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Full Length Research Paper

Biochemical assessment of the mineral and some anti-nutritional constituents of *Aspergillus niger* fermented *Chrysophyllum albidum* seed meal

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Effect of fermentation using *Aspergillus niger* on some mineral and anti-nutritional constituents of *Chrysophyllum albidum* seed meal was carried out. Levels of the some minerals and anti-nutritients (like tannin, oxalate, saponin, phytate and cyanide) of the raw and fermented seed meal were determined. The percentage nutritional concentrations (%) in the raw seed were: tannin (6.23 ± 0.01), oxaloacetate (48.39 ± 0.14), saponin (1.58 ± 0.01), phytate (0.76 ± 0.03) and cyanide (1.07 ± 0.32). While level of mineral element concentrations (%) of significantly ($p<0.05$) high values in the raw seed where: sodium (74.13 ± 0.07), potassium (61.15 ± 1.25), phosphorus (40.76 ± 0.28) and iron (43.10 ± 0.35ppm). Fermentation however, significantly reduced levels of the anti-nutrients (like phytate, oxalate, tannin, saponin and cyanide) and minerals (like phosphorus, sodium, copper and zinc), but increased significantly, levels of minerals (like calcium, potassium, magnesium and iron) in the *C. albidum* seed meal. Hence the fermentation significantly ($p<0.05$) improved the nutritional quality of *C. albidum* seed meal.

Key words: Fermentation, *Aspergillus niger, Chrysophyllum albidum* seeds, minerals, anti-nutrients.

INTRODUCTION

Chrysophyllum albidum (G. Don) or African star apple belongs to the family Sapotaceae. The African star apple fruit is a large berry containing 4 to 5 flattened seeds or sometimes fewer due to seed abortion (Keay, 1989). The plant has in recent times become a crop of commercial value in Nigeria. The fleshy pulp of the fruits is eaten especially as snack and relished by both young and old (Cenrad, 1999). It is reported to be an excellent source of vitamins, iron, flavours to diets and raw materials to some manufacturing industries. Nevertheless, while the fleshy pulp is eaten, the seeds are thrown away (Okafor and Fernandes, 1987; Bada, 1997; Umelo, 1997; Adisa, 2000). Seeds are prominent features in the peasant's diet, especially in the developing countries and oilseeds are becoming valuable sources of nutrients for man, especially in countries where the diet is plant-based (Agbede, 2000). Ignorance of their food value has resulted in their wastage in terms of economic returns and (or) post harvest losses (Akubugwo and Ugbogu, 2007). The presence of anti-nutrients is equally a major problem facing the proper utilization of the nutrients in plant seeds. Insoluble oxalate, phytate and tannins (particularly the condensed tannins) have been reported to be heat stable (Aderibigbe et al., 1997; Osagie, 1998; Reddy and Sathe, 2002) and are therefore not eliminated by heat during processing. Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins and various other organic compounds including amino acids and alkaloids.

The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine (Reed, 1995;

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Giner-Chaver, 1996; McGee, 2004). Generally if ingested in excessive quantities, tannins inhibit the absorption of minerals such as iron which may, if prolonged, lead to anemia (Brune et al., 1989). This is because tannins are metal ion chelators, and tannin-chelated metal ions are not bioavailable. Many plants employ tannins to deter animals. Animals that consume excessive amounts of these plants fall ill or die. The lethal dose is said to be around 6% of the animal's body weight. Humans would usually find the bitter taste of foods containing high amounts of tannins unpalatable. Phytate is the principal storage form of phosphorus in many plant tissues, especially bran and seeds (Klopfenstein et al., 2002). Phosphorus in phytate form is, in general, not bioavailable to non-ruminant animals because they lack the digestive enzyme phytase, which is required to separate phosphorus from the phytate molecule. Phytate in plants are usually chelated with cations, protein and (or) starches and this chelated form called phytin constitutes between 1 and 3% by weight of many of the cereals and oilseeds used in animal feeds (Cheryan, 1980).

