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Effect of Fermentation and Heat Treatment on the Nutrient and Antinutrient Composition of African Walnut (*Tetracarpidium conophorum*)

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Abstract: Conophor plant (*Tetracarpidium conophorum*) is a tropical climbing shrub that belongs to the family Euphorbiaceae. It is one of the lesser known and underutilized oilseeds. Mature conophor nuts were processed in three ways namely cooking for 2 h, roasting in hot sand at 130°C for 1 h and natural open air fermentation for three days. Both raw and processed samples were dried and milled into flour for the determination of proximate and mineral composition and certain antinutrients. The raw nuts showed high content of protein and fat. Cooking and fermentation further significantly ($p<0.05$) improved the protein level. Fat was significantly ($p>0.05$) reduced by fermentation. Ash, crude fibre and carbohydrates were significantly ($p<0.05$) reduced by the three processing methods. Fermentation significantly ($p<0.05$) reduced all the minerals determined. However, Ca content was significantly increased ($p<0.05$) by cooking and roasting. The sodium: potassium ratio in the raw sample (1.02) was reduced by the three processing methods. The level of antinutrients were generally reduced by cooking and fermentation with the latter being more effective than the former in the case of alkaloid content. Roasting increased the tannin content. The study thus reveals cooking and fermentation as good methods of processing the conophor nut in order to harness its nutrient.

Key words: Nutrient, antinutrient, *Tetracarpidium conophorum*

INTRODUCTION

Conophor plant (*Tetracarpidium conophorum*) is one of the lesser known and underutilized perennial crops grown and consumed in the forest zones of Nigeria. It is a tropical climbing shrub that belongs to the family Euphorbiaceae. Conophor plant is cultivated principally for the nuts which are cooked and consumed as snacks, along with boiled corn. The nuts have been reported to have high nutrient potentials by various researchers (Oke and Fatunso, 1975; Ogunsua and Adebona, 1983; Nwokolo, 1987). Enujiugha and Ayodele-Oni (2003) reported some significant concentrations of oxalates, phytates and tannins in raw conophor nut. The presence of antinutritional factors has been a major limitation in the utilization of some of the unconventional oilseeds that have been found to be highly rich in protein and calories. Many processing methods have been shown to reduce antinutrients and improve the nutritive quality of plant foods for human nutrition (Obizoba and Atti, 1991). In particular, boiling which is currently the principal method of processing conophor nut has been found useful in this regard (Enujiugha, 2003). Roasting has been shown to enhance the level of tannin and phytate (Enuijiagha, 2003). Although fermentation have been employed in the preparation of pearl millet-conophor weaning diet (Akeredolu *et al*., 2005), there is no report on how fermentation specifically affects the antinutrient content of conophor nut hence the need for this comparative study which looks at how the oilseed's nutrient and antinutrient are affected by boiling, fermentation and roasting.

MATERIALS AND METHODS

Collection and preparation of samples: Matured freshly harvested conophur nuts were purchased from Iruekpen market in Esan West Local Government Area, Edo State, Nigeria. Identification was carried out by an agronomist and botanist from the Department of Crop Science and Botany respectively, Ambrose Alli University, Ekpoma, Nigeria.

Processing of the seeds: The shelled nuts were removed from their pods and divided into four portions. One portion, in the raw state, was deshelled and cut into tiny pieces. The second portion was deshelled, cut into pieces and mixed with distilled water (1:2 w/v). It was allowed to stand for three days at room temperature (25°C) for natural fermentation to take place. The third portion was boiled in water at 100°C for 2 h before deshelling and cutting into pieces. The fourth portion was roasted in hot sand (while stirring continuously) at 130°C for 1 h before deshelling and cutting into pieces. All the samples were dried in a hot air oven at 60°C for 24 h before milling into flour. They were then packaged and kept in a refrigerator at 4°C for use.

Proximate chemical composition: Proximate composition was determined by standard procedures (AOAC, 2000). Minerals were determined after wet ashing using concentrated nitric acid and perchloric acid (1:1, v/v), using Atomic Absorption Spectrophotometer (Hitachi model 170-10) (AOAC, 2000).

