

Changes in nutrient and antinutrient composition of *Vigna racemosa* flour in open and controlled fermentation

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Abstract This study was conducted to investigate the effect of open and controlled fermentation on the proximate composition, mineral elements, antinutritional factors and flatulence-causing oligosaccharides in *Vigna racemosa*. 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 proximate composition of the *Vigna racemosa*, some anti-nutrients and the mineral elements were analyzed using standard procedures. The protein content was increased by 12.41 ± 1.73 % during open fermentation while it decreased by 29.42 ± 0.1 % during controlled fermentation. The lipids, carbohydrates, crude fibre and ash content were all reduced in both types of fermentation except the moisture content which increased in controlled fermentation. Apart from calcium, the other elements (Fe, Na, Mg, Zn, and K) suffered reduction in both types of fermentation. The phytate, tannin, alkaloids, hydrogen cyanide, lectins, trypsin inhibitors and oxalate content all had drastic reductions in both types of fermentation. Open and controlled fermentation reduced the levels of both raffinose and stachyose. The percentages of reduction due to controlled fermentation were higher than those of open fermentation in the antinutrients studied. Fermentation is an efficient method for detoxifying the antinutrients in the *Vigna racemosa* studied in this work.

Keywords Fermentation · Flatulence oligosaccharides · Minerals · Proximate · Antinutrients · *Aspergillus niger*

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Introduction

Legumes are the most important sources of proteins, carbohydrates and vitamins in the diet of many populations, especially in developing countries (Philips and McWatters 1991). They range from the highly utilized legumes such as *Vigna unguiculata*, *Glycine max* and *Arachis hypogaea* to the lesser known ones such as *Sphenostylis stenocarpa*, *Mucuna flagellipes* and *Vigna racemosa* (Ndidi et al. 2014). Due to the high demand of the popular legumes, there is rise in their prices which necessitated the need to look for alternatives such as the wild legumes. Indeed, plant tissues of wild *Vigna* spp. are used for human consumption (seeds and tubers) (Padulosi and Ng 1990). However, the high levels of antimetabolites may decrease the nutritional value of legumes especially wild legumes, reducing protein and starch digestibility and mineral bioavailability (Carnovale et al. 1991). Some of these antimetabolites, also called antinutrients, include protease inhibitors, lectins, saponins, alkaloids and flatulence factors. However, they could be eliminated or reduced by some processes such as soaking, dehulling and fermentation, although fermentation has been said to be the best in maximizing the nutrient content.

Microorganisms in nature are useful for the degradation of plant polysaccharides and polyphenols (de Vries et al. 2003). Their actions have been reported to enhance the digestibility and bioavailability of nutrients (Difo et al. 2013). They are usually applied in the process of making nutrients available through fermentation (Madeira et al. 2011). Purnama (2004) found that *Aspergillus niger* isolated from cacao pod reduced tannin levels by up to 79.3 % (wt/wt). 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 including enzymes and bioactive components that enhance the biofunctionality of food products and develop properties such as flavor (Yadav et al.

2011). The fermentation technology depends on the microbial components and produces different molecules from small laboratory scale to large industrial scale.

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 (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).

Vigna racemosa are under-exploited wild *Vigna* spp. belonging to the kingdom Plantae, order Fabales, family Fabaceae, Sub-family Faboideae, genus *Vigna* and species *racemosa* (Marconi et al. 1996). Very few studies have been carried out to reveal the nutritional aspect of *Vigna racemosa* seeds. Marconi et al. (1996) evaluated some chemical composition in under-exploited wild *Vigna* spp. seed including *Vigna racemosa*. However, there are no reports known to us on the effect of open and controlled fermentation on the chemical composition of this *Vigna* spp. Indeed, this is the first report known to us in which open and controlled fermentation were carried out on a wild *Vigna* spp. to determine the changes in the nutrient and antinutrient composition of the wild *Vigna* spp. in relation to the unprocessed wild *Vigna* spp. Therefore, this work was aimed at studying the effects of open and controlled fermentation on proximate composition, some antinutritional factors, some mineral elements and flatulence factors of *Vigna racemosa* flour.

