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Effect of Traditional Methods of De-bittering on the Proximate and Vitamin Contents of Fresh and Squeezed-Washed Bitter Leaf

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Abstract: Bitter leaf is a leafy vegetable that is widely consumed and cherished in South-Eastern Nigeria. The effect of traditional methods of de-bittering of bitter leaf (*Vernonia amygdalina*) on the proximate and vitamin contents was studied using potash, palm oil, and salt and boiling process in squeeze-washing at 3 pre-processing methods of squeeze-wash and periods of 3 to 8 minutes. The percentage retention and losses of nutrients increased simultaneously during squeeze-washing. The sample squeezed-washed with palm oil had nutrient retention ranging between 55 to 100% of moisture, ash, crude fibre, fat, vitamin A and vitamin C than other squeeze-washed samples. This could be due to the rigidity of the cells of the sample squeezed-washed with palm oil which did not allow much nutrient to leach into the squeezed leaf water; whereas, the loss of nutrient was practically of the same magnitude (27.3 to 80.5%) in all other samples. The loss of 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 nutrient initially present. Palm oil should be used in the squeeze-washing of bitter leaf for better nutrient retention.

Keywords: Bitter Leaf, Palm Oil, Potash, Salt, Squeeze-Washing

1. Introduction

Leafy vegetables constitute an indispensible constituent of human diets in Africa generally and in Nigeria in particular (Okudu, 2008). They could be eaten either raw or cooked and contain both essential and toxic elements with wide range of concentrations (Kola, 1999). Leafy vegetables have high water content and an abundance of cellulose though not digestible, serves a useful purpose in the intestine as roughages, thus, promoting normal elimination of wastage (Adeboye and Babajide, 2007).

In Nigeria as in other tropical countries of Africa, where the daily diet is dominated by starchy staple foods, leafy vegetables are the cheapest and most readily available sources of important vitamins and minerals with some of them containing protein (Okafor, 1983). Leafy vegetables as a group are low in fat, energy and appreciably high in ascoibic acid (Ejoh*et et al.*, 2007).

Bitter leaf (*Vernonia amygdalina*) is a perennial plant belonging to the compositae family (Igile *et al.,* 1995,). Their leaves are dark green coloured with a characteristic odour and a bitter taste (Aina and Uko, 1990). The plant is grown in the garden around the homestead in Nigeria for a quick supply (Ajebesone and Aina, 2004). Unlike any other leafy vegetable, bitter leaf is cherished in some Igbo Speaking area of South-Eastern Nigeria because of the distinctive flavour it imparts to any food of which it is a component (Morah and Obiegbuna, 2002). According to Latham (1997), the consumption of bitter leaf and how it is prepared in most localities is greatly influenced by social factors and cultural practices of the people. The leaves are eaten after crushing and squeeze washing them thoroughly to remove the bitterness (Mayhew and Penny, 1998). Processing most times if not always, modifies the nutrient composition especially vegetables which easily lose their water soluble or heat labile nutrient. The process of squeeze- washing with palm oil,

potash and salt has not been studied and comparing the normal squeezed – washed leaves with the ones squeeze-washed with palm oil, potash and salt will show clearly which method optimally preserve the overall nutrient composition.

This work therefore investigates the proximate and vitamin contents of fresh and squeeze-washed bitter leaf as affected by traditional methods of de-bittering.

2. Materials and Methods

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

3. 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 processing 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 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. 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.

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 3 rounds of squeeze –wash. The fifth 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 it in a mortar with pestles.

4. Proximate Analysis

The proximate compositions of the vegetables were determined on the edible portions of bitter leaf, according to the association of official analytical chemist (A.O.A.C., 2005). All analysis was carried out in triplicates.

