## Full Length Research Paper

# Effect of post-harvest processing on the nutrient and anti-nutrient compositions of *Vernonia amygdalina* leaf

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Vernonia amygdalina leaves are used as soup condiment in Nigeria either in the processed or unprocessed forms. This study assessed the effect of some conventional post-harvest treatments on the proximate, vitamins, minerals and anti-nutrient compositions of the leafy vegetable. Fresh leaves of V. amygdalina were subjected to two different processing methods; abrasion with salt (AWS) and abrasion without salt (AWOS). The proximate, β-carotene, vitamins B<sub>1</sub>, B<sub>3</sub>, C and E, minerals sodium (Na), potassium (K), calcium (Ca), phosphorus (P), iron (Fe), manganese (Mn), magnesium (Mg), zinc (Zn), cupper (Cu), aluminium (Al), selenium (Se), cobalt (Co), arsenic (As), cadmium (Cd) and lead (Pb) and anti-nutrients (phytate, tannin, oxalate and cyanide) contents of the vegetable were subsequently evaluated by standard methods. The results showed that unprocessed V. amygdalina had 34.56% protein, 6.04% fat, 9.08% fibre, 5.60% ash and 44.73% carbohydrate on dry weight (DW) basis. However, processing techniques employed caused a significant decrease (p<0.05) in the protein, fat and ash contents of V. amygdalina leaf; while there was no significant difference in the fibre and carbohydrate contents. Processing with or without salt significantly decreased (p<0.05) the levels of vitamins B<sub>1</sub>, B<sub>3</sub>, C, E and  $\beta$ -carotene compared with the unprocessed leaf. Elemental analysis revealed varied activities for all the elements except As and Cd that were non-detectable. The processing also depleted the levels of all the minerals detected in the fresh leaf. Phytate, tannins, oxalate and cyanide were detected in the fresh leaf and were notable to decrease by processing steps. In conclusion, V. amygdalina has very high nutrient and anti-nutrient potentials; however, the various conventional post-harvest processing tend to cause a significant decrease (p<0.05) in these parameters. Abrasion with minimal salt is most advisable.

Key words: Vernonia amygdalina, proximate, vitamins, minerals, anti-nutrients, processing.

## INTRODUCTION

Vegetables play significant role in human nutrition, especially as a source of vitamins (C, A, B and E), minerals and dietary fiber (Aletor and Adeogun, 1995). Vegetables contain compounds that are valuable antioxidant and protectants. The main protective action of vegetables has been attributed to the presence of antioxidants, especially antioxidant vitamins including the

ascorbic acid,  $\alpha$ -tocopherol and  $\beta$ -carotene (Amic et al., 2003).

In Nigeria, unlike the western worlds where green leafy vegetables are usually consumed in their unprocessed forms, green leafy vegetables are usually subjected to various post-harvest treatments such as blanching, soaking, abrasion with salt (AWS) or abrasion without salt (AWOS) (Oboh et al., 2005), in order to improve their palatability and to remove the bitter taste and some of the acids present in the vegetables. The various processing techniques have been reported to alter both the nutrient, anti-nutrient and antioxidant properties of some commonly consumed plant foods in Nigeria (Oboh and Akindahunsi, 2004; Oboh et al., 2005).

**Abbreviations**: **ANOVA**, Analysis of variance; **LSD**, least significant difference; **DW**, dry weight; **HCN**, hydrogen cyanide; **AWS**, abrasion with salt; **AWOS**, abrasion without salt.

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Vernonia amygdalina Delile, popularly known as bitter leaf is a shrub 2 to 5 m tall with petiolate green leaves of about 6 mm diameter. The leaves are characteristically bitter but the bitterness can be abated by boiling or by soaking in several changes of clean water (Burkill, 1985). It is known in Nigerian local languages as ewuro (Yoruba), etidot (Efik), onugbu (Igbo) and chusar duki (Hausa). Elsewhere in Africa, it is called muop or ndole (Cameroon), tuntwano (Tanzania), and mululuza (Uganda) (Owolabi et al., 2008).

