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Full Length Research Paper

Mineral and phytochemical contents in leaves of *Amaranthus hybridus L* and *Solanum nigrum L.* subjected to different processing methods

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Mineral and phytochemical contents of leaves of *Amaranthus hybridus L.* and *Solanum nigrum L.* Subjected to different processing methods were evaluated. Processing procedures adopted include shredding, sun–drying, oven–drying, steaming and a combination of these. Minerals examined are Na, K, Ca, Mg, Fe, P, and Zn while the phytochemicals are alkaloids, flavonoids, hydrocyanic acid, phenols, phytic acid and tannins. Oven – drying was the most effective method for retaining the studied minerals in *S. nigrum* but only for Na, Ca, Fe and Mg in *A. hybridus L.* Na/K and Ca/P ratios ranged between 0.13 – 0.14 and 1.24 - 1.28 *A. hybridus L.* while values for *S. nigrum L.* were 0.70 – 0.80 and 0.21 – 0.24 respectively in all the treatments. Sun–drying was the most effective method for retaining the phytochemicals. For retention of alkaloids, flavonoids and saponins, oven–drying proved the second best method while steaming with sun – drying elicited the greatest reduction in the levels of hydrocyanic acid, phenols, phytic acid and tannins. Processing methods employed when utilizing these leaves should therefore reflect the desired effect.

Key words: *A. hybridus L.*, *S. nigrum L.*, processing, minerals, phytochemicals.

INTRODUCTION

Trees and shrubs with medicinal and nutritional potentials abound in Nigeria (Burkill, 1985). Vegetables are indispensable constituents of human diets. They supply the body with minerals, vitamins and certain hormone precursors in addition to proteins and energy (Oyenuga and Fetuga, 1975).

The medicinal values of plants and vegetables are dictated by their phytochemical and other chemical constituents (Fallah et al., 2005). This explains why efforts have been expended in studies aimed at elucidating their levels in many plants both in Nigeria and elsewhere (Galitsikil et al., 1997; Edeoga et al; 2005; 2006).

Amaranthus hybridus L. (popularly called Amaranth or "pig weed") and *Solanum nigrum L.* are two herbaceous plants that have found large applications in food and medicinal industries. In Nigeria and other parts of Africa, the leaves are used to prepare soup, salad or eaten as spinach. Amaranthus' high content of squalene makes it preferred natural candidate over marine animals as source for squalene (Oliveria and Decarvalho, 1975; Martin and Telek, 1979; Oke, 1983: Dhellot et al., 2006; Mepha, 2007). Leafy vegetables are highly perishable agricultural products. They require special processing methods to prevent post harvest losses. Processing causes some changes in nature and content of constituents. Little seems to have been done to address and document the effect of processing methods commonly employed in Nigeria on chemical constituents of leafy vegetables. The present study is aimed at filling this scientific gap with respect to two commonly consumed leafy vegetables – *A. hybridus L.* and *S. nigrum L.*

MATERIALS AND METHODS

Plant sample collection and identification

The leaves of the two plants were harvested from cultivated farm located in Afikpo North local Government Area, Ebonyi State, Nigeria. They were authenticated by a taxonomist and voucher specimen deposited at the herbarium of, Abia state University, Uturu. The plant materials were cleaned, rinsed with deionized

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Table 1. Mineral composition in differently processed *Amaranthus hybridus L.* leaves