5. Determination of Vitamin Contents

Vitamin A: In vitamin A determination, the method described by Okwu (2004) was employed. A measured mass (5kg) of each prepared sample was dispensed in 30ml absolute alcohol. Three millilitres (3ml) of 50% KOH solution was added to it and boiled under reflux for 30 minutes. After rapidly cooling in running water, 30ml of distilled was added to it and the mixture was transferred to separation funnel. three portions of 50ml of either was used to wash the mixture, thus extracting the vitamin A the lower aqueous layer was discarded, while the vitamin A extract was washed with 50ml distilled water. Care was taken to avoid formation of emulsion. The extract was then evaporated to dryness and dissolved in 10ml of isopropyl alcohol and its absorbance of vitamin A was measured at 325mm wavelength. Meanwhile, the standard vitamin A was dissolve in isopropyl alcohol and its absorbance was measured at 325mm. the vitamin A content was calculated using the relationship below:

$$\text{Vitamin A (iu/100g)} = \frac{100}{W} \times \frac{AU}{AS} \times C$$

AU = absorbance of sample, W= weigh of sample

AS= Absorbance of standard, C =concentration of vitamin

Vitamin B_1 (thiamine): Thiamine content of the samples was determined using the dischromate colourimetric method describe by Okwu and Ndu (2006). A weighed portion of each part of a sample (5g) was extracted by homogenate was filtered to obtain the extract. An aliquot of the extract (10ml) was treated with 1ml of moral potassium dichromate solution. Standard thiamine solution was prepared and treated the same way as the sample. A reagent blank consisting of the extracting solution alone was also treated the same way. The reagent blank was used to set to the instrument at zero. The absorbance of the standard solution and test extracts were read in a jenway model spectrophotometer at a wavelength of 360mm.

The thiamine content was calculated as show below:

$$\text{Thiamine (mg/100g)} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{vf}{va}$$

Where W = weigh of sample analyzed

Au= absorbance of sample

Us= absorbance of the standards

C= concentration of the standard

Vf= total volume for extract

Va= volume of extract analyzed

Vitamin B_2 (riboflavin): A measured weight (50g) of the sample was treated with 100ml of 50% ethanol solution and shaken for one hour for extraction. This was filterer into a 100ml flask. A measured volume (10ml) of the extract was pipetted into a 50ml volumetric flask. To this, 10mls of 5%potassium permanganate and 10mls of 30% H_2O_2were added and allowed to stand over a hot water bath for about 30mins. Afterword 2mls for 40% sodium sulphate solution

was added and a yellowish pale colour was formed. This was made up to 50ml mark and the absorbance measured at 510nm wavelength in a jenway spectrophotometer.

Vitamin B_3 (Niacin): The method of the association of chemists described by Okwu (2004) was employed. Five grams (5g) of the sample was treated with 50ml of 1N sulphuric acid and shaken for 30mins. Three drops of ammonia solution was added to the sample and filtered. A measured volume (10ml) of the filtrate was pipetted into a 50ml volumetric flask and 5mls of potassium cyanide was added. This was acidified with 5mls of 0.02N sulphuric acid and its absorbance was measured in the Jenway model spectrophotometer at 47nm wavelength.

Ascorbic Acid (Vitamin C): Vitamin C was determined using the method for vitamin assay (inter-science publishers, 2006). This method is based on the reduction of the dye (2, 6 dichlorophenolindophenenol) by an acid solution of ascorbic acid. The capacity of an extract of the sample to reduce a standard solution of the dye, as determined by titration is directly proportional to the ascorbic acid content. Two hundred grams (200g) of each sample was blended with 6% HPO_3 to yield homogenous slurry. 10g of each sample slurry was weighed into a 100ml volumetric flask and diluted to 100ml with 3% meta phosphoric acid solution (0.0033M EDTA). The diluted samples were filtered, pipetted into a small flask and titrated immediately with a standardized solution of 2.6 dichlorophenol-in dephenol to a faint pink end point which persisted for about 15sec. The ascorbic acid content of the fruit was calculated from the relationship below.

$$\frac{V}{W} \times T \times 100 = \text{mg ascorbic acid per 100g sample.}$$

Where, V= ml dye used for titration of aliquot of diluted sample.