The stem and root divested of the bark of *V. amygdalina* are used as chew-sticks in Nigeria. More importantly, the leaves are a very popular soup vegetable and have even been reported to be consumed by goats in some parts of Nigeria (Aregheore et al., 1998). Nutritionally, *V. amygdalina* is used mainly in soup making in the tropics and also as an appetizer and febrifuge (Ijeh et al., 1996; Iwu et al., 1996). In Nigeria, as in other tropical countries of Africa where the daily diet is dominated by starchy staple foods, vegetables are the cheapest and most readily available sources of important proteins, vitamins, minerals and essential amino acids (Okafor, 1983). The importance of *V. amygdalina* in animal nutrition in Nigeria has also been well documented (Onwuka et al., 1989; Aregheore et al., 1998).

Studies on the nutritional composition of Vernonia are numerous and often limited to one species: amygdalina. Faboya (1990) demonstrated that ascorbic acid level in V. amygdalina decreases with storage time. Oshodi (1992) found that the dried leaves of V. amygdalina were rich in minerals, especially in phosphorus (P), and that the content of ascorbic acid was temperature dependent. Akindahunsi and Oboh (1999) reported a reduced zinc (Zn) bioavailability from Celosia ergentea, Talinum triangulare L., Corchorus olitorious, V. amygdalina, Basella alba and Amaranthus hybridus by such post-harvest treatments as sun-drying and ovendrying compared to the blanching. Ejoh et al. (2005) noted a general decrease in the vitamins A and C content of V. amygdalina, V. calvoana var. and V. colorata, following squeeze-washing, boiling and squeeze-washing with natron. Ejoh et al. (2007) also reported the nutrient components of V. amygdalina, V. calvoana var. and V. colorata. However, there is a dearth of information on the effect of the conventional post-harvest processing techniques (AWS and AWOS) on the elaborate nutrient and anti-nutrient contents of *V. amygdalina* leaf. Thus, this study was aimed at evaluating the proximate, mineral, vitamin and anti-nutrient potentials of the leaf of V. amgdalina. More so, we assessed the effects of these commonly practised post-harvest processing techniques on these parameters.

#### **MATERIALS AND METHODS**

The leaves of *V. amygdalina* were collected from the staff quarters of Babcock University, Ilishan-Remo, Ogun State, south-western

Nigeria. They were authenticated by an Ethno-botanist, Professor E.B. Esan of the department of chemical and environmental sciences, Babcock University.

#### Sample processing

The leaves of *V. amygdalina* were then subjected to some conventional food processing techniques. A portion (500 g in each case) of the sample was squeezed mechanically (abrasion) with or without sodium chloride (NaCl) (20 g) until the foaming of the vegetable stopped and the bitterness abated. De-ionized water was used for the processing. The remaining served as the control (fresh). Both the processed and unprocessed leaves were subsequently analyzed.

## Chemical analysis

*V. amygdalina* samples were analyzed in triplicates for moisture, protein, lipid, fiber and ash using standard methods of analysis. The chemical composition was evaluated according to the following (AOAC, 2005) methodology: moisture (930.04), protein by Kjeldahl (954.01), fat by Soxhlet (2003.06), fiber (978.10) and ashes (930.05). Carbohydrate was estimated by difference.

β-carotene (970.64), vitamins  $B_1$  (942.23),  $B_3$  (961.14), C (967.21) and E (971.30) concentrations of the V. amygdalina samples were determined according to AOAC (2005). ) and Na were determined using Jenway flame photometer (956.01). P was determined by vanado-molybdate spectrophotometric method. Mg, Fe, Zn, Cu, Mn, Al, Co, As, Cd, Pb and Se were determined spectrophotometrically using Buck 210 atomic absorption spectrometer (975.03). All mineral determinations were done after dry ashing of the samples and according to AOAC official method (2005).

Phytate (986.11) and oxalate (974.24) were determined according to the standard methods of AOAC (2005); tannin was evaluated by the method outlined by Hagerman and Ler (1983), whereas the hydrogen cyanide was determined by the method of Bradbury et al. (1999).