Mineral composition (Mg/100)									
Processing Variables	**Sodium (Na)**	**Potassium (K)**	**Calcium (C)**	**Magnesium (Mg)**	**Iron (Fe)**	**Zinc (Zn)**	**Phosphorus (P)**	**Na/k**	**Ca / P**
SHD	7.12^{a}	52.97^{b}	42.93^{b}	229.80^{b}	13.20^{b}	3.51^{b}	34.17^{a}	0.13^{a}	1.26^{a}
SND	7.43^{b}	54.20^{a}	44.15^{a}	231.22^{a}	13.58^{a}	3.80^{a}	34.91^{a}	0.14^{a}	1.26^{a}
OVD	7.48^{b}	54.01^{a}	44.31^{a}	231.59^{a}	13.70^{a}	3.78^{a}	34.94^{c}	0.14^{a}	1.28^{a}
STM	7.19^{a}	53.65^{a}	43.01^{a}	229.74^{b}	12.81^{c}	3.61^{b}	33.90^{a}	0.13^{a}	1.27^{a}
STM+SND	7.17^{a}	51.82^{C}	42.05^{d}	229.57^{b}	13.18^{b}	3.50^{b}	33.82^{b}	0.14^{a}	1.24^{b}
SHD+ OVD	7.20^{a}	52.06^{b}	42.49^{c}	229.58^{b}	13.20^{b}	3.53^{b}	34.20^{a}	0.14^{a}	1.24^{b}
STM + SND	7.11^{a}	53.41^{a}	43.54^{b}	229.54^{b}	12.76^{c}	3.59^{b}	33.88^{b}	0.13^{a}	1.27^{a}
STM+ OVD	7.19^{a}	53.69^{a}	42.85^{c}	229.79^{b}	12.82^{c}	3.55^{b}	33.80^{b}	0.13^{a}	1.26^{a}

Values are means of three replicates.
Figures followed by the same alphabet along the columns are not significantly different at $P < 0.05$ using Duncan multiple range test (DMRT)
SHD = shredding; SND = sun drying; OVD = oven-drying;
STM = steaming.

water, and allowed to evaporate at room temperature.

Processing and analysis of samples

The leaves were subjected to seven different processing methods as detailed below.

Processing methods

About 600 g each of the *A. hybridus L.* and *S. nigrum L.* leaves collected were subjected to the following processing techniques:

(i) Shredding (SHD) of the leaves using a chopped knife to cut the leaves and then a Thomas-willey milling machine to blends the leaves into fine pieces.
(ii) Sun-drying (SND) for 2 - 3 days with constant turning over to avert fungal growth on clean papers.
(iii) Oven-drying (OVD) on aluminum trays at 80 -100°C for 24 h.
(iv) Steaming (STM) of the leaf sample over wire gauze placed on top of a boiling water for 30 min.
(v) Shredding and Sun-drying (SHD+SND) in which the Thomas-willey blended samples were further sun-dried for 2 - 3 days with adequate turning over to avert fungal growth.
(vi) Shredding and oven-drying (SHD+OVD) in which the Thomas – willey blended sample were now oven – dried on aluminum trays at 80 - 100°C for 24 h.
(vii) Steaming and sun-drying (STM+SND) in which the steamed leaf samples were further sun-dried for 2 - 3 days with adequate turning over to avert fungal growth.
(vii) Steaming and oven-drying (STM+OVD) in which the steamed leaf samples were further oven-dried on aluminum trays at 80 - 100°C for 24 h.
All the processing samples were packed into tightly sealed nylon bags and analyses commenced immediately with minimum delay to forestall a further change in the quality of the samples. Where the analysis could not be completed on the same day, samples were kept frozen at temperature of -4°C.

Preparation of fat free sample

Exactly 2.0 g of each of the processed sample were defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 h.

MIneral analysis

The mineral elements, comprising sodium, calcium, potassium, magnesium, iron, zinc and phosphorus were determined according to the method of Shahidi et al., (1999) and Nahapetian and Bassiri (1975) with some modifications. Exactly 2.0 g of each of the processed samples were weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5.0 ml of $HNO_3/HCl/H_2O$ (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5.0 ml of de-ionized water was added and heated until a colorless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through Whatman No.42 filter paper and the volume was made to the mark with de-ionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer. A 10 cm long cell was used and concentration of each element in the sample was calculated on percentage (%) of dry matter that is mg/100 g sample. Phosphorus content of the digest was determined calorimetrically according to the method described by Nahapetian and Bassiri (1975).