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Antinutrient determination: Tannin was determined by the vanillin-HCl method of Price *et al.* (1978). Phytate was estimated according to Wheeler and Ferrell (1971). Oxalate was analyzed by the procedure described by Ukpabi and Ejidoh (1989). Total alkaloids were determined by the spectrophotometric method of Shamsa *et al.* (2008) based on the reaction with bromocresolgreen (BCG). Saponin composition was estimated using the gravimetric method of Hudson and El-Difrawi (1979).

Statistical analysis: All analyses were done in triplicate and data expressed as Mean±SD. Data were subjected to the principle of single factor analysis of Variance (ANOVA) and the differences between means at 5% was determined by the Turkey Krammer's test using a software, Graphpad instat 3.1 (Graphpad Software Inc., USA).

RESULTS AND DISCUSSION

The proximate chemical composition (% dry weight) of both raw and processed samples of African walnut (*Tetracarpidium conophorum*) is shown in Table 1. The level of protein in the raw conophor nut sample was found to be relatively high (23.13%). This was significantly ($p<0.05$) increased by cooking and fermentation. The high protein content fairly compares with legumes and is much higher than those obtained from some Australian varieties (Harold and Tatura, 2002). The very high oil content (49.27%) agrees with previous findings (Ogunsua and Adebona, 1983; Oke and Fatunso, 1975). The fat content was significantly ($p<0.05$) reduced by fermentation. Ash (3.80%) and crude fibre (9.88%) were significantly ($p<0.05$) increased and decreased respectively by the three processing methods. The fibre is within the range reported for most oilseeds (Enujiugha and Ayodele-Oni, 2003). The ash content is reflective of the low mineral content of the oilseed. Carbohydrate content was significantly ($p<0.05$) reduced by cooking and fermentation. Cooking and fermentation therefore improves protein level if the aim is to obtain maximal protein from walnut.

The mineral content of raw and processed conophor nut is shown in Table 2. Fermentation significantly ($p<0.05$) reduced all the minerals determined due possibly to leaching. In addition, cooking and roasting significantly ($p<0.05$) reduced Na, Fe, Mn, Zn and P. However, Ca content was significantly increased ($p<0.05$) by cooking and roasting possibly due to gain of Ca from the hard shell during the both processing. Interestingly, fermentation was carried out without the shell which may have accounted for the reduction in Ca during fermentation. Earlier studies by Edem *et al.* (1994) had shown a 33.3, 28.6 and 60.0% decrease in sodium, iron and copper respectively due to cooking. The sodium: potassium ratio in the raw sample (1.02) was reduced by the three processing methods studied. However, cooking was most effective in this regard. It has been hypothesized that high protein-low potassium diets could induce a low-grade metabolic acidosis that could induce demineralization of bone, osteoporosis and kidney stones (Lemann, 1999) and epidemiological and metabolic studies have supported this suggestion (Tucker *et al.*, 1999).

Antinutrients are generally known to reduce nutrient utilization and or food intake (Osagie, 1998). As indicated in Table 3, the level of tannin recorded in the raw conophor nut sample was 27.83 mg/100 g. This was higher than the 0.9 mg/100 g reported by Enujiugha (2003). However, Osagie (1998) had earlier reported a relatively higher value of 40 mg/100 g for the same nut. Egbe and Akinyele (1990) found 59 mg/100 g tannic acid in raw lima beans (*Phaseolus lunatus)* while a range of 0.76 to 1.11% have been reported for certain underutilized crop seeds in Nigeria (Osagie, 1998). In this study, the level of tannin was significantly ($p<0.05$) reduced by cooking and fermentation and significantly ($p<0.05$) increased by roasting. After the processing, percentage retention of tannin in the cooked, fermented and roasted samples relative to raw was 48.3, 37.9 and 163.4%, respectively. These findings were in agreement with Enujiugha (2003) where the level of tannin reduced (from 0.9 to 0.3 mg/100 g) due to boiling and increased (from 0.9 to 1.1 mg/100 g) after toasting at 100°C for 30 min.