## Statistical analysis

Data were analyzed by one way analysis of variance (ANOVA) with statistical package for the social sciences (SPSS) version 15.0 and differences were considered to be statistically significant at P<0.05. Least significant difference (LSD) test was further carried out to establish the pairs that showed significant differences.

## **RESULTS AND DISCUSSION**

The proximate composition of *V. amygdalina* as influenced by the various processing techniques is presented in Table 1. The unprocessed sample had 34.56, 6.04, 9.09, 5.60 and 44.73% dry weight (DW) for protein, fat, fiber, ash and carbohydrates, respectively. The protein value reported here is higher compared with 19.23, 14.6 and 22.99% DW reported by Ejoh et al. (2007), Mensah et al. (2008) and Asaolu et al. (2010), respectively. The high protein content may be as a result of more and active nitrogen fixing bacteria in the soil, where the *V. amygdalina* tree was grown; giving rise to high nitrogen content, which could result in high amount of protein in

**Table 1.** Effect of processing on the proximate content (%DW) of *Vernonia amygdalina* leaves.

| Sample | Protein                 | Fat                    | Fiber                  | Ash                    | Carbohydrate            | Moisture <sup>*</sup>   |
|--------|-------------------------|------------------------|------------------------|------------------------|-------------------------|-------------------------|
| Fresh  | 34.54±0.61 <sup>a</sup> | 6.04±0.25 <sup>a</sup> | 9.09±0.21 <sup>a</sup> | 5.60±0.03 <sup>a</sup> | 44.73±3.98 <sup>a</sup> | 83.27±0.05°             |
| AWOS   | 29.42±0.65 <sup>b</sup> | 6.27±0.14 <sup>a</sup> | 9.30±0.26 <sup>a</sup> | 5.51±0.04 <sup>b</sup> | 49.50±1.79 <sup>a</sup> | 85.48±0.04 <sup>a</sup> |
| AWS    | 29.69±0.40 <sup>b</sup> | 5.53±0.12 <sup>b</sup> | 9.11±0.21 <sup>a</sup> | 5.31±0.03 <sup>c</sup> | 50.36±2.41 <sup>a</sup> | 84.65±0.03 <sup>b</sup> |

Values with different superscript along the same column are significantly different (p<0.05). Values are expressed as mean ± standard deviation; n= 3; \*determined on a wet weight basis; Fresh, unprocessed *V. amygdalina* leaf; AWOS, *V. amygdalina* leaf processed by abrasion without salt; AWS, *V. amygdalina* leaf processed by abrasion with salt.

the *V. amygdalina* leaf collected for this study. The various processing methods significantly depleted (p<0.05) the protein content of the vegetable, with similar pattern. This may be due to the leaching off of this nutrient by water when they were mechanically squeezed (Oboh et al., 2005).

The fat of *V. amygdalina* (6.04% DW) was also slightly higher than the 4.70 and 5.37% DW reported by Ejoh et al. (2007) and Asaolu et al. (2010), respectively. AWS caused significant decrease (p<0.05) in the fat content (5.53% DW), while AWOS had a non significant increase (6.27% DW). The salt seems to have interacted with the hydrophobic component of the leaf to enhance further leaching of fat.

The fiber (9.09% DW) is similar to the report of Mensah et al. (2008), higher than 6.5% DW reported by Akindahunsi and Salawu (2005), but far lower than 25.47 and 28.41% DW reported by Ejoh et al. (2007) and Asaolu et al. (2010), respectively. This differences might be due to variation in the environmental factors and agronomic practices at the various locations that the analyzed V. amygdalina leaves were harvested. 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, pectin, gum and mucilage (Wardlaw and Hampl, 2007). Non-starchy vegetables are the richest sources of dietary fiber (Agostoni et al., 1995) and are employed in the treatment of diseases such as obesity, diabetes, cancer and gastrointestinal disorders (Saldanha, 1995). The fiber content as shown in this study makes the vegetable viable enough to prevent colon cancer and other gastrointestinal disorders, even after the processing steps. A slight increase in dietary fiber was observed when the fresh sample was subjected to the different processing techniques. Slight increase in dietary fiber has been reported for different leafy vegetables during culinary and industrial treatments. This increase could be due to the fact that as samples were subjected to the different processing treatments, all soluble components were lost in the process thereby increasing the fiber contents (Nyman et al., 1987; Lintas and Cappelloni, 1988).