T= ascorbic acid equivalent of dye solution expressed as mg per ml of dye

W= gram of sample in aliquot titrated.

6. Statistical Analysis

The proximate and vitamin 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 Wahua (1999) method and was reported as means of three replicate.

7. Result and Discussions

The data obtained as shown in **table** 1, reveals that, the moisture content of the bitter leaf samples which ranged from 80.3% to 92.1% was within range of moisture content in fresh green leafy vegetables, that ranged from 72% in cassava leaves to 92.93% in indian spinach and water leaf. It has also been reported that the amount of moisture content in individual samples of green leafy vegetables depends on several factors which includes age, freshness and agronomical practices prevailing during cultivation (Oguntona, 1998; Uwaegbute, 1989).

Ejoh *et al.* (2007a) stated that the amount of moisture content in a food affects the packaging, keeping quality, nutrient provided and the types and rate of microbial spoilage that occur. Hence, vegetables such as, this should be consumed fresh after harvest or preserved by freezing or by drying in order to increase the shelf–life, prevent microbial growth and enzymatic activities in the vegetables (Enwere, 1998).

The ash contents of bitter leaf obtained in this study (2.27-3.24%) is similar to the value reported by Saidu and Jideobi (2001), Ajayi *et al* (2006) in pump kin (2.01%) and waterleaf (2.50%). The results also compare to 3.72% for "Ukazi" as reported by Okafor (1979) but lower than that of conventional and popular *Amaranthus sp and moringa oleifeira vegetables* (Adepoju *et al.,* 2006). The ash content reflected the amount of minerals in specific bitter leaf samples. Bitter leaf squeeze – washed with potash had the highest ash content (3.24%) which was probably responsible for its high mineral content.

Leafy vegetables are known to be poor sources of lipids, thus the low fat content (0.99-2.34%) of the bitter leaf samples was in agreement with reports that vegetables as a group are low in fat content (Ejoh*et al.*, 1996; Ifon, 1980). However, the values obtained were comparable with that of Baobab (1.68%) and Okra leaves (0.97%) (Ihekoronye and Ngoddy, 1985). According to Okafor (1995), Lewu *et al.* (2009) and Gupta *et al*. (2005), the fat content of *Colosia argenta, Gnetum africanum, Colocasia esculenta L*. were 0.7, 1.2 and 1.85%, respectively, which are in line with the results obtained in this study. Fats and oil are recognized as essential nutrients in human and animal diets. They provided concentrated energy, supply essential fatty acids and are carriers of the fat soluble vitamins A, D, E and k. More so, they serve to make the food more palatable, giving a sense of fullness following a meal. However, the American Dietetic Association suggested that diets should contain less than 30% fat (Smith, 1983). Therefore, increasing the consumption of these vegetable would naturally lower the percentage of total fat intake. More importantly, no cholesterol is found in fruits and vegetables (Lymido *et al.,* 1991).

The fibre content (9.3-20.12%) of the bitter leaf samples were within the range when compared with *Gnetum africanum* (12.50%), *pterocarpies soyauxii* (14.2%), *amaranthus hybridus* (8.61%) and *colocasia esculenta L.* (26.4%)vegetables (Chimaand Iygor 2007; Akubugwo *et al.,* 2007; Lewu *et al*., 2009). It is significant in human nutrition as the therapeutic effects of fibre in the prevention of heart disease, colon cancer and diabetes and its role in the treatment of digestive disorders (diverticulosis and constipation) are generally recognized as reported by Lund (1982) and Kelsay (1985).

Overall, fresh green leafy vegetables are low in protein content (Ifon and Bashir, 1987). However, the protein content of the bitter leaf samples was high (3.216-6.04%) when compared to mean values of crude protein of 4.2% for

seventeen of such vegetables as reported by Aletor and Adeogum (1995). This can be attributed to the maturity stage of the vegetables. Thus, adequate consumption of these vegetables and proteins from other sources will help in supplying amino acids for growth, replacement of damaged tissues, and formation of body enzymes, hormones and antibodies.

