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Article

Chemical Changes during Open and Controlled Fermentation of Cowpea (*Vigna unguiculata*) Flour

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Abstract: The effect of open and controlled fermentation on the proximate composition, some mineral elements, antinutritional factors and flatulence- causing oligosaccharides of a domesticated bean (*Vigna unguiculata*) was studied. The open fermentation was carried out using the microorganisms present in the atmosphere, while the controlled fermentation was carried out using *Aspergillus niger* as a starter. The two types of fermentation brought about more than 20% increase in the protein content. The lipids, carbohydrates, crude fibre and ash content were all reduced by less than 75% by both processing techniques, except the level of moisture content which increased in controlled fermentation. Apart from calcium, the other elements (Fe, Na, Mg, Zn, and K) suffered from less than 90% reduction by the two types of fermentation. The phytate, tannin, alkaloids, hydrogen cyanide, lectins, trypsin inhibitors and oxalate content all had more than 20% reduction by the two types of fermentation. The percentages of reduction due to controlled fermentation were higher than those of open fermentation in the eight antinutrients studied. Fermentation is, therefore, an efficient method for detoxifying the antinutrients in the cultivated beans studied in this work.

Keywords: *Vigna unguiculata*; minerals; flatulence factors; antinutrients; proximate composition; fermentation.

1. Introduction

Microorganisms used for fermentation process of food products are capable of growing on a wide range of substrates and can produce a remarkable spectrum of products and bioactive components that enhance the biofunctionality of food products and develop properties such as flavor (Yadav et al., 2011). The relatively recent advent of in vitro genetic manipulation has extended the range of products that may be produced by microorganisms and has provided new methods for increasing the yields of existing ones (Chambers and Pretorius, 2010). The term fermentation is derived from the Latin verb fervere, to boil, which describes the appearance of the action of yeast on extracts of fruit or malted grain during the production of alcoholic beverages. In physiological terms, fermentation is defined as the type of metabolism of a carbon source in which energy is generated by substrate level phosphorylation but to the microbiologist, the term fermentation describes a form of energy yielding microbial metabolism in which an organic substance, usually, carbohydrate is incompletely oxidized and an organic carbohydrate acts as election acceptor (Adams, 1990). However to a biochemist, the term fermentation can be technically defined as the chemical transformation of organic compounds with the aid of enzymes particularly those made by microorganisms. Fermentation process is a process which involves the conversion of large molecules to small molecules or molecular oxidation/ reduction mechanisms mediated by selected microorganisms (Yadav et al., 2011). Thus fermentation in food processing is defined as the conversion of carbohydrates to alcohol and carbon dioxide or organic acids using yeast and/or bacteria, under anaerobic conditions (William and Dennis, 2011). Fermentation technology depends on the microbial components and the production of different molecules from small laboratory scale to large industrial scale.

Fermentation is also seen as one of the oldest and most economical methods of food production and preservation (Oyewole and Isah, 2012). Several experiments have demonstrated that fermentation of legumes enhances their nutritive value and antioxidant properties; reduces some anti-nutritional endogenous compounds such as phytic acid, and exerts beneficial effects on protein digestibility and biological value of legumes (Oboh et al, 2012; Oyewole and Isah, 2012). Some anti-nutritional factors such as trypsin and cystatin inhibitors and lectins are heat-labile compounds and their negative effects are, therefore, markedly reduced by cooking (Adegunwa et al., 2012), while tannins and phytic acid are heat-stable compounds that retain negative effects on mineral and protein bioavailability after cooking (Ogun et al., 1989).