It also acts as an acid, chelating the vitamin niacin, which is basic, causing the condition known as pellagra (Anderson, 2005). In this way, it is an anti-nutrient, despite its possible therapeutic effects. For people with a particularly low intake of essential minerals, especially young children and those in developing countries, this effect can be undesirable (Anderson, 2005). These antinutrients have been reported to cause several deleterious effects some of which are protein indigestibility, chelation of inorganic mineral elements and even death (Liener, 1989; Osagie, 1998; Muhammad and Oloyede, 2004). Adequate processing techniques (physical, chemical and biological) could be used to reduce or eliminate some of these antinutritional factors. One of the processing methods that have been shown to significantly reduce or eliminate the level of anti-nutrients and also improve the nutritional quality of plant materials is solid fermentation using fungi (Bressani, 1973; Gloria et al., 1995; Jacqueline et al., 1996; Obiozoba, 1998; Muhammad et al., 2000; Muhammad, 2007; Adeyemi, 2008). Fermentation enhances the quantity, quality and bioavailability of nutrients, mineral elements, vitamins, essential amino acids and protein, by improving protein and fibre digestibility. Fermentation gradually changes the characteristics of the food by the action of enzymes, produced by some bacteria, moulds and yeasts (Bray et al., 1999; Adeyemi, 2008*).*

There are a number of roles that micro-organisms play in food processing, which can be either positive or negative. The negative effects include spoilage of food products and contamination by pathogenic micro-organisms (Bray, 1999). The positive effects are generally regarded as part of the fermentation process namely product preservation, flavour development and reduction of antinutrients (Eka, 1980; Campbell-Platt, 1987; Deacon, 2005). *C. albidum* seed, though a lesser known plant seed, is readily available in Nigeria and inexpensive when compared to other plant crops like soybean. It is reported to be rich in protein and may serve as a good substitute for soybean (which is expensive) as a protein source in animal feed. To this day however, there has not been to our knowledge information on the mineral element of fermented *C. albidum* seed. In view of the above highlighted problems therefore, this study was aimed at investigating the effect of fermentation by *Aspergillus niger* on some anti-nutrients and the mineral elements present in both the raw and fermented *C. albidum* seed meal.

MATERIALS AND METHODS

Five hundred healthy fruits of *C. albidum* of the same variety were collected from 'Odo Oba' in Oyo state, Nigeria 'Ipata' and 'Oja Oba' markets in Ilorin, Kwara State, Nigeria. *C. albidum* seeds were obtained by carefully slicing open the fruit pulp and then sun dried. They were dehulled (seed coat removed), oven dried at 60°C for 72 h and ground to powder using a local grinder.

Fermentation of *C. albidum* seed meal

Dried milled *C. albidum* seed meal (that is, the substrate) was autoclaved at the 121°C and 15 psi for 30 min. It was thereafter inoculated with the cultured fungus, *A. niger.* After this, the sterilized substrate was transferred and spread out on a tier of crate to a depth of 5 cm to give room for application of an inoculum. Inside the crate, the sterilized *C. albidum* seed meal was watered at the rate of 2 ml/100 g of sample (Belewu and Adeniyi, 2001). The spores of *A. niger* were harvested using a cork borer (this has a diameter of 13 cm), which was initially sterilized with methanol and subsequently passed over flames before it was used to pick 8 spots of the colonies of the fungi. Two of such picks were put into 100 ml sterile distilled water and shaken vigorously to release the spores of the fungi from the agar medium into the water. The spore suspension (5 ml) containing 2.21 x 10^4 of (*A. niger*) per ml was used to inoculate 25 g of the already autoclaved *C. albidum* seed meal. The inoculum was well mixed with the *C. albidum* seed meal and each tier properly labelled was covered round with a dark polythene sheet to provide the necessary darkness for the fermentation exercise (Belewu et al., 2002). The spent substrate was oven dried at 60°C for 48 h (Belewu et al., 2002). Portions of the dried fermented sample were ground into very fine particles using the moulinex AW9 optimum blend 2000, manufactured in France blender, then tightly packed for further analysis.

Proximate analysis

Proximate composition of the raw and fermented *C. albidum* seed meal as well as all formulated diets that is, soybean meal and *C. albidum* seed meal based diets), were carried out as follows.

Determination of moisture content

The method described by Pearson (1976) was used. Moisture content was determined as the loss in weight due to evaporation from sample at a temperature of 105°C. This method measures free water, to a lesser extent, the bound water. A clean petridish was

dried in an oven at 105°C for 30 min. It was allowed to cool in a desiccator and weighed (W_1). The test sample was measured into the empty, clean petridish and weighed (W_2). The sample was dried in an oven at 105°C for 30 min and thereafter transferred into a desiccator to cool and weighed again (W_3). The weight loss in each case represented the amount of the moisture present in the sample. The percentage moisture was computed as follows:

$$\text{Percentage moisture} = \frac{\text{loss in weight due to drying}}{\text{Weight of original sample}} \times 100$$

$$= \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Determination of crude protein

This was determined by AOAC (1980) method. Protein is the major compound containing nitrogen (amino acids, purines, ammonium salts, etc), which is an indicator of the protein content termed "crude protein" as distinct from true protein (Oyeleke, 1984; Jacobs, 1999). The nitrogen content of protein varies from 15 to 18%. An average of 16% nitrogen was assumed to be present, hence the nitrogen content was multiplied by 6.25 (conversion factor) to obtain the percentage protein. The procedure was carried out in four stages.

