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Proximate, Mineral and Antinutritional Composition of Fermented Slimy Kolanut (*Cola verticillata*) Husk and White Shell

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Authors' contributions

This work was jointly carried out by the authors using laboratory facilities within the Federal University of Technology, Akure, Nigeria. The authors jointly wrote the first draft of the manuscript and managed literature searches. They have both read and approved the final manuscript.

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Short Communication

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ABSTRACT

This study evaluates the proximate, mineral and antinutritional composition of slimy kolanut husk and white shell also known as obi olooyo in Yoruba language, a tribe in Nigeria. The samples were subjected to liquid and solid state fermentation and were dried, milled, sieved and analysed. Unfermented samples served as the control. The analyses revealed the presence of protein, fiber, ash, fat, moisture and carbohydrate, with observed reduction in fiber content for proximate analyses. Magnesium, potassium and calcium measured in percentage were observed to be more prominent among the minerals analysed for. Other minerals determined were sodium, phosphorus, iron and zinc. Antinutritional analyses showed the presence of phytate, oxalate, tannin, alkaloid, flavonoid and cyanide. There was an increase and decrease in the nutritional and antinutritional composition of the samples respectively, when compared with the control samples. The study has shown the samples to be useful in the fortification of animal feeds.

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Keywords: Slimy kolanut; fermentation; proximate; mineral; antinutritional quality.

1. INTRODUCTION

Kolanut seeds has found usefulness in some industries for the production of commodities such as drugs, wines beverages, liquid soap and formulation of animal feed just to mention a few [1]. The husk and white shell on the other hand has been regarded as waste until recently, when its nutritive quality was reported [2,3]. Kolanut husk was analysed for its nutritive value by Hamzat and Adeola for the formulation of poultry feeds [4]. The waste was nutritive but concluded that it's not suitable for poultry feed due to its high fiber content. A study carried out by Arotupin et al. on microorganisms, proximate and antinutrient content of *Cola nitida* white shell also indicated the nutritive value of kolanut white shell, showing that these agro-wastes can find usefulness [5].

In the agricultural sector, tonnes of waste are generated yearly and they are ignored causing nuisance to the environment resulting to environmental pollution and aesthetic nuisance [6]. These wastes pose a disposal problem and will even be more problematic with increase in industrial production without converting the waste to useful forms. Limitations to the use of crop waste include; low digestibility, low protein content and excessive crude fiber [7]. Fermentation has been identified as a technique that is inexpensive for the detoxification and increase in protein quality of some of such products [6]. Classification of is based mainly on the type of substrate been used. The bioactive compounds have been found to be produced more in the solid state fermentation while some others are produced more in the liquid state fermentation [8].

The objective of this study is to analyse for the nutritive values present in husk and white shell of slimy kolanut.Liquid and solid state fermentation were employed to increase the nutritive value and to reduce the limitation of excessive crude fiber, low digestibility and low protein attributed to crop residue or waste. The analyses were carried out to provide alternative for materials used in the production or fortification of animal feeds.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Kola nut was obtained from a farm in Ilogbo Ekiti, Ekiti state and transported to the laboratory for analysis. The kolanut was rinsed to get rid of sand particles after which the nut was removed from the pods using sterile knife. The white shell was removed from the kola seed by soaking in water for 24 hours. The samples were then fermented for ten days, after which they were dried, grounded, sieved and analysed.

2.2 Determination of Proximate Composition of the Samples

Proximate composition which include; moisture, crude fibre, ash, crude protein and carbohydrates were determined by the methods of Association of Official Analytical Chemists [9].

2.3 Fermentation Procedure

Five hundred grams of husk and three hundred grams of white shell of the species were weighted into clean bowls with lids labelled appropriately. The samples was subjected to liquid and solid state fermentation for ten days using same measurement of sample. For the liquid state fermentation, one thousand five hundred and one thousand milliliters of distilled water was used to ferment the husk and white shell samples respectively, while water was not added to solid state medium. The solid-liquid ratio is 1 :0, weighing five and three hundred grams of husk and white shell respectively. The samples were just weighed and covered in bowels for ten days. The fermented samples were dried, grounded and sieved for analyses.

2.4 Determination of Mineral Content of Kolanut Husk and White Shell Samples

The mineral composition of samples was determined by wet-ashing method followed by reading of the level of mineral. Triplicate sample of one gram each were weighed into porcelain crucible and placed in muffle furnace. The temperature was raised gradually to 450ºC. The sample was ashed at 550ºC for 5 - 6 hours. After cooling to room temperature, the ash was dissolved in one millilitre (l ml) 0.5% HNO_3. The sample volume was made up to 100ml and the level of mineral present was analyzed by Atomic absorption spectrophotometer Buck 201 VGP [10].