Determination of the phytochemical content of the leave samples

Alkaloids, phenols and saponins were determined by the method of Harbone, (1973) as detailed by Obadoni and Ochuko (2001). Flavonoids were estimated by the method of Bohm and Kocipal (1974).Tannin determination was according to Van-Burden and Robinson (1981) and determination of Hydrocyanic acid was quantified by the method of Bradbury et al. (1991) while Determination of Phytic Acid was by the method of Wheeler and Ferrell (1971).

Statistical analysis

All values are means of three replicate determinations. Data for all determinations were subjected to analysis of variance (ANOVA) using Duncan Multiple Range Test (DMRT) for mean separation (Sokahl and Rholf, 1969).

RESULTS

Tables 1 and 2 show the effects of different processing

Table 2. Mineral composition in differently processed *S. nigrum L.* leaves

	Mineral composition (MG/100								
Processing variables	Sodium (Na)	Potassium (K)	Calcium (C)	Magnesium (Mg)	Iron (Fe)	Zinc (Zn)	Phosphorus (P)	Na/k	Ca / P
SHD	2.69^{b}	39.83^{c}	16.09^{b}	245.01^{b}	12.91^{a}	0.01^{b}	74.97^{a}	0.07^{a}	0.21^{a}
SND	2.90^{a}	41.69^{a}	17.83^{a}	246.04^{a}	13.01^{a}	0.09^{b}	75.22^{a}	0.07^{a}	0.24^{a}
OVD	3.15^{a}	41.82^{a}	18.07^{a}	246.65^{a}	13.52^{a}	0.12^{a}	75.36^{a}	0.08^{a}	0.24^{a}
STM	2.67^{b}	40.05^{b}	15.64^{c}	244.88^{c}	11.61^{c}	0.04^{b}	74.83^{b}	0.07^{a}	0.21^{a}
SHD+SND	2.56^{b}	39.21^{c}	15.43^{c}	244.10^{c}	12.61^{b}	0.03^{b}	74.53^{b}	0.07^{a}	0.21^{a}
SHD+OVD	2.60^{b}	39.75^{c}	16.11^{b}	244.99^{b}	12.98^{a}	0.05^{b}	74.80^{b}	0.07^{a}	0.22^{a}
STM+SND	2.38^{b}	39.96^{c}	16.07^{b}	244.10^{c}	12.12^{b}	0.01^{b}	74.17^{c}	0.06^{a}	0.22^{a}
STM+OVD	2.57^{b}	39.60^{c}	15.60^{c}	244.68^{c}	12.45^{b}	0.04^{b}	74.11^{c}	0.07^{a}	0.21^{a}

Values are means of three replicates
Values bearing different superscripts along the columns are significantly different (P<0.05) using Duncan Multiple range Test (DMRT).
SHD = Shredding: SND = Sun – drying; OVD Oven – drying;
STM = Steaming.

methods on the mineral composition of *A. hybridus L.* and *S. nigrum L.* leaves respectively subjected to different processing methods.

In both *A. hybridus L.* and *S. nigrum L.,* oven drying resulted in the retention of highest amount of Na and Ca, Mg, Fe, Zn while steaming with sun-drying caused the greatest losses in these minerals.

However, steaming with oven-drying produced leaves with the lowest phosphorus content amounting to 33.80 and 74.11 mg/100 g for *A. hybridus L* and *S. nigrum L.* respectively, while oven-drying still produced leaves with the greatest amount of phosphorus (34.94 and 75.36 mg/100 g) for *A. hybridus L.* and *S. nigrum L.* The Na/K ratio in both *A. hybridus L* and *S. nigrum L.* are less than 1. The Ca/P ratio in *A. hybridus L.* > 1 while that in *S nigrum* L< 1.

The phytochemical contents of the leaves of *A. hybridus* and *S. nigrum* following the different processing methods are shown in Tables 3 and 4. Table 3 indicates that in *A. hybridus*, shredding with sun-drying caused the greatest reduction in the amounts of flavonoid and Phenols while steaming with oven- drying resulted in the lowest values in tannins, phytic acid and saponins.