Materials and methods

Collection and preparation of samples

Vigna racemosa seeds were harvested from bushes in Zaria metropolis, a city located in Kaduna state (Northern Nigeria). The matured fruits were harvested with their pods, sun-dried and the seeds were removed from the pods, threshed and winnowed. Broken seeds, dust, and other foreign materials were removed to obtain clean seeds. The plant was identified at the Herbarium of the Department of Biological Science, Ahmadu Bello University-Zaria, Nigeria and the voucher number 1463 was deposited at the unit. The seeds were then stored in a plastic container at room temperature (27–30 °C) for subsequent analysis. Bambara nuts (*Vigna subterranea*) seeds used in the isolation of *Aspergillus niger* for controlled fermentation were purchased from local farmers in Zaria, Kaduna State, Nigeria and also identified at the Herbarium

unit of the Department mentioned above where the voucher number as deposited in the unit is 1321.

Open fermentation

Raw beans were washed with distilled water and dried in an oven at 55 °C for 24 h. After drying, bean samples were grinded in a laboratory bench mill (*Thomas-WILEY*, Laboratory mill, Model 4, Arthur H. Thomas Company, Philadelphia, PA., U.S.A.) and sieved, and the 1 mm fraction was collected. The bean flour was suspended in distilled water at 300 g/l according to the method of 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 °C in oven for 24 h (Fadahunsi 2009) and re-ground using the laboratory bench mill.

Controlled fermentation

About 250 g of bean flour was weighed into 500 ml flat bottom flask and autoclaved at 121 °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 °C) for 48 h. After the fermentation, the fungal growth was terminated by drying at 55 °C in oven for 24 h (Fadahunsi 2009) and re-ground using kitchen blender.

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 starter culture was adjusted to 1.064×10^7 spores/mL and the harvested *A. niger* spores were centrifuged at $3000 \times g$ for 2 min, washed in sterile distilled water and re-centrifuged. The washed cells were then used as inoculum singly in the Solid State Fermentation (SSF) of *Vigna racemosa*.

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 (Ogun et al. 1989).

Anti-nutritional factors analysis

Trypsin inhibitor was analyzed using the spectrophotometric method described by Arntfield 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) and lectin by the method describe by Onwuka (2005).

Mineral content analysis

The following minerals: magnesium, calcium, zinc, iron, potassium, and sodium were determined using atomic absorption spectrophotometry (Shimadzu AA-6200) as described by AOAC (1990) using nitric acid and hyperchloric acid (6:1) as the digestion mixture.

Flatulence factors analysis

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

Statistical analyses

Results are presented as mean ± standard deviation (SD) except where otherwise stated. The student’s t-test was used to determine the significance level between the raw sample and the processed samples where $P < 0.05$ was considered significant. These statistical analyses were carried out using the SPSS software package (IBM SPSS statistics desktop v20.0). The relative change in percentage between a raw sample and a processed sample is:

$$\text{Relative change} = \text{OF (CF)} - \text{raw/raw} \times 100.$$

Where OF means open fermentation; CF means controlled fermentation; raw signify raw sample.

Results and discussion

Effects of fermentations on composition

Table 1 shows the proximate composition of raw and fermented *Vigna racemosa* flour and the percentages of

Table 1 The effect of open and controlled fermentation on percentage proximate composition of *Vigna racemosa* seeds

Processing methods	Protein	Lipid	Moisture	Ash	Fibre	Carbohydrate
Raw	27.12±0.44	7.40±0.20	3.91±0.07	2.77±0.14	2.82±0.04	55.88±0.49
Open fermentation	30.49±0.84	6.68±0.25	2.79±0.03	2.43±0.09	2.30±0.06	55.31±0.73
Controlled fermentation	19.14±0.40	2.75±0.02	4.75±0.03	1.91±0.03	2.80±0.03	68.65±0.38
Relative Δ due to OF (%)	12.41* ±1.73 [†]	9.76±1.62 [↓]	28.67* ±1.82 [↓]	12.28±1.12 [↓]	18.56* ±1.02 [↓]	1.02±0.44 [↓]
Relative Δ due to CF (%)	29.42* ±0.10 [↓]	62.77* ±1.25 [↓]	21.46* ±2.09 [↓]	30.79* ±2.26 [↓]	0.93±0.41 [↓]	22.88* ±0.87 [↓]