2.5 Antinutrient Determination

2.5.1 Determination of Tannin of kolanut husk and white shell samples

Gravimetric determination of tannin was done according to the method of Makkar et al. [11] Zero-point-two millilitres (0 20 ml) of the kolanut husk and white shell samples were weighed into test tubes and tannin was extracted in ten millilitres (10 ml) 70% acetone. The test tubes were then placed in cold water bath for 10minutes to allow for complete extraction of tannin. Zero-point-two millilitres (0.2 ml) was filtered into test tubes and made up to one millilitre (1 ml) with distilled water. Two-point-five millilitres (2.5 ml) of 20% Na_2CO_3 and zero-point-five millilitres (0.5 ml) with distilled water were added and the content was mixed properly. The solution was incubated for 45 min at room temperature to develop colour (blue colour). The absorbent of each samples were read at wavelength 700 nm using a Coring colorimeter 253, Corning Ltd, Essex, England.

2.5.2 Determination of phytate of kolanut husk and white shell samples

Phytate was determined according to the method of Young and Greaves [12]. Four grams (4g) of the kolanut husk and white shell samples was soaked in one hundred millilitres (100 ml) of 2% HCl for 3 hours and then filtered, twenty-five millilitres (25 ml) of the filtrate was placed in a conical flask. Five millilitres (5 ml) of 0.3% ammonium thiocyannate (NH_4SCN) solution was added as indicator and diluted with distilled water. This was titrated with standard $FeCl_3$ solution until a brownish yellow colour persisted for 5minutes.

2.5.3 Determination of oxalate of kolanut husk and white shell samples

The determination was done according to the method of Day and Underwood [13]. One gram (1 g) of the kolanut husk and white shell samples were placed into labelled plastic bottles followed by the addition of seventy-five millilitres (75 ml) of H_2SO_4. The content was mixed properly and allowed to extract for one hour with constant agitation using a mechanical shaker. This was then filtered and twenty-five millilitres (25 ml) of the filtrate was titrated with zero-point-one millilitres (0.1 ml) of $KMnO_4$ while hot (80–90°C) until a purple colour was observed. The titre value was then multiplied by 0.9004 to give the result expressed as mg/g.

2.5.4 Flavonoid determination of kolanut husk and white shell samples

Ten grams (10 g) of the kolanut husk and white shell samples were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through what man filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight [10].

2.5.5 Alkaloid determination of kolanut husk and white shell samples

Five grams of the kolanut husk and white shell samples were weighed into a 250ml beaker and two hundred millilitres (200 ml) of 10% acetic acid in ethanol was added, covered and allowed to stand for four hours to allow it extract. This was filtered and filtrate was placed in a water bath to allow it concentrate to one quarter of the original volume. To the concentrated samples, ammonium hydroxide was added drop wisely until the precipitation was completed. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed [14].

2.5.6 Determination of cyanide of kolanut husk and white shell samples

Four grams (4 g) of kolanut husk and white shell samples were soaked in a mixture of forty millilitres (40 ml) of distilled water and two millilitres (2 ml) of orthorphosphoric acid. The samples were thoroughly mixed and covered and left overnight at room temperature. Forty-five millilitres of the distillate was collected in a receiving flask containing four millilitres (4 ml) distilled water containing one gram (1 g) of sodium hydroxide pellet. The distillate was transferred into 50ml volumetric flask and made up with distilled water. Twenty millilitres (20 ml) of the distillate was collected and then placed in the conical flask and one-point-six millilitres (1.6 ml) of 5% potassium iodine solution was added to the samples and titrated against $AgNO_3$ [10].

2.5.7 Statistical analysis

Mean and standard errors of the mean of triplicate readings obtained in the study were subjected to statistical analysis using SPSS

version 16 Microsoft windows and descriptive one way analysis of variance (ANOVA).

3. RESULTS

Proximate composition of fermented and unfermented (control) *Cola verticillata* husk and white shell were observed to be significantly different when compared (Table 1). In comparing the values of the fermented and unfermented (control) samples, husk and white shell samples subjected to liquid state fermentation had more nutritive and lesser antinutritive value as compared to those subjected to solid state fermentation.