The carbohydrate values obtained (6.02%-10.05%) were high and could compare favourably with that of *Gentum africanum and Pterocarpies soyauxii* vegetables (Chima and Iygor, 2007). The result also agrees with those reported for *Colocasia esculenta*, which was between 12-15% according to Lewu *et al.* (2009). Carbohydrates are needed majorly for providing energy. Though green leafy vegetables are not good dietary sources of energy, yet the body uses the calories contained in all foods (including vegetables) to produce energy and warmth to the system. Excess calories are stored in the adipose tissue for use at the time when sufficient calories would not be available.

The low gross energy value of the bitter leaf samples (1.02%-2.11%) could have resulted from their low crude protein, lipid and carbohydrate contents. This is a reflection on the low dry matter content of many of these leaves (Oguntona, 1998). These contribute to the energy needs of the body for growth, development and metabolic processes.

Vitamin A is present in plant food as B- carotene and in animal source as retinol (Lean, 2006) and is found as a constituent of the red cells in the retina, where it helps proper vision in dim light.

Table 2 shows that the vitamin A content of bitter leaf obtained in this study is similar to the value reported by Atangwho *et al.* (2009). International unit is used to express the potency of drugs or particular nutrients. One international unit (IU) is equivalent to 0.6 µg (Micro gram) of B –carotene. One microgram is equivalent to 103 milligram. Some literature (Lean, 2006; Nkafamiya *et al.*, 2010) reported the vitamin A contents of some vegetables in micrograms. The equivalent value of 356.841U in micrograms is 214.ug. The vitamin A content of the bitter leaf used in this study is 214.104ug. This value was quite high when compared to the value of some vegetables reported by Lean (2006). It is higher than the vitamin A values reported for*B. cosatum and M. Oleifera* by Nkafamiya *et al.* (2010). Lean (2006) reported a reference nutrient intake of 700ug of carotene \day for adult male and 600ug of carotene /day for adult females; bitter leaf with a vitamin A content of 214.104ug/100g could serve as a good source of this nutrient and consequently aid in prevention of impaired vision associated with vitamin A deficiency.

B –carotene (Vitamin A precursor) is found to be present in the small amount of fat present in green leafy vegetables and is the squeeze- washing leaches very little or no vitamin A into the washing water. This is in conformity with the report of Hildreth (1971) that vitamin A being a fat soluble vitamin is less readily leached out into the washing water.

The vitamin A content of the sample subjected to squeeze –washing and boiling was significantly lower ($p<0.0.5$) than that of the sample subjected to only squeeze-washing. Lean (2006) had earlier reported that vitamin a is susceptible to heat. This report also agrees with the work of Uwaegbute *et al.* (2010) that heating of vegetables significantly reduced their vitamin A content. It is also established that bleaching of vegetable oils by heating decolourize the oil, greatly destroys the B – carotene content of the vegetable oil.

There is no significant difference between samples squeeze- washed with palm oil and the normal squeeze-washed one. This is due to the fact that palm oil contains vit A. (Lean 2006).

Vitamins C are highly soluble in water, susceptible to heat, sensitive to light and oxidation (Hildeth, 1971; Julie, 2003; Ruttledge, 1991; Lean, 2006).

The value of the ascorbic acid content of the *Vernonia amygdalina* used in this work compared very well with that obtained by Atangwho *et al.* (2009) and Ejoh *et al.* (2005) for *V. amygdalina* and other *Vernonia* species. This value is high enough to meet the reference intake (RNI) of 40mg of vitamin C /day for males and females (Lean, 2006) vitamin C is referred to as anti –scorbutic vitamin (anti-scurvy acid). It assists in the proper formation of collagen, a fibrous structural protein found in joints. It is needed for healthy gums, teeth and a clear skins, vitality and endurance (Hildreth, 1971; Lean, 2006). Its deficiency in diets leads to the disease, scurvy (Said and Mohammed, 2006). The values obtained for squeeze –washed bitter leaf samples used in this work were significantly lower ($p>0.05$) than the fresh sample because ascorbic acid is very soluble in water. The operation of squeeze – washing to remove bitterness, damages the tissues of the leafy vegetable, rupturing the cells and exposing more areas of the sample to washing water.

