

Effect of fermentation on the proximate, mineral and antinutritional composition of slimy kola (*Cola verticillata*) husk and white shell.

ABSTRACT

This study evaluates the proximate, mineral and antinutritional composition of slimy kolanut husk and white shell also known as obi olooyo or abidan in Yoruba language. The analysis was carried out on dried, milled and sieved samples subjected to liquid and solid state fermentation for ten days, with unfermented samples serving as the control. Result of the proximate analysis revealed the presence of protein, fiber, ash, fat, moisture and carbohydrate, with observed reduction in fiber content. Magnesium, potassium and calcium measured in percentage were the most prominent minerals in the samples. Others determined were sodium, phosphorus, iron and zinc. Antinutritional analysis shows the presence of phytate, oxalate, tannin, alkaloid, flavonoid and cyanide reduced by both methods of fermentation. In comparing the effectiveness of the fermentation methods employed, liquid state fermentation yielded a more positive result. The presence of essential nutrients, minerals and reduction of antinutrients in the samples are an indication that it can be used in the production or fortification of animal feeds.

Key words: Slimy kolanut, fermentation, proximate, mineral, antinutritional quality.

INTRODUCTION

Kolanut, indigenous to tropical Africa with its center of diversity in West Africa (Asogwa *et al.*, 2006) belongs to the family Sterculiaceae (Adebiyi, 2009). About one hundred and twenty-five (125) species of kolanut has already been identified, out of which not less than 40 species are indigenous to the tropical rainforest of Africa countries (Ndagi *et al.*, 2012). Cultivation of kolanut in Nigeria is

ecologically limited to the rain forest zone of the south and riverine areas of the savannah regions, with *Cola acuminata* (obi abata) and *Cola nitida* (gbanja or goro) being the commonly cultivated species of kolanut (Asogwa *et al.*, 2006; Asogwa *et al.*, 2008). *Cola verticillata* (slimy kola) is one of the numerous species of kolanut not usually cultivated due to its slimy nature when chewed (Esther *et al.*, 2010).

Kolanut seeds contain about one percent (1%) protein, forty-five percent (45%) starch and one point three five percent (1.35%) of fats, indicating that they are nutritious. Kolanut seeds have been used industrially for the production of drugs, soft drinks, wines beverages, liquid soap, dyes and formulation of animal feed (Olubamiwa *et al.*, 2002). It is a good source of dye in textiles and thread because of its tannin content (Tim, 2012). The husk and white shell of kolanut are not considered important by farmers and are left to rot at farm site. The nutritive quality of kolanut husk has been reported (Oluokun and Olulokun, 1999; Babatunde and Hamzat, 2005)

Kolanut husk was analysed for its nutritive value by Hamaz and Adeola to determine if the waste can be used to formulate feed for poultry birds. From the study, they were able to find out that the waste is 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.*, 2012 on microorganisms, proximate and antinutrient content of *Cola nitida* white shell also indicated the nutritive value of kolanut white shell. The reason for working out alternative measures to augment the available food is due to an increase in competition between man and animal for food such as cereals which is partly responsible for high cost in livestock feeds. In the agricultural sector, tonnes of waste are generated yearly causing nuisance in the environment and are usually ignored and these wastes will cause environmental pollution and aesthetic nuisance if left in processing site. Over 10million tonnes of cassava are processed for garri in Nigeria alone annually (Ubalua, 2007). These wastes pose a disposal problem and will even be more problematic with increase in industrial production without converting the waste to something useful.

Agricultural waste can be used to provide potential cleaning adsorbent for the environment such as activated carbon which appears to be of interest from a view point of large scale application using simple technology such as subjecting the parent material to either chemical or thermal process to remove non carbon atoms from the material leading to the development of internal porosity and increased surface area (Adeyi, 2010).

Major limitations to the use of crop residues include; low digestibility, low protein content and excessive crude fiber (Abdel-Azim, 2011). Fermentation has been identified as a technique that is inexpensive for the detoxification and increase in protein quality of some products such as cassava (Ubalua, 2007). Solid state fermentation for example uses solid substrates, making recycling process of nutrient rich waste materials easier (Subramaniam, 2012).

The objective of this study is to analyse for the nutritive values present in kolanut husk by employing liquid and solid state fermentation. Fermentation was employed to reduce the limitation of excessive crude fiber, low digestibility and low protein attributed to crop residue or waste. The analysis was carried out firstly to; reduce effect of these wastes on the environment because they cause nuisance in the environment. Secondly, to provide for an alternative to materials used for the production or fortification of animal feed to help augment available supply, by making use of waste obtained from the environment

MATERIALS AND METHODS

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 dried and grounded on which analysis was carried out.