Table 2 shows mineral analysis carried out on dried, milled and grounded fermented and unfermented (control) husk and white shell samples. Potassium (K %), phosphorus (P %) and magnesium (Mg %) were observed to be higher in fermented husk samples as compared to unfermented (control) husk samples. These same minerals were observed to be higher in the unfermented (control) white shell samples as compared to the fermented. While on the other hand, husk and white shell samples subjected to solid state fermentation had higher calcium (Ca), iron (Fe) and zinc (Zn) content than those subjected to liquid state fermentation.

Reduction was observed inphytate (mg/g), tannin (mg/g) and cyanide (mg/g) content of the husk and white shell samples under both methods of fermentation. An increase was observed in oxalate (mg/g) content of samples fermented when compared with unfermented (control) samples while alkaloid and flavonoid increased in samples subjected to liquid state fermentation as presented in Table 3.

4. DISCUSSION

High moisture content reported in the study could be due to intake of water during fermentation by the process of imbibition [15]. This allows for the growth of microorganisms thus enabling them to make use of nutrients in the husk sample, reducing the nutrient but allows and facilitates movement and exchange of nutrient between cells [16]. This suggests that the period of fermentation should be reduced. Production of extra cellular enzymes by microorganisms could be the resulting effect of high fat content [17] also, fat content of samples subjected to liquid state fermentation is lower probably due to its wash into the infusion. High ash content observed for unfermented samples could be pointing to the fact that the kolanut husk and white shell has a reasonable quantity of mineral elements and also, could be due to metabolic activities of organisms involved in the fermentation process [18].

Fibre content of the fermented samples which was observed to be high could be attributed to the fermentation process. This is because during fermentation, carbohydrate including cellulose, pectin, lignocelluloses and starch are broken down by fermenting microorganisms. The microorganisms use them as their sole carbon source, converting it to microbial biomass thereby reducing the fiber content of the sample [19]. Decrease in fibre content might be attributed to production of various lignocelluloses enzymes by the fermenting microorganisms [20].

Secretion of certain proteineous extra cellular enzymes into fermenting medium, their breakdown and subsequent metabolism might be the reason for increased protein content of the samples. It also could be as a result of hydrolysis of starch into glucose and its use by organisms as carbon source [21]. Structural proteins being an integral part of microbial cells of organisms involved in fermentation could increase protein content of the samples [22].

Reduction of carbohydrate during fermentation as observed in the fermented samples could be as a result of utilization of sugar and its bioconversion. This generates energy for cell metabolism during fermentation, thereby enhancing microbial growth [23,24]. Increase in carbohydrate as recorded in few cases of fermented samples in this study could be an indication that most of the microorganisms involved in the fermentation might have produced metabolites which are carbohydrates [25].

Magnesium which was observed to be the most abundant mineral in the study has been reported to be an activator of many enzyme systems and maintains electrical potential of the nerves [26]. It is of benefit therefore that magnesium is high in the husk and white shell samples. Increase in mineral content of the samples as reported could be due to break down of some plant compound by microorganisms, thereby releasing such minerals and reduction might be due to the microorganism's ability to assimilate (use) such minerals [27].

Table 1. Proximate composition of fermented and unfermented husk and white shell

Samples	Moisture (%)	Fat (%)	Fibre (%)	Ash (%)	Protein (%)	CHO (%)
Husk LF	11.853^{d}±0.035	6.330^{d}±0.100	10.390^{c}±0.096	6.433^{b}±0.099	6.726^{e}±0.200	58.270^{c}±0.095
Husk SF	11.856^{d}±0.076	7.666^{f}±0.140	12.533^{e}±0.059	7.300^{c}±0.090	5.360^{d}±0.130	55.283^{a}±0.129
Husk UF	4.230^{a}±0.060	7.000^{e}±0.981	19.067^{f}±0.059	10.100^{f}±0.092	4.127^{c}±0.055	55.477^{b}±0.891
White shell LF	10.400^{c}±0.089	4.610^{a}±0.020	8.066^{a}±0.061	5.273^{a}±0.200	8.436^{f}±0.125	63.213^{e}±0.186
White shell SF	10.420^{c}±0.050	5.150^{c}±0.140	10.073^{b}±0.078	7.500^{d}±0.120	4.047^{b}±0.042	62.813^{d}±0.081
White shell UF	4.470^{b}±0.110	4.830^{b}±0.020	12.286^{d}±0.115	10.046^{e}±0.050	2.250^{a}±0.090	66.116^{f}±0.040

Note: values followed by similar alphabet along the same column are not significantly different at P<0.05
KEY: LF- liquid state fermentation, SF- solid state fermentation, UF – Unfermented, CHO- carbohydrate

Table 2. Mineral analysis of fermented and unfermented dried kolanut husk and shell and infusion