Vitamin C like other nutrients is contained inside the cytoplasm of the cells. Thus, for any dissolution of ascorbic (vitamin C) from the cell into the washing water, there must be movement through the cell wall down a concentration gradient. For leafy vegetables and other food materials containing vitamin C, ordinary soaking or washing in water without rupturing of the cells by shredding, dicing or crushing results to lower vitamin losses than when the cells are ruptured. Ejoh *et al.* (2005) reported significant reductions in the vitamin C contents of *V. amygdalina* and *V. calvoana var bitter* when squeeze- washed.

The bitter leaf sample subjected to squeeze – washing and boiling was significantly lower ($p> 0.05$) in ascorbic acid contents than that of the sample subjected to only squeeze-washing. The combined effect of squeeze- washing and boiling leached out more ascorbic acid. This is because solubility increases with increase in solubility of solvent (water). The lower value of vitamin C in this sample was due to the leaching of vitamin into the washing water and the subsequent dissolution of the vitamin in the boiling water. The heating also contributed to higher level of losses in vitamin C. Hildreth (1971) had earlier reported that the mechanism of degradation of ascorbic acid in three leafy vegetables studied (*C.olitorius, A. hybrius, T. occidentalis*) appears to be dependent on thermally induced energy

intensity on physico-chemical changes and the ability to sustain cell stability and turgor pressure rather than the composition of the vegetable. They also reported that losses as high as 48 to 74% of ascorbic acid would occur when vegetables are blanched which is also in line with the result obtained.

Squeeze- washing increases the surface area of the leafy vegetable to the water thereby increasing leaching losses. If vegetables are put in cold water which is heated to boil, the dissolved oxygen in the water will in the presence of oxidases (oxidizing enzymes), destroy a substantial amount of the ascorbic present. The oxidases are most active at about 60-80^{0}C and above these temperatures are quickly inactivated (Lean, 2006). The same reason is in the case of squeeze –washing and boiling with salt and potash. Hildreth (1971) opined that high alkalinity destroys Vit C. therefore; high alkaline nature of the squeeze water affected the Vit. C content of the sample squeeze- washed with salt and potash.

Thiamine is a water soluble vitamin and controls the liberation of energy from carbohydrate foods by way of complete oxidation of glucose in the system (Julie, 2003). Absence of vitamin B1 in diet leads to a loss of appetite, fatigue, indigestion and constipation, with prolonged deficiency leading to beriberi (Hildreth, 1971; Lean, 2006). The thiamine content of bitter leaf in this study compares very well with value reported by Lean (2006) for some vegetable. The value of the thiamine content (0.19+0.005mg/100g) is lower than values reported by Akubugwo *et al.* (2007) and Nkafaiya *et al.* (2010) for *A. hybridus and M. oleifera*, respectively. Lean (2006) stated that the Reference Nutritional Intake (RNI) of thiamine is dependent on the amount of carbohydrate intake. He reported a reference (RNI) of 0.4mg of thiamine/1000kcal. Considering the amount of carbohydrate consumed in Nigeria, the thiamine content of the bitter leaf in this study is not enough to meet the RNI.

Thiamine content of the bitter leaf in this study was affected by different de-bittering methods. The bitter leaf sample subjected to squeeze-washing and boiling had the lowest thiamine content. Thiamine is very soluble in water and as much as 50 percent may be lost when vegetables are boiled (Lean, 2006). The significant loss of thiamine for squeeze –washed bitter leaf as reported in this study also agreed with that reported by Lean (2006). Squeeze –washing operation increasing the surface area, thereby making more of vitamin to come in contact with the boiling water.