Digestion

2 g of the sample was carefully measured into a digestion flask, 0.5 g of copper sulphate pentahydrate, 5 g $NaSO_4$ anhydrous, a speck of selenium and 25 ml Conc. sulphuric acid was added to the flask. The mixture was slowly heated for 2 h using a bunsen burner, until sample frothed. When frothing subsided, the solution changed from black to brilliant green coloration (expectedly), indicating a complete digestion. The digest was diluted to 250 ml with distilled water in a volumetric flask after cooling.

$$R\text{-}NH_2 + H_2SO4 \xrightarrow[\text{(Catalyst)}]{\text{Selenium}} (NH_4)_2SO_4 + CO_2 + H_2O$$

Distillation

5 ml aliquot of the digest was pipetted and delivered into the Markam distillation apparatus. 10 ml of 60% NaOH was added and distilled with 5 ml 2% boric acid in the receiving flask to trap the liberated ammonia. The distillate was collected in a 50 ml flask as represented by the equation:

$$(NH_4)_2SO_4 + 2\ NaOH \longrightarrow Na_2SO_4 + 2NH_3 + 2H_2O$$

Absorption

The liberated ammonia was trapped by boric acid to form ammonium borate.

$$3NH_3 + H_3BO_3 \longrightarrow (NH_4)_3\ BO_3$$

Titration

The distillate obtained was titrated against 0.01M hydrochloric acid (HCl) and the titre value recorded. The reaction is represented by the equation:

$$(NH_4)_3BO_3 + 3\ HCl \longrightarrow 3NH_4Cl + H_3BO_3$$

Calculation

1000 ml of 1MHCl = 14 g Nitrogen, 1ml 0.01MHCl = 0.00014 g N_2. 5 ml of digest contains (0.00014 x 5) g of N_2. If the titre value is Vml, then 250 ml of the digest will contain:

$$\frac{(1.4 \times 10^{-4} \times 250 \times V \times 1)g}{5} \times \frac{N_2}{w};$$

Wg of sample will contain

$$\frac{(1.4 \times 10^{-4} \times 250\ v \times 1)g}{5} \times \frac{N_2}{w}$$

$$\% \text{ protein} = \frac{1.4 \times 10^{-4} \times 250}{5}\ v \times 100 \times \frac{6.25}{w}$$

(6.25 = crude protein factor)

Determination of lipid content

The method described by AOAC (1980) was used. Non polar components of the sample are easily extracted into organic solvent (petroleum ether in this case). Direct extraction gives the proportion of free fat but gives no clue to the particular fatty acids involved.

Procedure

A known weight of the dried sample (2 g) was placed into a fat-free extractor thimble that had been previously dried in an oven and weighed (W_1). It was plugged with cotton wool and the weight was noted as W_2. The thimble was then placed in the extractor and fixed with a reflux condenser and a previously weighed round bottom flask (W_3). The flask was half-filled with petroleum ether. This was heated at 50 to 60°C and allowed to reflux for 6 h. The thimble was thereafter weighed after removal from the extractor and the solvent evaporated; the gain in weight of the round bottom flask after extraction was due to lipid deposited in it (W_4).

Calculation

$$\text{Lipid (\%) (w/w)} = \frac{W_4 - W_3}{\text{Weight of sample}} \times 100$$

Determination of crude fibre content

The crude fibre content was carried out using the method described by AOAC (1980). The bulk of roughage in food is referred to as the fibre and is estimated as the crude fibre. Milled sample was dried and then defatted with ethanol-acetone (fat-off). Protein determination was carried out on defatted sample (protein–off). The remaining sample was extracted using acid and alkali (carbohydrate off), after which the sample was ashed (ash-off). The remnant of the original sample was referred to as the crude fibre and not 'true' fibre.