The bean plants (legumes) belong to the genus Vigna savi (Willis, 1985) and the family Leguminosae-papilionoidae and the tribe Phaseoleae which is made up of about 80-100 species. They grow in the tropics and Asia. Cowpea (*Vigna unquiculata* L. Walp) is a diploid species and a dicotyledonous leguminous food crops (Ogbemudia et al., 2010). *Vigna unguiculata* has 11 subspecies

that includes ssp. unguiculata having the cultivated forms (var. unguiculata) and wild forms (var. spontanea) and 10 wild perennial subspecies (Pasquet 1999; Maxted et al., 2004). In general, beans are important sources of macronutrients, micronutrients and antioxidant compounds with a great potential for human and animal nutrition (Porres et al., 2003). However, they contain several anti-nutritional factors which limit their consumption and affect the digestibility and bioavailability of nutrients (Bressani et al., 1993). Therefore, this work aims to study the effects of open and controlled fermentation on proximate composition, some antinutritional factors, some mineral elements and flatulent factors of *Vigna unguiculata* flour.

2. Materials and Methods

2.1. Collection and Preparation of Samples

Matured *Vigna unguiculata* seeds were purchased from local farmers in Zaria, Kaduna State, Nigeria. The seeds were taken to the laboratory of Biochemistry Department, Ahmadu Bello University, Zaria, Nigeria where they were picked clean of all debris and broken seeds. The plants were identified at the Herbarium of the Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria. The voucher number 1461 was assigned to *Vigna unguiculata*. The seeds were then stored in a plastic container at room temperature (27-30 ^{o}C) for subsequent analysis. Bambara nuts (*Vigna subterranea*) used in the isolation of *Aspergillus niger* for controlled fermentation were purchased from local farmers in Zaria metropolis and also identified at the Herbarium unit mentioned above where the voucher number as deposited in the unit is 1321.

2.2. Open Fermentation

Raw beans were washed with distilled water and dried in an oven at 55 ^{o}C for 24 h. After drying, bean samples were grinded in a laboratory bench mill (Thomas-WILEY, Laboratory mill, Model 4, Arthur H. Thomas Co, PA., USA) and sieved, and the 1 mm fraction were collected. The bean flour was suspended in distilled water at 300 g/L concentration which was found to be the optimal concentration for fermentation according to Doblado et al. (2002). The suspension was allowed to ferment naturally with the microorganisms present in the seeds and in the surrounding atmosphere for 48 h. After the fermentation, the microbial growth was terminated by drying at 55 ^{o}C in oven for 24 h (Fadahunsi, 2009) and re-ground using the laboratory bench mill.

2.3. Controlled Fermentation

About 250 g of bean flour was weighed into 500 mL flat bottom flask and autoclaved at 121 ^{o}C

for 15 min. Moisture content of the samples was adjusted to 25% before aseptic inoculation with spore suspension of *Aspergillus niger*, containing 1.064×10^7 spores/25 g of flour (Bhat et al., 1997), and incubated at room temperature (29±3 ^oC) for 48 h. After the fermentation, the fungal growth was terminated by drying at 55 ^oC in oven for 24 h (Fadahunsi, 2009) and re-ground using kitchen blender.

2.4. Selection of Simultaneous Tannin and Phytate Degrading Aspergillus niger Isolate

The tannin and phytate degrading *Aspergillus niger* isolate used for the controlled fermentation was obtained from red color seed coat Bambara nuts as reported earlier by Difo et al. (2013). *Aspergillus niger* was isolated from mouldy Bambara nut seeds according to the method of Pang and Ibrahim (2004). The method of Ellis (2006) was employed for identification of the *Aspergillus niger*. The volume of *A. niger* spores' suspension from a fully sporulated start culture was adjusted to 1.064×10^7 spores/mL and the harvested *A. niger* spores were centrifuged at 3000 *g* for 2 min, washed in sterile distilled water and re-centrifuged. The washed cells were then used as inoculums singly in the solid state fermentation (SSF) of *Vigna unguiculata*.

2.5. Proximate Analysis

The different samples (unfermented and fermented) were analyzed for moisture, ash, crude fat, crude protein and crude fibre in proportions of 1 g each, by standard methods recommended by AOAC (1980). Carbohydrate was calculated by difference based on the total seed composition (Olegunde et al., 1990; Onwuliri and Obu, 2002).