Samples	Na (%)	K (%)	P (%)	Mg (%)	Ca (%)	Fe (ppm)	Zn (ppm)
Husk LF	0.507^{c}±0.006	7.640^{e}±0.130	0.253^{a}±0.025	9.036^{c}±0.023	4.066^{d}±0.076	0.633^{b}±0.029	1.443^{d}±0.040
Husk SF	0.490^{b}±0.010	9.103^{f}±0.080	0.250^{a}±0.000	9.200^{d}±0.303	5.500^{f}±0.090	1.343^{e}±0.309	1.620^{e}±0.104
Husk UF	0.510^{c}±0.086	5.653^{b}±0.181	0.263^{b}±0.015	11.066^{e}±0.070	5.100 ^{e}±0.390	0.963^{c}±0.075	1.096^{a}±0.080
White shell LF	0.603^{d}±0.049	5.480^{a}±0.227	0.326^{c}±0.035	7.000 ^{a}±0.290	2.100^{a}±0.390	0.430 ^{a}±0.053	1.353^{c}±0.439
White shell SF	0.826^{e}±0.222	6.423^{c}±0.271	0.393^{d}±0.035	7.997 ^{b}±0.045	3.000^{c}±0.272	1.533^{f}±0.542	1.770^{f}±0.080
White shell UF	0.433^{a}±0.189	7.720^{d}±0.220	0.470^{e}±0.030	10.000^{e}±0.280	2.233^{b}±0.899	1.316^{d}±0.445	1.320^{b}±0.270

Note: values followed by similar alphabet along the same column are not significantly different at P<0.05
KEY: LF- liquid state fermentation, SF- solid state fermentation , UF – Not fermented, Na- sodium, K-potassium, P- phosphorus, Mg- magnesium, Ca- calcium, Fe- iron, Zn-zinc, ppm- part per million

Table 3. Antinutritional component of fermented and unfermented dried kolanut husk and shell

Samples	Phytate (mg/g)	Oxalate (mg/g)	Tannin (mg/g)	Alkaloid (mg/g)	Flavonoid (mg/g)	Cyanide(mg/g)
Husk LF	43.13^{c}±0.26	0.70^{c}±0.07	4.17^{b}±0.10	0.07^{b}±0.01	0.07^{b}±0.01	2.19^{a}±0.07
Husk SF	48.41^{d}±0.26	0.55^{a}±0.06	4.26^{c}±0.66	0.02^{a}±0.01	0.02^{a}±0.00	2.24^{a}±0.41
Husk UF	53.36^{e}±0.36	0.52^{a}±0.07	5.46^{e}±0.11	0.03^{a}±0.00	0.03^{a}±0.01	3.26^{b}±0.03
White shell LF	28.17^{a}±0.33	0.70^{c}±0.03	4.26^{c}±0.07	0.09^{b}±0.02	0.09^{b}±0.01	4.34^{c}±0.18
White shell SF	28.31^{a}±0.68	0.72^{c}±0.08	3.31^{a}±0.42	0.04^{a}±0.01	0.05^{a}±0.01	4.34^{c}±0.08
White shell UF	35.16^{b}±0.29	0.61^{b}±0.03	5.31^{d}±0.35	0.07^{b}±0.01	0.08^{b}±0.01	4.44^{c}±0.19

Note: values followed by similar alphabet along the same column are not significantly different at P<0.05
KEY: LF- liquid state fermentation, SF- solid state fermentation , UF – Not fermented (control)

Antinutrients are natural or synthetic compounds that interfere with the absorption of nutrients. One common example is phytic acid which forms insoluble complexes with calcium, zinc, iron and copper [28]. Tannin chelate metals and reduces the absorption of nutrients and also inhibits digestive enzymes and may also precipitate proteins. However, tannins have anticancer properties, so drinks such as green tea that contain large amounts of these compounds might be good for the health of some people despite its antinutritional property [29]. Phytate reduction could be attributed to the action of enzyme released by microorganisms.

The ability of microorganisms to break down tannins has been attributed to the secretion of tannase, an enzyme capable of catalyzing gallotannins to gallic acid and glucose [30].The environment influence the morphology and expression of compounds in plants and those particular compounds may be produced only at certain times or under certain conditions [31,32]. This could be the reason for variations in antinutritional composition of fermented and unfermented samples in the study.

5. CONCLUSION

The effect of fermentation was evident on the samples when compared with the unfermented samples. The samples subjected to liquid state fermentation had more nutritional value than those subjected to solid state fermentation. The husk and white shell of slimy kolanut could substitute some quantity of grains/cereals used for the formulation of animal feed or used to fortify animal feed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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