Procedure

2 g of sample was defatted using petroleum ether for 8 h. The defatted sample was boiled under reflux for 30 min with 200 ml (1.25%) H_2SO_4. It was further filtered and washed with boiling water until the washing was no longer acidic. The residue was boiled in a

round bottom flask with 200 ml (1.25%) NaOH for another 30 min filtered and washed with boiling water until the washing was no longer alkaline. The residue was scraped into a previously weighed crucible (W_1) and dried at 100°C. It was left in a desiccators to cool and weighed (W_2). It was thereafter incinerated in a muffle furnace at about 600°C for 3 h, left in a desiccator to cool and then weighed (W_3).

Calculation

$$\text{Weight of fibre} = (W_2 - W_3)\ \text{g}$$

$$\%\ \text{Fibre} = \frac{W_2 - W_3}{\text{Weight of original sample}} \times 100$$

Determination of ash and organic content

This was determined according to the method described by Pearson (1976). The ash of a biological material is an analytical term for the inorganic residue that remains after the organic matter has been burnt off (Oyeleke, 1984). The ash is not usually the same as the inorganic matter present in the original food since there may be losses due to volatilization or chemical interaction between the constituents. The value is for assessing the quality or grading certain edible materials. The importance of ash content is that it gives an idea of the amount of mineral element present in the sample while the organic matter gives an estimate of proteins, lipids, carbohydrates plus nucleic acid in the sample. A dried porcelain crucible that had been heated in a muffle furnace for 1 min was transferred to a desiccator to cool and weighed to a constant weight (W_1). A known weight of the sample (2 g) was placed into the crucible and weighed (W_2). The crucible with sample was gently heated on the bunsen flame until smoke ceased, and then transferred into a muffle furnace where it was burnt at 600°C to white ashes. The crucible and its contents were then removed and placed in a desiccator to cool after which it was weighed to a constant weight (W_3).

Calculation

$$\text{Ash}\ (\%) = \frac{\text{Weight of ash}\ (W_2 - W_3)}{\text{Weight of sample}\ (W_2 - W_1)} \times 100$$

Carbohydrate estimation

Carbohydrates represent a major fraction of the typical diet of developing countries. When the total of protein and lipid content are subtracted from the organic matter, the remainder will be accounted for by the carbohydrate and nucleic acid levels.

Calculation

Carbohydrate = Organic matter (%) – {Protein (%) + Lipid (%)}. This was referred to as estimation of carbohydrate by difference method (Jacob, 1956).

Determination of antinutrients

Tannin content

The method described by Joslyn (1970) was used. 2 g of the sample was poured into a beaker containing 50 ml distilled water and heated to 60°C. It was thereafter filtered and the residue discarded. 10 ml of 4% copper acetate solution was added to the hot filtrate and boiled again for 10 min.

The precipitate was filtered and the filtrate was discarded. The residue was dried using filter paper and the dried sample scraped from filter paper into a pre-weighed crucible. The weight was recorded as W.

The crucible (which contained the sample) was incinerated in a muffle furnace at 550°C, cooled in a dessicator and then re-weighed as W_1. The difference between the weight of sample before ashing and the ash residue after incineration represents the tannin content.

Phytate

The Wheeler and Ferrel (1971) method was used to determine the phytate content. This method relies on the solubilization of phytate by dilute acid and the subsequent precipitation of the phytate with ferric ion ($Fe^{3\pm}$). 4 g of the sample was soaked in 100 ml of 2% HCl for 3 h, and then filtered. 25 ml filtrate was dispensed into a conical flask and 5 ml of 0.3 ml ammonium thiocyanate solution was added as indicator.

Thereafter, 53.5 ml distilled water was added to the mixture to give it a proper acidity and this was titrated with standard iron III chloride solution, which contains about 0.00195 g of iron per millilitre (ml), until a brownish-yellow colour persisted for 5 min.

Oxalate

This was determined using the modified method employed by Iwuoha and Kalu (1995). The principle involves the digestion of sample containing oxalate and the precipitation of the oxalate to remove ferrous ions on addition of NH_4OH solution.

Digestion

2 g of the milled sample was suspended in 190 ml of distilled water contained in a 250 ml volumetric flask and boiled for 1 h. 10 ml of 6 M HCl was added and the suspension digested at 100°C. It was thereafter cooled, made up to 250 ml with distilled water, and then filtered.

Oxalate precipitation

Duplicate portions of 125 ml of the filtrate sample was measured into a beaker and four drops of methyl red indicator was added, followed by concentrated NH_4 OH solution (in drops) until the test solution changed from its salmon pink colour to a faint yellow colour.