2.6. Anti-nutritional Factors Analysis

Trypsin inhibitor was analyzed by using the spectrophotometric method as described by Amtfield et al. (1985). Hydrogen cyanide was analyzed by the method of AOAC (1980). Tannin content was estimated spectrophotometrically by Folin-Denis method (Makkar et al., 1993). Saponins and alkaloids were determined by the gravimetric method of AOAC (1984). Phytic acid was determined using the procedure described by Lucas and Markakas (1975). Oxalate was determined by using the method of Oke (1969). Lectins content was determined according to the method of Onwuka (2005).

2.7. Mineral Content Analysis

The following minerals: magnesium, calcium, zinc, iron, potassium, and sodium were determined using atomic absorption spectrophotometry as described by AOAC (1990). Nitric acid and hyperchloric acid (6:1) were used for the digestion of the mixture.

2.8. Flatulence Factors Analysis

Flatulence causing oligosaccharides (stachyose and raffinose) were extracted by the method of Borejszo and Khan (1992) as modified by Onyenekwe et al. (1999) and separated by TLC using the method described by De Stefanis and Ponte (1968) as modified by Onyenekwe et al. (1999). The spots were detected and quantified according to the method of Stahl and Kaltenbach (1961).

3. Results and Discussion

Table 1 shows the effect of fermentation on proximate composition of *Vigna unguiculata*. From the result obtained, we discovered that fermentation increased the percentage protein in relation to the raw flour. Open fermentation, however, is slightly higher (24.81%) than controlled fermentation (21.87%). However, the levels of percentage lipid, moisture, ash, fibre and carbohydrate were all reduced by open fermentation in relation to the raw flour. On the other hand, controlled fermentation increased the levels of percentage moisture and carbohydrate while reducing the level of percentage lipid, ash and fibre in relation to the raw seeds. Controlled fermentation reduced the level of percentage lipid and ash by more than 50%.

Table 1. The effect of open and controlled fermentation on proximate composition of *Vigna unguiculata*

Processing technique	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)	Fibre (%)	Carbohydrate (%)
Raw	24.49 ± 0.58	9.50 ± 0.21	5.45 ± 0.10	4.49 ± 0.15	4.45 ± 0.03	51.77 ± 0.88
OF	30.58 ± 0.90	7.10 ± 0.12	4.61 ± 0.05	3.96 ± 0.02	3.68 ± 0.04	50.14 ± 0.71
CF	29.82 ± 0.06	3.92 ± 0.04	6.09 ± 0.01	1.65 ± 0.03	3.17 ± 0.02	55.35 ± 0.09
Change due to OF (%)	24.81 ± 1.30$^{\uparrow}$	25.18 ± 2.30$^{\downarrow}$	15.28 ± 2.80$^{\downarrow}$	11.49 ± 2.60$^{\downarrow}$	17.23 ± 0.60$^{\downarrow}$	3.14 ± 1.04$^{\downarrow}$
Change due to CF (%)	21.87 ± 2.68$^{\uparrow}$	58.71 ± 1.34$^{\downarrow}$	11.88 ± 1.78$^{\uparrow}$	63.10 ± 1.87$^{\downarrow}$	28.68 ± 0.79$^{\downarrow}$	6.97 ± 1.74$^{\uparrow}$

Note: OF, Open fermentation; CF, Controlled fermentation; $^{\downarrow}$percentage decrease; $^{\uparrow}$percentage increase;
Values are means ± SD for triplicate readings.

The increase in protein content could be due to the increase in the biomass brought about by the fermenting microorganisms. It has also been shown that the increase in the protein susceptibility to proteolytic enzymes is due to partial protein denaturaion and pH decrease during fermentation (Czarneka et al., 1998). The lipid, carbohydrate, fibre, ash and moisture contents decreased during the open and controlled fermentation which is consistent with earlier works (Granito et al., 2002; Martin-cabrejas et al., 2004). The reduction in these parameters may be due to the metabolism of the microorganisms in the fermentation medium.