Thiamine is destroyed by high temperature of boiling water (Hildreth, 1971). The susceptibility of thiamine to heat was also reported by Lean (2006) as he opined that thiamine decomposes on heating. Nkafamiya *et al.* (2010) had earlier reported that thiamine (vitamin B1) contents of leafy vegetables are reduced drastically after blanching operation. Latunde- Dada (1990) had also reported that the processing operation of squeeze- washing ruptures the cells of leafy vegetables and paves way for leaching of thiamine into the washing and rinsing water.

The riboflavin content of the unprocessed raw bitter leaf, 0.22+0.0mg/100g is lower than values reported by Akubugwo *et al.* (2007) and Nkafamiya *et al.* (2010) for *A. hybridus, M. Oleifera* and *B. cosatum*. This value is however, higher than those reported by Lean (2006) for cabbage and potatoes. Lean (2006) stated that the reference nutrient intake (RNI) of riboflavin is dependent on basal metabolic rate. He gave the RNI of riboflavin as 1.3mg/day for adult males, 1.1mg/day for adult females.

The riboflavin content of the squeeze-washed bitter leaf is lower than the RNI, although, it could perform well as a fair source of the vitamin if the quantity of the bitter leaf consumed per day is increased. The importance of riboflavin is in the oxidation of carbohydrate for energy release as well as in the avoidance of inflammation of the mouth, tongue and cornea of the eye (Julie, 2003). Riboflavin is only slightly soluble in water with minimal losses occurring during boiling of foods containing the vitamin (Lean, 2006). The squeeze- washed bitter leaf sample had a riboflavin content that was significantly lower than that in the fresh sample ($p< 0.05$). Like other vitamins which are soluble, the process of squeeze-washing ruptures the cells of the leafy vegetable, leading to increased leaching losses of vitamin. This finding is in agreement with the report of Oguntona (1998) that squeeze-washing leaches some riboflavin into the washing water.

There were no significant differences ($p>0.05$) in the riboflavin contents of bitter leaf subjected to squeeze-washing process and that of squeeze-washed and boiled ($p>0.05$) due to the heat stability of vitamin. Studies by Eunok *et al.* (2005) showed that heating barely affects the riboflavin in foods. High amounts of riboflavin in roasted pork were retained after a heating process that significantly destroyed most other vitamins (Lassen *et al.,* 2002). Lean (2006) opined that little or no loss of riboflavin occurs during canning of food.

Niacin, a moderately water soluble vitamin is important in diets as anti-pellagra vitamin and in releasing energy from carbohydrate foods (Lean, 2006). It is fairly present in vegetable foods. The amino acid tryptophan in milk is usually converted by the body into the vitamin. The niacin content of bitter leaf reported in this study is 0.88+0.289mg/100g and compares well with the values reported by Nkafamiya *at al.* (2010). The niacin content of the studied bitter leaf is lower than the niacin content of potato (1.5mg/100g), which is regarded as a good source of the vitamin (Lean, 2006; Hildreth, 1971). Lean (2006) also stated that the RNI of niacin for good health is related to the energy content of the diet and the amount of tryptophan also present. He gave the RNI of niacin for all ages and both sexes (except lactating women) as 6.6mg niacin/1000kcal.

Squeeze –washing significantly reduced ($p<0.05$) the niacin content of bitter leaf. This is in agreement with the report of Lean (2006) that some amounts of the vitamin are lost to wash water. Niacin is a water soluble vitamin like other B-vitamin and is easily absorbed in water. The result of this study showed that the processing method had no significant difference ($p>0.05$) on the niacin content of leafy vegetable. Niacin is the most stable vitamin to the processing

methods given to *V. amygdalina* in this study. This agrees with report of Lean (2006) that niacin is the most stable vitamin among the B- vitamins.