Each portion was heated to 90°C and 10 ml of 5% calcium chloride solution was added while being stirred constantly. The solution was cooled and left overnight at 5°C. It was then centrifuged at a speed of 2500 rev/minute for 5 min. The supernatant was decanted and the precipitate completely dissolved in 20 ml of 20% v/v H_2SO_4 solution.

Permanganate titration

The total filtrate resulting from digestion and oxalate precipitation was dissolved in 20 ml of 20% v/v H_2SO_4 solution and was titrated against 0.05 M $KMnO_4$ solution to a faint pink colour, which persisted for 30 s.

Calculation

$$\text{Oxalate Content} = \frac{T \times (Vme)\ (DF)\ x10\ (mg/100g)}{ME \times M_f}$$

Where; T = Titre value of $KMnO_4$, DF = Dilution factor; V_T/A; Vme = Volume-mass equivalent (that is, 1 ml of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid). A = Aliquot used (125 cm^3), ME = Molar equivalent of $KMnO_4$; M_f = Mass of sample used, V_T = Total volume of filtrate (300 ml).

Saponin

The saponin content was determined using the modified method of Hudson and El-Difrawi (1979). Saponin was extracted with a polar solvent after removal of lipids with petroleum ether. Saponin was isolated into the polar solvent. 100 g of the milled defatted sample was added into 200 ml of 20% aqueous ethanol in a conical flask and corked to prevent evaporation. The duplicate portions of the extracts were combined and put in a 250 ml separating funnel after the collection of the solvent, 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether discarded. The process of purification was continued until a colourless aqueous solution was at pH 4.5. Thereafter NaCl, 60 and 30 ml portions of n-butanol were added to the solution and shaken vigorously following each addition. The combined butanol extract was washed using 5% aqueous NaCl and evaporated to dryness to give crude saponin, which was weighed (Saponin content = weight of sample before extraction – loss in weight after extraction).

Cyanide content determination

The AOAC (1990) method was used. 40 ml distilled water was added to release bound hydrocyanic acid after which it was distilled and titrated against $AgNO_3$ solution. 4 g of the sample was soaked in a mixture containing 40 ml distilled water and 2 ml of orthophosphoric acid. The mixture was stirred, stopped and left overnight at room temperature to set free all bounded hydrocyanic acid. The resulting sample was transferred to a distillation flask and a drop of paraffin added (as an anti-foaming agent) together with broken chips (as anti-bumps). The distillation flask was fitted into another distillation apparatus before distillation. About 5 ml of distillate was collected in the receiving flask containing 40 ml of distilled water and 0.1g NaOH pellets. The distillate was then transferred into 25 ml volumetric flask and made up to mark with distilled water. 20 ml aliquot of the distillate was measured into a conical flask and 1.6 ml 0f 5% potassium iodide solution was added to the flask. The resulting mixture was titrated against 0.01M $AgNO_3$ until the end point was indicated by a faint but permanent turbidity. The blank was also prepared using distilled water instead of the distillate.

Calculation

$$\text{Cyanide (mg/kg)} = \frac{13.5\ (V_1 - V_0)}{M}$$

Where; V_1 = Titre value for sample, V_0 = Titre value for blank, M = Mass of sample.

Mineral content determination

The mineral analysis of *C. albidum* was carried out according to the process described by AOAC (1980). The mineral content of a food sample is what is left after the sample has been ashed. A dry porcelain crucible that had been heated in a muffle furnace for 1 min was transferred to a desiccator to cool and weighed. A known weight of the dried, milled sample (2 g) was placed into the crucible and weighed, transferred to a muffle furnace and burnt for 3 h at 600°C to white ashes. The crucible was removed and placed in a desiccator to cool and weighed to a constant weight. Extraction of ash was done by half filling the crucible with 3 ml of 2 M HCl and gently warmed (50 to 60°C) for 3 min. The suspension obtained was transferred into a 50 ml beaker using a Pasteur pipette. The procedure was repeated four times and thereafter 15 ml hot distilled water was used. The content of the beaker was then transferred into a volumetric flask and the resulting precipitate was washed (using distilled water), into the filtrate. After which the solution was made up to the 50 ml mark with distilled water. Analysis for mineral content in the solution was carried out using the Atomic Absorption Spectrophotometer (AAS) model 420: Perkin-Elmer, Norwalk CT.

Statistical analysis

To test the level of significance, data was expressed as mean ± standard error of the mean and was subjected to analysis of variance (ANOVA). Significant differences between treatment means were determined at 5% level using the Duncan Multiple Range Test (Duncan, 1955).