From the results obtained in this study, cooking and fermentation led to a 21.6 and 24.4% reduction, respectively while roasting brought about an increase of 5.2% in phytate content. The level of alkaloid in the raw conophor nut sample was found to be 30.90 mg/100 g which was significantly ($p<0.05$) reduced by the three processing methods employed in this study. Remarkably, fermentation produced the highest level of reduction amounting to a loss of 43.9% in alkaloid content. Ekwe and Ihemeje (2013) recently reported higher alkaloid content in raw conophor nut as 40.91

Table 1: Proximate composition of raw and processed conophor nut (%)

Component	Raw	Cooked	Roasted	Fermented
Crude protein	23.13±1.13^{c}	27.77±1.24^{ab}	25.52±1.12^{bc}	29.38±0.54^{a}
Crude fat	49.27±0.99^{a}	48.00±0.60^{ab}	47.46±0.71^{ab}	46.93±0.91^{b}
Ash	3.80±0.20^{b}	6.00±0.20^{a}	7.40±1.40^{a}	6.10±0.09^{a}
Crude fibre	9.88±0.87^{a}	7.43±0.60^{b}	7.29±0.78^{b}	6.24±0.16^{b}
Carbohydrate	13.92±1.33^{a}	10.79±1.33^{b}	12.33±0.17^{ab}	11.35±0.59^{b}

Means on the same row with different superscripts are significantly ($p<0.05$) different

Table 2: Mineral content of raw and processed conophor nut (mg/100 g)

Mineral (mg/100 g)	Raw	Cooked	Roasted	Fermented
Magnesium	110.7±1.24[ab]	125.5±15.11[a]	118.0±14.06[a]	86.0±5.30[b]
Sodium	110.2±0.83[a]	90.9±6.68[b]	95.2±8.79[b]	65.8±0.89[c]
Potassium	108.8±0.28[ab]	115.5±20.78[a]	119.6±14.09[a]	79.6±0.28[b]
Calcium	100.3±0.76[b]	129.1±5.95[a]	132.8±5.24[a]	79.1±4.68[c]
Iron	32.6±7.67[a]	19.8±0.53[b]	20.0±0.57[b]	14.66±0.25[b]
Manganese	5.7±0.57[a]	3.0±0.32[b]	3.3±0.43[b]	2.7±0.33[b]
Zinc	11.4±0.71[a]	5.4±1.54[b]	4.5±1.42[b]	3.0±0.00[b]
Copper	0.3±0.14[a]	0.3±0.00[a]	0.3±0.00[a]	ND
Phosphorus	27.1±0.03[a]	25.2±0.00[b]	22.3±0.02[d]	24.1±0.03[c]

ND: Not detected. Means on the same row with different superscripts are significantly ($p<0.05$) different

Table 3: Antinutrient composition of raw and processed conophor nuts (mg/100 g dry matter)

Antinutrient	Raw	Cooked	Roasted	Fermented
Tannin	27.83±2.42[b]	13.43±3.69[c]	45.47±2.73[a]	10.54±1.27[c]
Phytate	6.68±0.27[a]	5.24±0.22[b]	7.03±0.50[a]	5.05±0.51[b]
Alkaloid	30.90±1.75[a]	22.80±1.38[b]	18.97±2.60[bc]	17.33±1.72[c]
Saponin	0.38±0.04[a]	0.25±0.03[b]	0.43±0.04[a]	0.30±0.05[b]
Oxalate	61.17±7.46[a]	45.43±3.76[b]	67.13±4.96[a]	48.63±5.85[b]

Means on the same row with different superscripts are significantly ($p<0.05$) different

mg/100 g which was reduced to 0.03 mg/100 g and 0.1 mg/100 g upon cooking and toasting, respectively. Saponin content in the raw sample was 0.38 mg/100 g which is much lower than the 1.27 g/kg and 98.30 mg/100 g, respectively reported by Osagie (1998) and Ekwe and Ihemeje (2013). Cooking and fermentation brought about significant ($p<0.05$) reduction in the saponin level while roasting had no significant ($p>0.05$) effect.

The level of oxalate in the raw conophor nut was found to be 61.17 mg/100 g which was significantly ($p<0.05$) reduced by 25.7% after cooking. This agrees with the study by Edem *et al.* (1994) where a 72.4% reduction in total oxalate level was observed after cooking conophor seeds. Similarly, a 20.5% reduction was observed after fermentation. By and large, fermentation brought about maximal antinutrient reduction followed by cooking. Roasting is definitely not a good choice as the antinutrients were generally increased.

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