The lowest amount in alkaloid and phytic acid occurred with steaming combined with sun-drying while sun-drying alone caused the highest retention of the studied phytochemicals in *A. hybridus L*.

In *S. nigrum L*. steaming with sun-drying (STM + SND) produced the greatest reductions in the amounts of alkaloids, saponins, phenols, hydrocyanic acid and phytic acid. Shredding with sun-drying (SHD + SND) resulted in the lowest level of flavonoids. However the least value for tannins was recorded with steaming + oven drying treatment. Sun drying alone caused the greatest retention of these phytochemicals.

For each parameter studied, the values obtained for the treatment that produced the least effect when compared with the treatment that produced the greatest effect significant (P<0.05) differences were observed.

DISCUSSION

Mineral content of the processed leaves:

Sodium and potassium are important intracellular and extracellular cations The Na/K ratio are important in determining the health status of an individual. A ratio of less than one has been recommended to prevent high blood pressure (FND, 2002) the processing method produced Na/K ratio less than one which is in consonance with recommended ratio. The result show that a number of the processing methods elicited significant losses in Na and K when compared with one another. Oven-drying, and sun-drying are perhaps the methods of choice when high levels of these two minerals are desirable.

Some of the processing methods employed caused significant changes in the level of Ca and P in the two leaf samples. The Ca/P ratio elicited by the different treatments where > 1 in *A. hybridus L*. and < 0.5 in *S. nigrum*. *L*. Calcium and Phosphorus are important in bone, teeth and muscle metabolism (Dosunmu, 1997; Turan, 2003). Food is considered “good “if the Ca/P ratio is > 1 but poor if < 0.5 (Niemans et al., 1992). The implication is that *A. hybridus L*. is “good” while *S. nigrum L*. is “bad” in this respect. This also implies that the processing methods did not improve the food value of the two plant leaf with respect to Ca/P ratio.

Magnesium content of the two vegetables varied with the processing methods but were still >200mg/100 g (DW). The Fe content varied between 11.61 - 13.58 mg/100 g, while the Zn content varied between 0.01 to 0.12 in *S. nigrum L*. Shredding as well as steaming with sun-drying caused the greatest losses in this mineral. Magnesium is an important mineral element in connection with circulatory diseases such as ischemic heart

Table 3. Chemical constituents in differently processed *Amaranthus hybridus L.* leaves.

	Chemical composition (Mg/100 g)						
Processing variables	**Alkaloid**	**Flavonoid**	**Saponin**	**Tannin**	**Phenols**	**Hydrocyanic acid**	**Phytic acid**
SHD	3.40 [a]	0.46 [b]	1.59[a]	0.40 [a]	0.23 [a]	16.10 [a]	1.17 [a]
SND	3.54 [a]	0.83 [c]	1.68 [a]	0.49 [a]	0.35 [a]	16.99 [a]	1.32 [a]
OVD	3.39 [a]	0.68[C]	1.62 [a]	0.39 [a]	0.30 [a]	13.07[c]	1.01[b]
STM	3.34 [a]	0.49 [b]	1.56 [a]	0.32 [a]	0.26 [a]	12.87 [c]	0.98 [b]
SHD+SND	3.24[b]	0.38 [b]	1.50 [a]	0.31 [a]	0.17 [a]	15.48 [b]	1.12[a]
SHD+OVD	3.24 [b]	0.38 [b]	1.50 [a]	0.31 [a]	0.17 [a]	15.48 [b]	1.12 [a]
STM+SND	3.07 [c]	0.43 [b]	1.51 [a]	0.29 [a]	0.20 [a]	11.40[d]	0.79[b]
STM+OVD	3.20 [b]	0.41 [b]	1.45[b]	0.29 [a]	0.23 [a]	11.00 [d]	0.87 [b]

Values are means of three replicates.
Values bearing different superscripts along the columns are significantly different (P < 0.05) using Duncan Multiple Range Test (DMRT).
SHD = Shredding; SND = Sun-drying; OVD = Oven-drying; STM = Steaming.