RESULTS

The effect of 3 weeks of fermentation, using *A. niger* on the proximate composition *C. albidum* seed meal is shown in Table 1. Fermentation was observed to have significantly increased ($p < 0.05$) the levels of the crude protein, crude fibre and the ash content of the samples and significantly reduced ($p < 0.05$) the levels of the ether extract. The effect of fermentation, using *A. niger* on the levels some antinutritional factors in *C. albidum* seed meal is shown in Table 2. Findings showed significant reductions ($p < 0.05$) in the phytate, tannin, cyanide and oxalate contents of the treated sample. The reduction was progressive throughout the 3 week fermentation period. The effect of fermentation with *A. niger* for 3 weeks on the level of inorganic mineral elements of *C. albidum* seed meal is shown in Table 3. Findings show that with the exception of potassium, magnesium and iron which were significantly increased ($p < 0.05$) fermentation was observed to have significantly reduced ($p > 0.05$) all the micro-nutrients in the *C. albidum* seed meal throughout the fermentation period.

DISCUSSION

Findings show a significant increase ($p < 0.05$) in the crude protein content of the *A. niger* fermented *C. albidum* seed meal (Table 1), this is an indication that the fungus synthesizes and adds fungal protein to the substrate. Fungi have been reported to synthesize fungal proteins and subsequently add these to the protein content of the substrate (Jacqueline et al., 1996;

Table 1. Proximate composition of *C. albidum* seed meal.

Parameter (%)	**RCA**	**FCAW 1**	**FCAW 2**	**FCAW 3**
Crude protein	13.70 ± 0.06^{d}	13.95 ± 0.27^{c}	15.07± 0.24^{b}	15.15 ± 1.25^{a}
Ether extract	7.25 ± 0.072^{a}	5.88 ± 0.25^{b}	5.58 ± 0.46^{d}	5.86 ± 0.04^{c}
Crude fibre	1.55 ± 0.01^{d}	2.81 ± 0.27^{c}	4.00 ± 0.35^{b}	4.18 ± 0.45^{a}
Ash content	6.38 ± 0.07^{d}	8.03 ± 0.25^{c}	8.55 ± 0.35^{b}	8.93 ± 0.45^{a}
Nitrogen free extract	71.12 ± 0.35^{a}	69.33 ± 0.41^{b}	66.80 ± 0.39^{c}	65.88± 0.30^{d}

Values are means of three determinations ± SEM. Values along the same row with different superscripts are significantly different ($P < 0.05$). Where; RCA = Raw *C. albidum* sample, FCAW1 = Fermented *C. albidum* seed meal week 1. FCAW2 = Fermented *C. albidum* seed meal week 2, FCAW3 = Fermented *C. albidum* seed meal week 3. To test the level of significance, data was expressed as mean ± standard error of the mean and was subjected to analysis of variance (ANOVA). Significant differences between treatment means were determined at 5% level using the Duncan Multiple Range Test (Duncan, 1955).

Table 2. Levels of antinutrients (mg/g dry weight) in *C. albidum* seed meal fermented for 3 weeks.

Antinutrient	**RCA**	**FCAW1**	**FCAW2**	**FCAW3**
Tannin	6.23 ± 0.01^{a}	3.20 ± 0.14^{b}	2.15 ± 0.07^{c}	1.09 ± 0.01^{d}
Oxalate	48.39 ± 0.14^{a}	32.17 ± 0.01^{b}	26.03 ± 0.02^{c}	21.75 ± 0.04^{d}
Saponin	1.58 ± 0.01^{a}	1.13 ± 0.10^{b}	0.97 ± 0.03^{c}	0.92 ± 0.10^{d}
Cyanide	1.07^{a} ± 0.32^{a}	0.23 ± 0.06^{b}	0.21 ± 0.06^{b}	0.07 ± 0.02^{c}
Phytate	0.76 ± 0.03^{a}	0.33 ± 0.01^{b}	0.27 ± 0.02^{c}	0.15 ± 0.03^{d}

Values are means of three determinations ± SEM. Values along the same row with different superscripts are significantly different ($P < 0.05$). Where; RCA = Raw *C. albidum* sample, FCAW1 = Fermented *C. albidum* seed meal week 1. FCAW2 = Fermented *C. albidum* seed meal week 2, FCAW3 = Fermented *C. albidum* seed meal week 3. To test the level of significance, data was expressed as mean ± standard error of the mean and was subjected to analysis of variance (ANOVA). Significant differences between treatment means were determined at 5% level using the Duncan Multiple Range Test (Duncan, 1955).