Methods

Proximate composition which include; moisture, crude fibre, ash, crude protein and carbohydrates, was determined by the methods of Association of Official Analytical Chemists (AOAC, 2000). Mineral content of each sample were determined by atomic absorption spectrophotometry after dry ashing the samples using method indicated in AOAC, 2000. Antinutrients which include; tannin, phytate, oxalate, flavonoid and alkaloid were determined according to the method of Makkar *et al.*, (1993), Young and Greaves (1990) and Day and Underwood (1986).

RESULTS

The samples subjected to analysis were sun dried, grounded and sieved after the fermentation process. Proximate composition of fermented and unfermented (control) *Cola verticillata* husk and white shell were observed to be significantly different as presented in Table 1. Moisture content of kolanut husk subjected to both liquid and solid state fermentation was high measuring 11.853 ± 0.035^d and 11.856 ± 0.076^d respectively (no significant difference) as compared to the unfermented which measured 4.230 ± 0.060^a . The difference between the fermented and unfermented husk samples was significant. For the white shell samples, samples subjected to liquid and solid state fermentation measured $10.400\% \pm 0.089^c$ and $10.420\% \pm 0.050^c$ respectively (no significant difference) while the unfermented measured $4.470\% \pm 0.110^b$. Moisture content of both samples fermented has high moisture content when compared with the unfermented samples.

The husk samples analysed for fat content measured $7.666\% \pm 0.140^f$ and $6.330\% \pm 0.100^d$ while that of the white shell samples measured $5.150\% \pm 0.140^c$ and $4.610\% \pm 0.020^a$ for liquid and solid state fermentation respectively. The result has shown that fat content of husk samples subjected to both methods of fermentation is more than that of the white shell samples. Although, unfermented husk and white shell samples had higher fat content measuring $7.000\% \pm 0.981^e$ and $4.830\% \pm 0.020^b$ respectively. In comparing the values of the fermented and unfermented samples, husk and white shell samples subjected to liquid state fermentation had more fat content than those subjected to solid state fermentation.

Unfermented husk sample had as high as $19.067\% \pm 0.059^f$ of fiber while that fermented using liquid and solid state fermentation was observed to have a reduced fiber content of $10.390\% \pm 0.096^c$ and $12.533\% \pm 0.059^c$ respectively. White shell samples that were not fermented had a fiber content of $12.286\% \pm 0.115^d$, the fermented measured $8.066\% \pm 0.061^a$ and $10.073\% \pm 0.078^b$ for liquid and solid state fermentation respectively. Liquid state fermentation showed a more reduced fiber content in the samples as compared to samples subjected to solid state fermentation.

A reduction was observed in the ash content of the husk and white shell samples when compared with the unfermented samples. The ash content of husk samples subjected to liquid state fermentation measured $6.433\% \pm 0.999^c$ and solid state fermentation measured $5.273\% \pm 0.200^a$ while the white shell samples was $7.300\% \pm 0.090^b$ and $7.500\% \pm 0.120^b$ for samples analysed under liquid and solid state fermentation respectively. The values obtained for unfermented husk and white shell sample is $10.100\% \pm 0.092^d$ and $10.046\% \pm 0.050^d$ accordingly, with no significant difference. Liquid state fermentation reduced the ash content in both the husk and white shell samples than the solid state fermentation but not with a big margin.

A value of $6.726\% \pm 0.200^c$ and $5.360\% \pm 0.130^d$ was recorded for protein content present in the husk sample subjected to liquid and solid state fermentation respectively. White shell sample subjected to liquid and solid state fermentation has $8.436\% \pm 0.125^f$ and $4.047\% \pm 0.042^b$ measure of protein in it. An increase in protein content of the samples is evident from the result as that of unfermented husk and white shell measured $4.127\% \pm 0.055^c$ and $2.250\% \pm 0.090^a$ respectively. Fermentation increased the protein content of the husk and white shell samples.

Carbohydrate content of husk and white shell samples subjected to solid state fermentation recorded a reduction as compared to the ones subjected to liquid state fermentation. Liquid and solid state fermentation measured $58.270\% \pm 0.095^c$ and $55.283\% \pm 0.129^a$ for husk while the amount of carbohydrate recorded for white shell sample was 63.213 ± 0.186^c and 62.813 ± 0.081^d for samples under liquid and solid state fermentation respectively. Unfermented husk and white shell samples were recorded to have $55.477\% \pm 0.891^b$ and $66.116\% \pm 0.040^f$ of carbohydrate content respectively.

