10% calcium chloride solution was added and stirred well for calcium oxalate precipitate to appear, and allowed to settle overnight. The clean supernatant liquid was carefully decanted off through Whatman No. 42 filter paper, without disturbing the precipitate. The precipitate was dissolved in HCI (1:1) acid and precipitated by adjusting the pH with ammonium hydroxide solution. The content was boiled and allowed to settle overnight. Oxalic acid was determined by titrating against 0.05N KMnO4 solution.

Calculation:

1ml of KMnO4 = 0.00225 anhydrous Oxalic acid

$$= \%\text{Oxalic acid} = \frac{\text{titre value} \times 0.00225 \times 100}{2 \qquad 1}$$

$$= \text{titre value} \times 0.1125.$$

Statistical Analysis

The mineral and anti-nutrient compositions were determined by different methods. The data obtained were subjected to analysis of variance (ANOVA) to determine any significant difference at 5% level ($p<0.05$) using SPSS version 16 and was reported as means of three replicate.

RESULTS

Table 1: Mineral composition of different samples of *Vernonia amydalina* on wet basis (mg/100g)

Parameter	Unpro-cessed	Normal squeeze washed washed	Squeeze washed with salt with	Squeeze washed with boiling	Squeeze washed with palm potash	Squeeze oil
Calcium (Ca)	13.11±.99^a	9.01±0.01^b	6.99±0.02^c	5.22±0.02^d	6.01±0.01^c	10.11±0.01^b
Magnesium (Mg)	19.01±0.03^a	15.06±0.03^b	10.20±0.16^c	10.10±0.11^c	20.16±0.69^a	11.02±0.09^c
Potassium (K)	9.00±0.01^a	6.90±0.10^c	5.7±0.20^d	5.10±0.14^{cd}	6.2±0.13^{cd}	7.3±0.18^b
Sodium (Na)	12.01±0.01^b	7.210±0.05^a	15.29±0.26^a	7.00±0.16^c	11.30±0.31^b	12.11±0.14^b
Manganese (Mn)	1.01±0.01^b	2.06±0.03^c	0.99±0.02^b	0.86±0.04^b	1.88±0.12^a	2.11±0.01^a
Iron (Fe)	21.59±0.03^a	12.99±0.01^c	10.98±0.14^d	18.64±0.12^b	7.21±0.05^e	6.01±0.01^e
Zinc (Zn)	2.08±0.02^a	1.81±0.05^b	2.01±0.01^a	2.10±0.02^a	1.87±0.03^b	1.09±0.02^c
Copper (Cu)	0.75±0.13^b	0.69±0.33^b	0.31±0.01^d	0.416±0.04^c	0.19±0.01^e	0.85±0.03^a

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Values are means and standard deviations of three replicates; values with the same superscript are not significantly different ($p<0.05$)

Table 2: Anti-nutrient composition of *V. amygdalina* in percentage (%)

Samples	Oxalate	Phytate
Unprocessed	3.72 ± 0.19^{a}	2.16 ± 0.67^{c}
Normal squeezed washed	2.99 ± 0.01^{b}	3.56 ± 0.02^{a}
Squeezed washed and boiled	1.34 ± 0.16^{d}	1.84 ± 0.14^{e}
Squeezed washed with salt	1.24 ± 0.13^{e}	1.92 ± 0.10^{d}
Squeezed washed with potash	2.01 ± 0.01^{c}	1.02 ± 0.09^{f}
Squeezed washed with palm oil	3.07 ± 0.14^{b}	3.04 ± 0.02^{b}

Values are means and standard deviations of three replicates; values with the same superscript are not significantly different ($p<0.05$)

DISCUSSION

The values of minerals analyzed showed that there were losses of minerals when bitter leaf was squeeze – washed. Squeeze-washing with salt, potash, palm oil and that squeeze-washed and boiled also caused significant ($p<0.05$) reductions in the K, Ca, Mg, Na, Fe and Zn contents of the vegetable. Similarly, Oladunmoye *et al.* (2005) reported significant reductions ($p<0.05$)in K, Ca, Mg, Na, Fe and Zn contents of blanched, cooked tender and matured cassava leaves. Selman (1994) also reported reductions in the Fe and Zn contents of scent leaf (*Ocimum gratissum)*, bitter leaf (*Vernonia amygdalina)*, bush okro and green pepper (*Piper guinensis*) that were used in soup preparation. This is in conformity with reports of Mepba *et al.* (2007) that blanching and cooking significantly reduced ($p<0.05$) the K, Ca, Na, Zn and Fe contents of *Amaranth*, tomatoes, fluted pumpkin, spinach, slippery vine and cocoyam leaves..