Table 3. Mineral composition of *C. albidum* seed meal fermented for 3 weeks.

Mineral	**RCA**	**FCAW 1**	**FCAW 2**	**FCAW 3**
Phosphorus (%)	40.76± 0.28^{a}	16.99± 0.14^{d}	33.24± 0.46^{b}	25.75± 0.30^{c}
Potassium (%)	61.15± 1.25^{d}	102.50± 0.15^{b}	107.60± 0.05^{a}	87.71± 0.35^{c}
Calcium (%)	11.93± 0.46^{d}	21.20± 0.01^{c}	21.80± 0.11^{b}	22.57± 1.25^{a}
Sodium (%)	74.13± 0.07^{b}	74.78± 0.01^{a}	63.21± 0.35^{c}	60.22± 0.45^{d}
Magnesium (%)	10.80± 2.14^{d}	11.10± 0.41^{c}	11.50± 0.24^{b}	11.50± 0.24^{a}
Copper (ppm)	0.17±0.06^{a}	0.09± 0.01^{b}	0.09± 0.01^{b}	0.03±0.01^{c}
Iron (ppm)	43.10± 0.35^{d}	67.90± 0.10^{c}	93.20± 0.39^{a}	81.70± 0.30^{b}
Zinc (ppm)	20.61± 0.08^{a}	16.62± 0.46^{b}	10.31± 0.24^{c}	9.75±1.25^{d}

Values are means of three determinations ± SEM. Values along the same row with different superscripts are significantly different ($P < 0.05$). Where; RCA = Raw *C. albidum* sample, FCAW1 = Fermented *C. albidum* seed meal week 1. FCAW2 = Fermented *C. albidum* seed meal week 2, FCAW3 = Fermented *C. albidum* seed meal week 3. To test the level of significance, data was expressed as mean ± standard error of the mean and was subjected to analysis of variance (ANOVA). Significant differences between treatment means were determined at 5% level using the Duncan Multiple Range Test (Duncan, 1955).

Muhammad et al., 2000). Thus *A. niger* fermented *C. albidum* seed may be a good source of protein in livestock feed. The significant reduction ($p < 0.05$) in the lipid content of the fermented *C. albidum* seed meal (Table 1), however, may be an indication that the fungus utilizes the lipid as a source of carbon and energy for growth and perhaps for the synthesis of protein. The reduction in the lipid content of the fermented sample would make it a favourable ingredient in animal feed. This is because it has been reported that high fat content in feed ingredients would cause difficulty in mixing the feed and also require antioxidants for preventing the feed from oxidative rancidity (Ewing, 1951; Muhammad et al., 2000).

The significant increase in the crude fibre content of *A. niger* fermented *C. albidum* seed meal falls within of 2.00 and 4.18% (Table 1). This is considered satisfactory since crude fibre content of not more than 7% is recommended in animal feed (NRC, 2007). As higher crude fibre is poorly digested by animals and interferes with other nutrients, thus making them unavailable for the use of the animal (Ewing, 1951; NRC, 2007).

The observed increase in the ash level of *A. niger* fermented *C. albidum* seed meal (Table 1) indicates an increase in the inorganic mineral elements of the sample. This confirmed the report of Oyeleke (1984) that the ash content of a sample indicates the inorganic element in the sample. Thus the significant increase in the ash content of the fermented *C. albidum* seed meal may be an indication of the increment in the inorganic element of the fermented sample. It is also possible that there was break down of some organic molecules within the fermented sample and consequently the release of the mineral elements from the organic phase into the inorganic phase. Therefore the present results showing a significant increase in the ash content of the fermented *C. albidum* seed meal may be an indication of a significant increase in the levels of the minerals of the fermented seed meal. This is in agreement with previous reports by Obizoba and Atti (1994) and Obizoba (1998). Fermentation of *C. albidum* seed meal using *A. niger* is permissible since, *A. niger* is well-known to produce enzymes some of which are pectinases, carbohydrases, phytases, amylase, amyloglucosidase, invertase, lactase and acid proteases (Bennett, 1985; Ward, 1989; Sani et al., 1992; Wyss et al., 1998), which play major roles in flavour development, increase of product preservation and reduction of antinutrients (Eka, 1980; Campbell-Platt, 1987; Deacon, 2005). Findings from this study showed that the dominating antinutrients in *C. albidum* are oxalate and tannin. Hence the significant reduction in all antinutrients in the *A. niger* fermented *C. albidum* sample (Table 2) may be an indication that *A. niger* had degraded the antinutritional factors or the complex formed by them.