The ash content (5.6% DW) was slightly lower than the value (7.72% DW) reported by Ejoh et al. (2007). AWOS seems to minimize the leaching of inorganic minerals

while the AWS depletes the ash further. This trend is a reverse to that reported by Oboh (2005a), in which AWS yielded higher ash content when he worked on *Cnidoscolus aconitifolus* with various processing methods. The depletion of minerals during AWS is clearly shown in the consistently lower values of the metals detected in this sample compared with sample processed with AWOS.

The carbohydrate level in our *V. amygdalina* was 44.73% DW slightly lower than the 47.9% DW reported by Mensah et al. (2008) and far different from 68.35% DW reported by Ejoh et al. (2007). The difference might be due to the physiological state of the plant before harvesting (Singhal and Kulkarni, 1988). carbohydrate sources are not generally used by humans because most of them remain undigested (Ejoh et al., 2007). AWS or AWOS affected a non significant increase in the carbohydrate content. This implies that the carbohydrate content of the leaf is not leached off, perhaps due to their complex and insoluble characteristics.

The vitamin contents of V. amygdalina are as summarized in Table 2. The  $\beta$ -carotene, vitamins  $B_1$ ,  $B_3$ , C and E concentrations in the unprocessed V. amygdalina were 187.23, 0.17, 16.72, 50.47 and 0.25 mg/100 g DW, respectively. There is a paucity of V. amygdalina vitamin reports.

Fruits and vegetables are rich sources of antioxidant compounds. Polyphenols, flavonoids and antioxidant vitamins are granted for these beneficial effects. Antioxidant activities can be supplied by  $\beta$ -carotene, vitamin C and E of the *V. amygdalina*.  $\beta$ -carotene and vitamin E are greatly depleted by the conventional processing technique; AWS being more. The lipophillic nature of these vitamins is likely to have made them to be depleted along with the fat as they show similar trend with the level of fat depletion.

The vitamin C content of the unprocessed sample (50.47 mg/100 g) was slightly lower than the value (52.0 mg/100 g) reported by Oboh (2005b) for *V. amygdalina*. This variation may be due to the different sources of the *V. amygdalina*. This value is also lower than the vitamin C contents of *Telfairia occidentalis* (148 mg/100 g), *Amaranthus cruentus* (70.0 mg/100 g), *B. alba* (64.6 mg/100 g) and *Ocimum gratissimum* (52 mg/100 g), but higher than the values of *Solanum macrocarpon* 

Table 2. Effect of processing on the vitamin content (mg/100 g DW) of Vernonia amygdalina leaves.

| Sample | β-carotene               | Vitamin B₁             | Vitamin B <sub>3</sub>  | Vitamin C               | Vitamin E              |
|--------|--------------------------|------------------------|-------------------------|-------------------------|------------------------|
| Fresh  | 187.23±2.87 <sup>a</sup> | 0.17±0.02 <sup>a</sup> | 16.72±0.35 <sup>a</sup> | 50.47±0.36 <sup>a</sup> | 0.25±0.02 <sup>a</sup> |
| AWOS   | 168.73±1.27 <sup>b</sup> | 0.15±0.02 <sup>a</sup> | 14.83±0.35 <sup>b</sup> | 47.57±0.70 <sup>b</sup> | 0.17±0.01 <sup>b</sup> |
| AWS    | 151.23±2.30 <sup>c</sup> | 0.13±0.01 <sup>b</sup> | 13.57±0.25°             | 45.57±0.40 <sup>c</sup> | 0.11±0.02 <sup>c</sup> |

Values with different superscript along the same column are significantly different (P<0.05). Values are expressed as mean  $\pm$  standard deviation; n= 3; Fresh, unprocessed *V. amygdalina* leaf; AWOS, *V. amygdalina* leaf processed by abrasion without salt; AWS, *V. amygdalina* leaf processed by abrasion with salt.