Calcium is probably mainly associated with the pectic substances of the cell wall and could significantly influence texture. Its high content in vegetables especially fruit vegetables is important in strengthening of bones and teeth in man and animals (Balakbir *et al.*, 1998) and necessary for blood clothing (Lean, 2006). The value of calcium content of the bitter leaf sample (13.11+0.99mg/100g) obtained (Table 1) is similar to the value reported for *V.amgydalina* by Ejoh *et al.* (2007a). The value is lower than the values reported for *V. amgydalin* and *M. charantia* by Ayoola *et al.* (2001), though it is higher than the Ca value reported for *A. hydrides* by Akabugwo *et al.* (2007). Murray *et al.* (2000) opined that macro-minerals are required in amounts greater than 100mg/100g. The value of calcium content of bitter leaf in this study is close to the values reported by Lean (2006) for bitter leaf and hence high enough to meet the requirement and could therefore be said to be a good source of the mineral. The availability of Ca in body fluids and in water shows that it is water soluble (Lean, 2006). This explains the significantly lower ($p<0.05$) value of the calcium content in the squeeze –washed bitter leaf. Ejoh *et al.* (2007b) reported a 50% loss of calcium in *V. Colorata* subjected to squeeze- washing, which was due to leaching of the calcium into washing water. Makobo *et al.* (2010) stated that blanched leafy vegetable (*Amaranthus conentus L.*) had lower calcium content than the fresh leaf, which was probably due to

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leaching/ loss of the calcium into the water. The squeeze- washed and boiled bitter leaf had the lowest values, which is due to the influence of heat in increasing dissolution of the solutes in water, enhanced by differences in the concentration of the mineral in the leaf and boiling water.

Magnesium is an active component of several enzymes. It is also a constituent of bones, teeth, enzymes co-factors and a constituent of chlorophyll (Murray *et al.*, 2000). The magnesium content of the studied bitter is similar to that reported for *A. hybidus* by Akubugwo *et al.* (2007). It is high enough to meet the RNI adult intake of 0.3g/day (300mg/day) for the mineral (Lean, 2006). It is therefore a good source of the mineral. Magnesium like most other minerals (inorganic substances) is soluble in water. Thus, some of the magnesium contained in the leafy vegetable will leach into the washing water. This explains the lower values of the magnesium content of the squeeze- washed bitter leaf. Squeeze- washing and boiling caused more significant reduction ($p<0.05$) in the magnesium content of bitter leaf. This was due to increased dissolution of the mineral into boiling water. Obiakor (2007) stated that cooked trifoliate yam had lower magnesium content than that of raw trifoliate yam. The sample squeeze –washed with potash had the highest result. This can be attributed to high value of magnesium in potash.

High potassium in the blood is life–threatening problem (Allison, 2001). Potassium is a primary electrolyte and a major cation inside the cell and low potassium in the blood is life–threatening problem (Allen, 2003). Potassium functions in acid-base balance, regulation of osmotic pressure, muscle contraction, particularly the cardiac muscle. Its deficiency affects the collecting tubules of the kidney. The potassium content of the bitter leaf in this study is similar to that reported for *V. amygdalin* and *M. charanti* by Ayoola *et al*. (2001). It is however; lower than that reported for *Amaranthus cruentus L.* by Makobo *et al*. (2010). Lean (2006) reported the RNI of potassium as 350mg/day. The potassium content of bitter leaf in this study is close to the values reported for *Amaranthus hybridus* by Akubugwo *et al.* (2007) and has potential of meeting this requirement with increased consumption of the quantity of the leafy vegetable daily. Therefore, bitter leaf could serve as a good source of potassium. The result showed a significant reduction ($p<0.05$) of the mineral with normal squeeze – washing treatment of the bitter leaf. This is due to leaching loss of the mineral into the washing water. Potassium being an important mineral in the body fluids is quite soluble in water. Hence the result obtained was expected.

Squeeze- washing and boiling of *V. amygdalina* resulted in a higher significant reduction ($p<0.05$) in the mineral, potassium. The result obtained for potassium was similar to that obtained for calcium probably due to dissolution of the potassium into the boiling water.

Sodium is a principal cation in extracellular fluids. It regulates plasma volume. It is involved in Na+/k-+ Tpase maintenance membrane potentials (Hays and Swenson, 1985). The sodium content of the bitter leaf in this study 12.001mg/100g (Table 1) is lower than that reported for *V. amygdalina* and *M. charantia* by Ayoola *et al*. (2001) and for *Amaranthus cruentus* by Makobo *et al.* (2010). This value is however, higher than that reported for *A. hybridus* leaves by Akabugwo *et al.* (2007). The sodium content of the studied bitter leaf is too low to meet the RNI of sodium (1600mg/day) according to Lean (2006) and therefore must be sourced from other sources, especially salt.