Table 2 shows mineral analysis carried out on dried fermented and unfermented husk and white shell samples. Sodium content was not significantly different in husk samples subjected to both methods of fermentation when compared with the unfermented samples. $0.507\% \pm 0.006^a$ and $0.490\% \pm 0.010^a$ was recorded for husk under liquid and solid state fermentation, while unfermented measured $0.510\% \pm 0.086^a$. There was significant increase in the sodium content of white shell samples subjected to both liquid and solid state fermentation, measuring $0.603\% \pm 0.049^b$ and $0.826\% \pm 0.222^c$ respectively. The unfermented for white shell measured $0.433\% \pm 0.189^a$. Solid state fermented is observed from the result presented to increase sodium content of the white shell sample than liquid state fermentation.

Potassium (K %), phosphorus (P %) and magnesium (Mg %) were recorded to be higher in fermented husk samples as compared to unfermented husk samples. Husk samples subjected to solid state fermentation had higher calcium, iron and zinc content than samples subjected to liquid state fermentation and unfermented samples. In the case of the white shell samples, zinc, calcium and iron were recorded to be increased in samples subjected to solid state fermentation when compared to unfermented samples. On the other hand, potassium, phosphorus and magnesium were observed to be high in unfermented samples.

Both liquid and solid state fermentation reduced phytate (mg/g), tannin (mg/g) and cyanide (mg/g) content of the husk and white shell samples. An increase was observed in oxalate (mg/g) content of both samples subjected to liquid and solid state fermentation when compared with the unfermented samples while alkaloid and flavonoid was observed to increase in samples subjected to liquid state fermentation in the husk and white shell samples as reported in Table 3.

141 **Table 1: Proximate composition of fermented and unfermented husk and white shell**

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

142 **Note:** values followed by similar alphabet along the same column are not significantly different at P<0.05

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145 **KEY**

146 LF- liquid state fermentation SF- solid state fermentation UF – Unfermented

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156 **Table2: Mineral analysis of fermented and unfermented dried kolanut husk and shell and infusion**

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

158 **Note:** values followed by similar alphabet along the same column are not significantly different at P<0.05

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160 **KEY**

161 LF- liquid state fermentation SF- solid state fermentation UF – Not fermented

162 Na- sodium K-potassium P- phosphorus Mg- magnesium Ca- calcium

163 Fe- iron Zn- zinc ppm- part per million

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168 **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±0.26 ^c	0.7±0.07 ^d	4.17±0.10 ^b	0.07±0.01 ^d	0.07±0.01 ^d	2.19±0.07 ^a
Husk SF	48.41±0.26 ^d	0.55±0.06 ^b	4.26±0.66 ^b	0.02±0.01 ^a	0.02±0.00 ^a	2.24±0.41 ^a
Husk UF	53.36±0.36 ^e	0.52±0.07 ^a	5.46±0.11 ^c	0.03±0.00 ^b	0.03±0.01 ^b	3.26±0.03 ^b
White shell LF	28.17±0.33 ^a	0.70±0.03 ^d	4.26±0.07 ^b	0.09±0.02 ^e	0.09±0.01 ^f	4.34±0.18 ^c
White shell SF	28.31±0.68 ^a	0.72±0.08 ^d	3.31±0.42 ^a	0.04±0.01 ^c	0.05±0.01 ^c	4.34±0.08 ^c
White shell UF	35.16±0.29 ^b	0.61±0.03 ^c	5.31±0.35 ^c	0.07±0.01 ^e	0.08±0.01 ^e	4.44±0.19 ^c

Note: values followed by similar alphabet along the same column are not significantly different at P<0.05

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KEY

LF- liquid state fermentation SF- solid state fermentation UF – Not fermented (control)

171 **DISCUSSION**

172 Moisture content is an important property measured in food products because it provides information on
173 the quality of food which helps to know what processing operation and storage method to employ, this is
174 because it affects the progress of chemical and microbiological spoilage reactions in foods (Alfred and
175 Patrick, 1985). The moisture content of the fermented sample was observed to be higher than that of the
176 unfermented (control) samples probably due to intake of water during fermentation by the process of
177 imbibition (*L. im-bibo*, to drink in), defined in the Steadman medical dictionary as the absorption of fluid
178 by a solid body. Water retention ability of fermented husk samples could be the reason for high moisture
179 content in husk samples as compared to white shell samples. High moisture content allows for the growth
180 of microorganisms thus enabling them to make use of nutrients in the husk sample, reducing the nutrient
181 but allows and facilitates movement and exchange between cells (Charles and Guy, 1999).