Table 1. *Proximate composition of different samples of Vernonia Amydalina on wet basis (g/100g).*

Parameter	Unpro-cessed	Normal squeeze washed	Squeezed washed and boiled	Squeezed washed with salt	Squeezed washed with potash	Squeezed washed with palm oil
Dry matter	9.2 ± 0.16^{a}	8.7 ± 0.20^{b}	7.8 ± 0.11^{c}	7.9 ± 0.10^{c}	7.3 ± 0.18^{d}	9.0 ± 0.01^{a}
Moisture	90.0 ± 0.16^{a}	$87. \pm 0.12^{b}$	86.4 ± 0.02^{b}	81.9 ± 0.10^{c}	80.3 ± 0.18^{c}	92.1 ± 0.02^{a}
Ash	2.39 ± 0.19^{c}	3.03 ± 0.02^{ab}	2.90 ± 0.18^{b}	2.27 ± 0.17^{c}	3.24 ± 0.04^{a}	3.04 ± 0.16^{b}
Crude fibre	1.55 ± 0.30^{d}	1.60 ± 0.30^{c}	1.44 ± 0.20^{c}	1.39 ± 0.02^{f}	2.67 ± 0.02^{a}	2.49 ± 0.27^{b}
Crude protein	1.01 ± 0.01^{a}	0.95 ± 0.01^{b}	0.55 ± 0.29^{c}	0.67 ± 0.30^{c}	0.43 ± 0.02^{d}	0.69 ± 0.20^{c}
Carbohydrate	1.87 ± 0.12^{b}	1.92 ± 0.11^{b}	1.88 ± 0.33^{b}	2.30 ± 0.18^{a}	1.33 ± 0.18^{c}	1.93 ± 0.13^{b}
Fat	2.34 ± 0.18^{a}	1.57 ± 0.30^{c}	1.47 ± 0.19^{d}	0.99 ± 0.20^{e}	1.15 ± 0.16^{e}	1.79 ± 0.03^{b}
Energy (kcal/g)	2.01 ± 0.02^{a}	1.02 ± 0.08^{c}	1.86 ± 0.30^{b}	2.06 ± 0.03^{a}	2.11 ± 0.99^{a}	1.82 ± 0.02^{b}

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

Table 2. *Vitamin contents of different samples of Vernonia amydalina on wet basis.*

Samples	Vitamin A (iu/100g)	Vitamin C (mg/100g)	Vitamin B1 (mg/100g)	Vitamin B2 (mg/100g)	Vitamin B3 (mg/100g)
Unprocessed	357.18 ± 0.05^{a}	163.20 ± 0.01^{a}	0.19 ± 0.008^{a}	0.22 ± 0.02^{a}	0.88 ± 0.02^{a}
Normal squeeze washed	355.92 ± 0.10^{a}	88.41 ± 0.69^{b}	0.099 ± 0.02^{b}	0.16 ± 0.01^{b}	0.53 ± 0.00^{b}
Squeeze washed and boiled	246.91 ± 0.02^{b}	49.28 ± 0.03^{c}	0.077 ± 0.01^{d}	0.15 ± 0.02^{b}	0.51 ± 0.01^{b}
Squeeze washed with salt	196.80 ± 0.03^{d}	42.10 ± 0.02^{d}	0.080 ± 0.01^{d}	0.148 ± 0.08^{b}	0.50 ± 0.01^{b}
Squeeze washed with potash	194.99 ± 0.01^{d}	38.29 ± 0.18^{e}	0.086 ± 0.01^{c}	0.140 ± 0.06^{c}	0.54 ± 0.02^{b}
Squeeze washed with palmoil	232.44 ± 0.02^{c}	48.10 ± 0.02^{c}	0.078 ± 0.02^{b}	0.151 ± 0.02^{b}	0.56 ± 0.03^{b}

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

8. Conclusion

Nutrient retention and losses 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%) nutrient uptake (moisture, protein, fibre, vitamin A e.t.c) than the remaining samples (38 to 65%), although the range of nutrient losses remained the same (27.3 to 80.5%) in all sample. By implication, losses of 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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