The effect of fermentation on the antinutritional factors in *Vigna unguiculata* is presented in Table 2. From the result, we observed decrease in all the antinutritional factors assessed for both open and controlled fermentation in relation to the raw flour. Controlled fermentation using *Aspergillus niger* reduced the levels of percentage tannin, saponin, lectin and the level of trypsin inhibitors by more than 90% in relation to the raw flour. Generally, controlled fermentation caused more than 60% reduction in the levels of all antinutritional factors assessed while open fermentation caused reduction in the levels of the antinutritional factors between 26 and 47%. This presents controlled fermentation as a much better means of reducing the levels of the antinutritional factors in the seeds of *Vigna unguiculata*.

Table 2. The effect of open and controlled fermentation on antinutritional factors of *Vigna unguiculata*

Processing methods	Phytate (%)	Tannin (%)	Alkaloids (%)	Lectins (%)	Oxalate (%)	Saponin (%)	Hydrogen Cyanide (mg/100 g)	Trypsin inhibitors (TIU/g)
Raw	0.19 ± 0.00	0.09 ± 8.82*	0.77 ± 0.02	2.13 ± 0.03	0.22**± 0.01**	0.25 ± 0.03	0.01 ± 0.00	0.84± 0.01
OF	0.10 ± 0.00	0.05 ± 5.77*	0.46 ± 0.01	1.40 ± 0.06	0.14**± 0.01**	0.18 ± 0.02	0.01 ± 0.00	0.45± 0.02
CF	0.06 ± 0.01	420.00*±0.01	0.18 ± 0.02	10.00* ± 1.45*	5.97**± 0.01**	42.00*±1.45*	0.01 ± 0.00	300.00*±0.01
Change due to OF (%)	44.64±1.52$^{\downarrow}$	36.78 ± 0.08$^{\downarrow}$	40.92 ± 1.22$^{\downarrow}$	34.42 ± 1.89$^{\downarrow}$	39.28 ± 0.37$^{\downarrow}$	26.56 ± 0.87$^{\downarrow}$	28.11± 1.68$^{\downarrow}$	47.09±0.61$^{\downarrow}$
Change due to CF (%)	67.59±0.49$^{\downarrow}$	95.06 ± 0.17$^{\downarrow}$	77.21 ± 1.47$^{\downarrow}$	99.99 ± 0.00$^{\downarrow}$	73.17 ± 0.29$^{\downarrow}$	99.83 ± 0.02$^{\downarrow}$	65.03± 1.45$^{\downarrow}$	99.67±0.01$^{\downarrow}$

Note: *10^{-5}; **10^{-9}; OF, Open fermentation; CF, Controlled fermentation; Values are means ± SD for triplicate readings; E: means time ten raised to power; $^{\downarrow}$ means percentage decrease; $^{\uparrow}$ means percentage increase.

The reduction of these complex and toxic molecules was attributed to degradation by microorganisms (Madeira et al., 2011). The higher percentages of reduction observed in controlled fermentation could be attributed to the fact that the presence of more than one microorganism in open fermentation might have resulted in competition. An undesired microorganism is often the faster growing species and consumes the fermentation media components, but does not give the desired product.

The mineral content of *Vigna unguiculata* was assessed after fermentation (Table 3). The results show that both open and controlled fermentation caused reduction in all the minerals assessed. Indeed, both fermentation processes caused over 90% decrease in the level of calcium in the seeds in relation to the raw plant while controlled fermentation still caused over 80% decrease in the level of potassium and over 50% reduction in the level of sodium. Generally, open fermentation resulted in lower reduction of the levels of mineral content in the *Vigna unguiculata* compared to the controlled fermentation in relation to the raw seeds except for the level of sodium which was reduced by 65.85% compared to the controlled fermentation that reduced the level of sodium by 52.87%. This in brief, suggests that open fermentation retains more minerals compared to controlled fermentation. The

reduction in the mineral content during fermentation could be attributed to the effect of concentration due to the increase in biomass.