182 Higher fat content of fermented samples could be linked to production of extra cellular enzymes by
183 microorganisms, thus increasing the fat content (Akindahunsi *et al.*, 1999) also, fat content of samples
184 subjected to liquid state fermentation might have being washed into the infusion. Ash is a rough
185 measurement of inorganic mineral elements in a sample. High ash content observed for the unfermented
186 samples could be an indication that the kolanut husk and white shell has a reasonable quantity of mineral
187 elements and also, could be due to the metabolic activities of the organisms involved in the fermentation
188 process which is in concordance with the report of Kuforiji, 2006. Ash content of unfermented samples
189 reported in this study is in variance with that obtained by Adeyi, 2010 whom studied the proximate
190 composition of some agricultural wastes in Nigeria with kola nut husk measuring 7.67% for ash content.

191 Fibre content of unfermented samples which was observed to be high as compared to fermented in this
192 study could be attributed to the fermentation process because during fermentation, carbohydrate including
193 cellulose, pectin, lignocelluloses and starch are broken down by fermenting microorganisms which use
194 them as their sole carbon source, converting it to microbial biomass thereby reducing the fiber content of
195 the sample (Bamidele, 2001). Decrease in fibre content might also be due to production of various
196 lignocelluloses enzymes (Jonathan, 2012). Oboh and Elusiyan, 2007 also reported that decrease in fibre of
197 fermented cassava flour could be due to the presence of micro-fungi which secretes hydrolytic enzymes
198 and the enzymes ability to hydrolyze fiber into simple sugars that the organism could use as its carbon,
199 transforming it to other macromolecules or metabolites such as protein and fat.

200 Secretion of certain proteineous extra cellular enzymes into fermenting medium, their breakdown and
201 subsequent metabolism might be the reason for increased protein content of the samples and also, could
202 be as a result of hydrolysis of starch into glucose and its use by some organisms as a carbon source to

synthesize fungal biomass rich in protein (Akinyele, 2003). Increase in protein content might also be due to the structural proteins being an integral part of the microbial cells of organisms involved in fermentation (Tortora, 2000). Mastika *et al.*, 2011 reported that fermentation of cocoa waste using *Aspergillus niger* improves the nutritional content and quality of feed thus suggesting that organisms present during fermentation process improves nutritional quality of food related materials.

Reduction of carbohydrate during fermentation as observed in the fermented samples in this study could be due to utilization of sugar and its bioconversion to generate energy for cell metabolism during fermentation and thereby enhancing microbial growth (Rainbult and Tewe, 2001; Oboh *et al.*, 2002). Increase in carbohydrate as recorded in exceptional cases of few fermented samples in this study could be an indication that most of the microorganisms involved in fermentation might have produced metabolites which are carbohydrates and could be partially due to decrease in moisture contents (Gabriel, 2009).

The mineral content results showed magnesium to be most prominent and potassium was found to be next to It., report has shown magnesium to be an activator of many enzyme systems and maintains electrical potential of the nerves (Ajiboye *et al.*, 2013). 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 (Aboloma, 2011).

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 (Gilani, 2005). 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 (Beecher, 2003). Badau (2005) reported that fermentation is one of the processes that decrease antinutritional level in food and food related materials. Phytate reduction could be attributed to the action of enzyme released by microorganisms (Eltayeb *et al.*, 2007).

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 (Mingshu *et al.*, 2006). 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 (Mubo *et al.*, 2009, Ogbe, 2012). This could be the reason for variations in antinutritional composition of fermented and unfermented samples in the study.

CONCLUSION

The nutritional composition of the sample increased and the antinutritional composition reduced considerably. The effect of fermentation was positive on the samples especially liquid state fermentation as compared to solid state fermentation. Although, some quantity of nutrient was lost into the fermenting medium (infusion). The challenge of liquid state fermentation in this study is that, disposal into the environment of the infusion which is slimy and coloured generated during fermentation process might pollute the environment. This is because it will allow for different types of organisms thrive due to nutrients released from the sample in to the infusion. There is a need to look in to an alternative and useful form the infusion generated can be transformed or converted into. Kolanut husk and white shell could be used to substitute some quantity of grains/cereals used for the production of animal feed or its formulation, and also could be used to fortify animal feed to increase its nutritional quality or value.

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