It is possible that these enzymes, in part are responsible for the degradation or possible usage of these nutrients as carbon and nitrogen sources for the production of energy and synthesis of proteins for the growth of the organism, hence reduction of antinutrients present in the *A. niger* fermented *C. albidum* sample (Table 2). Tannins are known to undergo hydrolysis by acids, bases or some hydrolytic enzymes. Therefore, the hydrolytic enzymes produced by *A. niger* might be responsible for degradation of the tannin content (especially pectin which could be degraded by pectinases) of the fermented sample. Tannins have been shown to precipitate proteins (Bate-Smith and Swain, 1962; Hemingway and Karchsy, 1989; Reed, 1995), which inhibits in some ruminant animals the absorption of nutrients from high-tannin grains such as sorghum. Tannins interfere with iron absorption through a complex formation with iron when it is in the gastrointestinal lumen which decreases the bioavailability of iron (Brune et al., 1989). In sensitive individuals, a large intake of tannins may cause bowel irritation, kidney irritation, liver damage, irritation of the stomach and gastrointestinal pain. A correlation has been made between esophogeal or nasal cancer in humans and regular consumption of certain herbs with high tannin concentrations (Elvin-Lewis et al., 1977). Hence, reduction in the level of tannins by the fermentation process is in agreement with earlier observations (Eka, 1980; Achinehwu, 1998; Obiazoba, 1998).

Phytic acid has a strong binding affinity to important minerals such as calcium, magnesium, iron, and zinc. When a mineral binds to phytic acid, it becomes insoluble, precipitates and will be inabsorpable in the intestines. This process can therefore contribute to mineral deficiencies in people whose diets rely on these foods for their mineral intake, such as those in developing countries (CFPFNBNRC, 1973; Hurrell, 2003). Contrary to that, one study correlated decreased osteoporosis risk with phytic acid consumption (López-González et al., 2008). Phytases are known to degrade phytate. The enzyme hydrolyzes phosphate groups from the phytin molecule potentially making the hydrolyzed phosphorus from within the phytin available to the animals (Applegate et al., 2003). *A. niger* had been reported to produce phytases (Wyss et al., 1998). Hence, the fungus used for the fermentation might have produced phytases causing the degradation of the phytate content of the fermented sample. Consequently, the treatment of the sample with *A. niger* would help in liberating phosphorus and other divalent ions from the complex and as a result make it available to the animals. This view is in consonant with the reported findings of Eka (1980); Achinehwu (1998) and Obiazoba (1998). Cyanide is a harmful ion that is hazardous to life. It forms a very stable complex with the active site of metals (for example, Fe and Mg) in enzymes, thereby inhibiting vital functions in cells such as respiration (ATSDR, 1997, 2004).

It is possible that the enzymes amyloglucosidase or one of the carbohydrases produced by *A. niger* degraded the cyanide containing compound to bring about the significant reduction of cyanide in the fermented feed. Given that very small quantities of cyanide may be harmless, as the cyanide (CN) radical is converted to the relatively non-toxic thiocyannate radical SCN and excreted as salts of this radical (West et al., 1966), thus the action on the cyanide content of the *A. niger* fermented *C. albidum* seed meal would render it non-toxic to the animal. The reduction in the level of antinutrients by the fermentation process is in agreement with earlier observations (Eka, 1980; Achinehwu, 1998; Obiazoba, 1998). Phytate, tannin and oxalate are known to chelate mineral elements especially the divalent ions of iron, calcium, zinc, manganese, magnesium and

potassium (Nwokolo and Bragg, 1977; Butler, 1992; Reed, 1995; Webb, 1999; Adeyemi et al., 2008) thereby rendering them unavailable in a biological system. The significant increase in the minerals observed (Table 3) would probably mean that fermentation with the fungus caused the breakdown of these antinutrients or the complexes they form with these ions led to the significant increases in their concentrations. Processing of these food materials may then be necessary if the minerals are to be made available for usage. Sodium and potassium appeared to be the major macro-elements, while iron appeared to be the major micro-element in the sample. The significant increase in the mineral elements; potassium, calcium, magnesium and iron of the *A. niger* fermented *C. albidum* sample (Table 3) may have been as a result of increase in the ash content of the fermented seed (Adeyemi, 2008). It may be adduced to the degradation of antinutritional factors like phytate and tannin (Table 3). Hence fermentation process significantly ($p < 0.05$) improved the nutritional quality of *C. albidum* seed meal.

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