Like other mineral elements, it is readily soluble in water and some sodium contents of bitter leaf leached into the washing water. The squeeze-washed and boiled *Vernonia amygdalina*

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showed the highest loss of sodium, which is due to dissolution losses into the boiling water enhanced by heating. The sample squeezed-washed with salt recorded the highest value and this is due to the salt concentration used in the process. The iron content of *V. amygdalina* obtained in this work is between 6.01 and 21.39mg/100g and compares favourably with those obtained by Ejoh *et al*. (2007) for *V. Amygdalina* and *V. calvonanavar bitter* as well as those obtained for *A. hybridus, L. occidentalis* and *Lycopersicon esculentum* (Mepba *et al.*, (2007). Iron is an integral constituent of haeme, of haemoglobin, myoglogbin and cytochromes (Chandra, 1990) it is also important in the transport of oxygen in blood. The iron content of bitter leaf in this study could be considered adequate when viewed against an RDA of 8mg fe/day of men of ages 19 and above, and women over 40years, 18mg Fe/day for girls and women between the ages of 11 and 50 (FNB,2001). Its deficiency causes anaemia (Awoyinka *et al.*, 1995). The iron content of the squeeze- washed, which was due to leaching losses of the mineral into washing water. This effect was observed by Ejoh *et al*. (2007). The squeeze-washed bitter leaf with palm oil had the lowest content of Fe.

Squeeze –washing and boiling retained the highest amount of Fe in the bitter leaf. This was probably due to a higher concentration of Fe in the boiling water than in the bitter leaf, which dissolved from the iron pot, as solute diffusion.

Zinc is among the mineral that has the lowest value in the samples of squeeze-washed bitter leaf analyzed with a 2.18mg/100g (Table 1). This value compares favourably withthe value reported for *Amaranthus hybidus* by Akubugwo *et al.* (2007) as well as with that reported for *V. amygdalina* by Ayoola *et al.* (2001). Lean (2006) states an RNI of 9mg of Zn/day. The zinc content of bitter leaf studied is good enough to meet the RNI of Zn and therefore is a good source of the mineral. Zinc is required for cell replication and gene expression as well as for tissue repair, wound healing and reticular development. It is also integral constituent of insulin (Murry *et al.*, 2000). Zinc deficiency in developing countries is becoming a growing concern because it has been shown that zinc deficiency is related not only to decrease growth, but also to increase morbidity and impaired immune function (Huffman, 1998).

The sample squeeze-washed with palm oil had the lowest retention of zinc while the highest zinc retention was observed in the normal squeeze –washed bitter leaf. Zinc like most other inorganic mineral elements exist in ionic forms for their involvement in biological functions and are therefore readily soluble in water.

The processing operation of squeeze –washing leaches out some of the zinc in the bitter leaf into the washing water.

A highly significant ($p<0.05$)reduction of zinc was observed for squeeze –washed with palm oil sample, probably due to dissolution of zinc into the boiling water.

The copper contents of the vegetable (0.85-0.19mg/100g) were higher than the result (0.06mg-0.21mg/100g) reported by Okafor (1979) and that of *Occimum gratissimum* (Adepoju and Oyewole., 2008) 1.8mg/day for women, respectively. Therefore, the vegetables are needed to enable the absorption and mobilization of iron and its utilization in haemoglobin synthesis.

Manganese is a metallo-enzyme involved in pyruvate metabolism and also required for glucose utilization. The values obtained (0.99-2.11mg/100g)were slightly lower than that of *Occimum gratissimum* reported by Adepoju and Oyewole (2008). Thus, adequate

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consumption would meet the needs of the body since adequate intake of manganese is 2.3mg/day.

From Table 2, the values of Oxalate in these vegetables (3.74%-1.26%) were higher than *pterocarpiessoyauxii and Genuetumafricanum* (Chinma and Iygor, 2007) and slightly above the toxic level of 2.5g reported by Munro and Basir (1969). Oxalates are known to form insoluble complexes with Ca, Mg and Fe thereby interfering with utilization of these mineral elements (Munro and Basir, 1969). However, since the vegetable are consumed after the squeeze-washing process, there appears to be no danger of toxicity arising from oxalate.

Although, literatures available on phytate content of green leafy vegetables are limited. High values of phytate (20.8 and 19.0mg/100g) have been reported for bitter leaf and fluted pumpkin leaf, respectively (Oguntona, 1998). Ingestion of 2.5g or more of phytate per day cause reduction in bioavailability of Ca, Fe and Zn. The mineral profile of the five samples showed that the values of the mineral varied depending on the substance used in the squeezing-washing process. More so, the low values of anti-nutritional contents in the different samples make them safe for consumption in high quantity and give these vegetables a place in the food list for recommendation, appropriate for good health.

CONCLUSION

Retention and losses of minerals and anti-nutrients in the bitter leaf samples varied from 23 to 61%, and 27.3 to 80.5% respectively. The sample squeeze-washed with palm oil exhibited a high (55-100%) mineral uptake (Calcium, copper, magnesium, e.t.c) than the remaining samples (38 to 65%), although the range of losses remained the same (27.3 to 80.5%) in all sample. By implication, losses of minerals and anti-nutrients depended on changes induced by the intensity of the squeeze-washing process to disintegrate the vegetable structurally rather than composition of each vegetable samples. Furthermore, nutrient gain of some of the samples is dependent on the compositions of the material of squeeze-washing (palm oil) and retention of its cellular structure. It is recommended that palm oil be used in the squeeze-washing of bitter leaf for better nutrient retention.

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