Table 3. Effect of processing on the mineral content (mg/100 g DW) of Vernonia amygdalina leaves.

| Sample | Na                      | K                       | Са                       | Р                       | Fe                     |
|--------|-------------------------|-------------------------|--------------------------|-------------------------|------------------------|
| Fresh  | 67.29±0.41 <sup>a</sup> | 98.35±0.34 <sup>a</sup> | 148.73±1.22 <sup>a</sup> | 43.47±0.43 <sup>a</sup> | 5.20±0.31 <sup>a</sup> |
| AWOS   | 54.83±0.29 <sup>b</sup> | 67.79±0.51 <sup>b</sup> | 136.21±2.20 <sup>b</sup> | 35.01±0.39 <sup>b</sup> | 4.19±0.47 <sup>b</sup> |
| AWS    | 43.51±0.34°             | 51.49±0.33°             | 126.35±0.35°             | 31.61±0.18 <sup>c</sup> | 3.63±0.46 <sup>c</sup> |
| Sample | Mn                      | Mg                      | Zn                       | Cu                      | Se                     |
| Fresh  | 68.03±0.46 <sup>a</sup> | 34.28±0.30 <sup>a</sup> | 15.82±0.30 <sup>a</sup>  | 17.39±0.22 <sup>a</sup> | 0.14±0.02 <sup>a</sup> |
| AWOS   | 59.59±0.45 <sup>b</sup> | 29.36±0.27 <sup>b</sup> | 13.82±0.15 <sup>b</sup>  | 15.02±0.24 <sup>b</sup> | 0.10±0.01 <sup>b</sup> |
| AWS    | 51.42±0.25°             | 23.94±0.32 <sup>c</sup> | 12.27±0.17 <sup>c</sup>  | 11.71±0.21 <sup>c</sup> | 0.08±0.02 <sup>b</sup> |
| Sample | Со                      | Al                      | Pb                       | As                      | Cd                     |
| Fresh  | $0.58 \pm 0.03^{a}$     | $0.36 \pm 0.02^{a}$     | $0.11 \pm 0.02^{a}$      | ND                      | ND                     |
| AWOS   | $0.51 \pm 0.02^{b}$     | $0.27 \pm 0.02^{b}$     | $0.07 \pm 0.01^{b}$      | ND                      | ND                     |
| AWS    | $0.39 \pm 0.02^{c}$     | $0.19 \pm 0.04^{c}$     | 0.06 ± 0.01 <sup>b</sup> | ND                      | ND                     |

Values with different superscript along the same column are significantly different (P<0.05). Values are expressed as mean ± standard deviation; n= 3; ND, non-detectable; Fresh, unprocessed *V. amygdalina* leaf AWOS, *V. amygdalina* leaf processed by abrasion without salt; AWS, *V. amygdalina* leaf processed by abrasion with salt.

(43.5mg/100g) and *Corchorus olitorus* (43.5 mg/100 g) reported by Oboh (2005b). This indicates that V. amygdalina is a good source of vitamin C. The treatments caused a significant depletion (p<0.05) in the vitamin C contents of the samples. This loss in vitamin C during processing with AWS or AWOS could be attributed to the fact that vitamin C is water soluble. Vitamin C contributes to the antioxidant properties of vegetables by protecting the membrane of erythrocyte, maintaining the blood vessel flexibility and improving blood circulation in the arteries of smokers as well as facilitating the absorption of iron in the body (Oboh, 2005b). The levels of vitamins B<sub>1</sub> and B<sub>3</sub> (water soluble) were also significantly (p<0.05) diminished by the two processing methods. However, AWS lowered the vitamins levels more.

The levels (mg/100 g DW) of minerals in *V. amygdalina* subjected to different processing techniques are shown in Table 3. This result shows non-detectable levels of heavy metals like As and Cd with a very low Pb and Al contents that diminished with abrasion. This vegetable had the essential minerals in the order of Ca> K> Mn> Na> P> Mg> Cu> Zn> Fe> Co> Se. Being rich in all these minerals, *V. amygdalina* can be very useful in supplying

them to its consumers, thereby facilitation various biochemical processes in humans and minimizing micronutrient deficiencies. The elemental contents of this vegetable were significantly diminished (p<0.05) by the treatments (AWS or AWOS). However, processing with salt had greater diminishing effect in virtually all the minerals analyzed. The diminishing effect of processing on *V. amygdalina* was similarly reported in *C. aconitifolus* that was processed by various methods (Oboh, 2005a). The Mn, Zn, Cu and Se content of this vegetable also make it a potential antioxidant source to its consumers. The trend of how the minerals were limited by the two processing methods is similarly reflected in the ash content results.