Table 3. The effect of open and controlled fermentation on the mineral content of *Vigna unguiculata*

Processing methods	Fe	Ca	Na	Zn	Mg	K
Raw (ppm)	2.35± 0.00	611.11±0.01	16.77 ± 0.02	0.69 ± 0.00	20.08±0.01	106.61±0.01
OF (ppm)	2.26± 0.00	11.19 ± 0.00	5.73 ± 0.02	0.54 ± 0.00	14.16±0.01	63.97 ± 0.01
CF (ppm)	1.61± 0.00	9.20 ± 0.00	7.90 ± 0.00	0.53 ± 0.00	12.43±0.00	13.50 ± 0.02
Change due to OF (%)	4.03±0.07$^{\downarrow}$	98.17± 0.00$^{\downarrow}$	65.85 ± 0.07$^{\downarrow}$	22.42±0.24$^{\downarrow}$	29.50±0.02$^{\downarrow}$	39.99 ±0.01$^{\downarrow}$
Change due to CF (%)	31.60±0.08$^{\downarrow}$	98.50±0.00$^{\downarrow}$	52.88 ± 0.04$^{\downarrow}$	23.28±0.27$^{\downarrow}$	38.09±0.02$^{\downarrow}$	87.33 ±0.02$^{\downarrow}$

Note: OF, Open fermentation; CF, Controlled fermentation; Values are means ± SD for triplicate readings; $^{\downarrow}$ means percentage decrease; $^{\uparrow}$ means percentage increase.

We also assessed the effect of fermentation on the levels of raffinose and stachyose in *Vigna unguiculata* (Table 4). Open and controlled fermentation reduced the levels of raffinose and stachyose in relation to the raw seeds. While open fermentation caused 84.81 and 59.04% reduction in the levels of raffinose and stachyose respectively, controlled fermentation caused 74.29 and 99.37% reduction in the levels of raffinose and stachyose respectively. Invariably, controlled fermentation is a better technique for the reduction of flatulence factors in the *Vigna unguiculata* compared to open fermentation. Since these oligosaccharides are fermented by intestinal bacteria (Granito et al., 2001), the present finding is of great interest, suggesting a simple method like open fermentation in order to reduce flatulence-causing factors.

Table 4. The effect of open and controlled fermentation on some flatulence factors of *Vigna unguiculata*

Processing methods	Raffinose	Stachyose
Raw (g/100 g)	2.035± 0.137	9.569± 0.87
OF (g/100 g)	0.305± 0.021	3.872± 0.121
CF (g/100 g)	0.517± 0.010	0.059± 0.0048
Change due to OF (%)	84.81± 1.71$^{\downarrow}$	59.04± 2.84$^{\downarrow}$
Change due to CF (%)	74.29± 2.11$^{\downarrow}$	99.37±0.049$^{\downarrow}$

Note: OF, Open fermentation; CF, Controlled fermentation; Values are means ± standard error of the mean for triplicate samples; $^{\downarrow}$ means percentage decrease; $^{\uparrow}$ means percentage increase.

4. Conclusions

In conclusion, fermentation is an efficient method for reducing (detoxifying) tannins, phytates, alkaloids, saponins, hydrogen cyanide, trypsin inhibitors, lectins and oxalate in cowpeas. The present research work has shown that controlled fermentation using *Aspergillus niger* as a starter is more efficient in detoxifying the above mentioned antinutrients in the cowpea studied here compared to open fermentation, although there is still need for further studies on the effect of fermentation on other antinutrients not studied. Moreover, other processing techniques as well as other types of fermentation methods needs to be employed on wild and other domesticated beans in order to further study the effect of processing on the level of reduction of antinutrients.

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