Table 4. Chemical constituents in differently processed *Solanum nigrum L.* leaves

Processing variable	Chemical composition (mg/100g)						
	Alkaloid	***Flavonoid***	**Saponin**	**Tanin**	**Phenols**	**Hydrocyanic acid**	**Phytic acid**
SHD	1.41[c]	0.33 [a]	0.28 [a]	0.09 [a]	13.00 [d]	9.21[e]	0.81[c]
SND	1.59[d]	0.71 [d]	0.30 [a]	0.14 [b]	14.13 [f]	9.96[f]	0.95[d]
OVD	1.40 [c]	0.53 [c]	0.28 [a]	0.13 [b]	13.98 [e]	6.43[c]	0.78[b]
STM	1.36 [b]	0.41 [b]	0.23 [a]	0.08 [a]	12.81 [c]	6.06[b]	0.65[a]
SHD+SND	1.38 [bc]	0.32 [a]	0.21 [a]	0.07 [a]	12.10 [b]	8.38[d]	0.71[b]
SHD+OVD	1.32 [b]	0.29 [a]	2.26 [a]	0.08 [a]	12.75 [c]	6.03[b]	0.63[a]
STM+SND	1.22 [a]	0.37 [b]	0.19 [a]	0.06 [a]	11.03 [a]	5.29[a]	0.52[a]
STM+OVD	1.25 [a]	0.36 [ab]	0.20 [a]	0.05 [a]	11.94 [a]	5.32[a]	0.57[a]

Values are means of three replicates.
Values bearing different superscripts along the columns are significantly different (P < 0.05) using Duncan Multiple Range Test (DMRT).
SHD = Shredding; SND = Sun-drying; OVD = Oven-drying; STM = Steaming.

disease. Zinc is involved in the normal functioning of the immune system while Fe is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and energy metabolism. (Shills and Young, 1988; Adeye and Otokiti, 1999; Ishida et al., 2000). Significant losses of these minerals caused by shredding, steaming and combination of these methods suggest that the best methods to preserve these vegetable samples when required for mineral supplement are oven and sun-drying.

Phytochemical content

Tables 3 and 4 contain details of the effects of processing on photochemical content of *A. hybridus L.* and *S. nigrum L.* leaves respectively. The results, especially for alkaloids indicate levels comparatively similar to many medicinal plants (Edeoga et al., 2005; Okwu and Josiah, 2006). This strongly supports their use in medicine. For example, *S. nigrum L.* has been reported to be useful in treatment of ulcer (Uanseoje et al., 2003).

Of the processing methods examined, sun drying was the most effective in retaining the photochemicals. Relative to sun drying, the other processing methods caused significant reductions in the studied photochemicals. Dupont et al., 2000, reported similar reduction in the photochemical content of the lettuce and endive subjected to a variety of processing methods.

Steaming with sun-drying resulted in the highest reductions in the levels of the ant nutrients- hydrocyanic acid (HCN), phenols, phytic acid and tannins in the two studied leafy vegetables. The findings in this study are also in consonance with reports by other researchers on some other plants. (Aletor, 1993; Bokanga, 1994; Nambiasan, 1994). Reduction in HCN levels in food and medicinal preparations is desirable because it has been implicated in cerebral damage and lethargy in man and animals (Sofowara, 1993; Starry, 1998).

Conclusion

A. hybridus L. and *S. nigrum L.* are important sources of mineral and phytochemicals. They are therefore, nutritionally and medicinally relevant. Processing methods produce diverse effects on the mineral and phytochemical content. Steaming with sun-drying caused the greatest loss of K while oven and sun-drying was most effective in retaining Na. The calcium and phosphorus levels also changed with different processing methods. However, the Na/K as well as the Ca/P ratios in each of the vegetable samples were similar in all the treatments.

Sun-drying is perhaps the preferred processing method to retain the phytochemicals while steaming with sun-drying should be employed to obtain samples having low levels of HCN, phenols, phytic acid and tannins among other anti-nutrients. Processing methods should be chosen to reflect any desired objective.

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