Table 4 shows the results of the anti-nutrient analysis of *V. amygdalina* subjected to different processing methods. Phytate, oxalate, tannin and hydrogen cyanide (HCN) were notably present in all samples, with higher amounts detected in the unprocessed sample. The levels of all anti-nutrients in the vegetable were significantly decreased (p<0.05) by AWS or AWOS. Treatment with salt limited more anti-nutrients.

There is paucity of information on the anti-nutrient contents of V. amygdalina as well. The phytate level in

**Table 4.** Effects of processing on the anti-nutrient content of *Vernonia amygdalina* leaves.

| Sample | Phytate (g/100g)    | Oxalate (g/100g)         | Tannin (g/100g)     | HCN (mg/kg)             |
|--------|---------------------|--------------------------|---------------------|-------------------------|
| Fresh  | $1.31 \pm 0.03^{a}$ | 0.96 ± 0.01 <sup>a</sup> | $0.09 \pm 0.00^{a}$ | $2.65 \pm 0.03^{a}$     |
| AWOS   | $1.15 \pm 0.02^{b}$ | $0.89 \pm 0.02^{b}$      | $0.07 \pm 0.00^{b}$ | 2.48± 0.07 <sup>b</sup> |
| AWS    | $1.05 \pm 0.02^{c}$ | $0.75 \pm 0.03^{c}$      | $0.06 \pm 0.00^{c}$ | 2.15± 0.02 <sup>c</sup> |

All values are reported on a DW basis. Values with different superscript along the same column are significantly different (P<0.05). Values are expressed as mean  $\pm$  standard deviation; n=3; Fresh, unprocessed V. amygdalina leaf; AWOS, V. amygdalina leaf processed by abrasion without salt; AWS, V. amygdalina leaf processed by abrasion with salt

the unprocessed sample (1.31 g/100 g DW) is quite lower than the value (1.47 g/100 g DW) previously reported of for V. amygdalina by Akindahunsi and Oboh (1999). It is also very much lower than the value (2.89 g/100 g DW) reported in C. aconitifolus (Oboh, 2005a). The cyanide level in the unprocessed leaf (2.65 mg/kg DW) was far lower than the value (10.84 mg/kg DW) reported in C. aconitifolus leaf (Oboh, 2005a). Thus, V. amygdalina can be considered safe with regard to acute cyanide poisoning since the cyanide levels were far below the detrimental levels of 30 mg/kg reported by Oboh and Akindahunsi (2003). The pattern by which the two processing techniques caused a significant decrease in the phytate (1.05-1.15g/100g DW) and cyanide (2.15-2.48mg/kg DW) levels of V. amygdalina leaf agrees with the earlier reports by Oboh et al. (2003) and Oboh (2005a) which show that processing will result in a decrease in these anti-nutrients. We also report similar decreasing effect of processing on oxalate (from 0.96 to 0.75g/100g DW) and tannins (from 0.09 to 0.06g/100 DW) after abrasion with or without salt. Tannins have diverse effects on biological systems, since they are potential metal ion chelators, protein precipitating agents and biological antioxidants (Hagerman, 2002).

## Conclusion

V. amygdalina leaf is very rich in protein, fibre, vitamins, minerals and anti-nutrients. However, the various conversional post-harvest processing caused a significant decrease in all the nutrients (except fibre and carbohydrate) and anti-nutrients investigated. It is very necessary to reach a compromise on the nutrients and anti-nutrients depletion vis-à-vis AWS or AWOS. Thus, abrasion with minimal salt is recommended to minimize nutrient depletion and ensure reasonable decrease of anti-nutrients in the leaf. A bioavailability study of the nutrients from the leaf can further enrich our knowledge on the better processing practice.

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