OF Open fermentation, CF Controlled fermentation, Δ represents change, [†] represents percentage decrease, [↓] represents percentage increase, Values are means±SD for triplicate readings. Means with asterisks ** are significantly different from the raw sample at $P < 0.05$. Relative change=OF (CF)—Raw/Raw× 100

change due to the two types of fermentation. From Table 1, open fermentation reduced the percentage composition of lipid, moisture, ash, fibre and carbohydrate but increased significantly ($P<0.05$) the percentage composition of protein. However, only the reduction of fibre and moisture were significant ($P<0.05$). On the other hand, controlled fermentation reduced significantly ($P<0.05$) the percentage composition of protein, lipid and ash but insignificantly ($P>0.05$) reduced fibre content while the composition of moisture and carbohydrate were significantly ($P<0.05$) increased. The little increase in the protein content during open fermentation 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 denaturation and pH decrease during fermentation (Czarnecka et al. 1998). Decrease in protein content in controlled fermentation could be due to the metabolism of *Aspergillus niger* with respect to other compounds present in *Vigna racemosa* and it might have produced some compounds capable of interfering with protein content of *Vigna racemosa*. The lipid, carbohydrate, fibre, ash and moisture content suffered from decrease during the open and controlled fermentation which is consistent with earlier workers (Martín-Cabrejas et al. 2004). The reduction in these parameters may be due to their metabolism by the microorganisms in the fermentation medium. The lower moisture content of the *Vigna racemosa* processed using open fermentation suggests that it will be a better technique for preservation (Biswas et al. 2001). This moisture content range has been reported to be suitable for storage and processing of flours without microorganism degradation of the triacylglycerol (James 1995).

Effects of fermentations on antinutritional factors

Table 2 shows the effects of open and controlled fermentation on antinutrients in the *Vigna racemosa* flour. From the table, controlled fermentation reduced the level of all antinutrients significantly ($P<0.05$) and the level of all the antinutrients were reduced by at least 70 % except for the level of alkaloids (29.76±2.20). On the other hand, open fermentation reduced the levels of all antinutrients significantly ($P<0.05$) except for the level of alkaloids. The concentrations of antinutrients in different foodstuffs may affect their nutritive values. Oxalate and phytate are known to precipitate or form insoluble complexes with calcium, magnesium, zinc and iron thus interfering with their utilization (Adegunwa et al. 2012). The reduction of the antinutrient complex and toxic molecules was attributed to degradation by microorganisms (Madeira et al. 2011). The higher percentages of reduction observed in controlled fermentation was attributed to the fact that the presence of more than one microorganism in open fermentation might have resulted in competition among the numerous microorganisms. An undesired microorganism is often the faster

Table 2 The effect of open and controlled fermentation on antinutritional factors of *Vigna racemosa* seeds

Processing methods	% Phytate	% Tannin	% Alkaloids	% Saponin	Hydrogen Cyanide (mg/100 g)	% Lectin	Trypsin inhibitors (TIU/g)	% Oxalate
Raw	0.31±0.004	0.19±5.770 ^a	1.90±0.004	0.87±0.030	0.07±58.000 ^a	2.60±0.060	1.21±0.008	4.72 ^b ±100.00 ^b
Open fermentation	0.20±0.003	0.14±5.770 ^a	1.85±0.010	0.53±0.030	0.04±33.000 ^a	2.10±0.060	0.93±0.006	1.93 ^b ±0.333 ^c
Controlled fermentation	0.09±15.000 ^a	0.02±15.000 ^a	1.33±0.040	64 ^{ab} ±1.730 ^a	0.01±8.820 ^a	24.00 ^a ±2.030 ^a	0.01±12.000 ^a	14.40 ^b ±3.330 ^c
Relative Δ due to OF (%)	33.49*±0.29 [↓]	25.82*±0.05 [↓]	2.53±0.72 [↓]	38.52*±0.98 [↓]	33.82*±0.75 [↓]	19.25*±0.43 [↓]	23.66*±0.83 [↓]	59.18*±0.16 [↓]
Relative Δ due to CF (%)	70.40*±0.35 [↓]	92.40*±0.07 [↓]	29.76*±2.20 [↓]	99.93*±0.01 [↓]	80.55*±0.05 [↓]	99.99*±98* [↓]	99.44*±0.01 [↓]	96.94*±58 ^{a1}

OF Open fermentation, CF Controlled fermentation, Δ represents percentage decrease, ↓ represents percentage increase, Values are means±SD for triplicate readings. Means with asterisks ** are significantly different from the raw sample at $P<0.05$. ^a = 10^{-5} ; ^b = 10^{-8} ; ^c = 10^{-12} ; Relative change = OF (CF)—Raw/Raw×100

Table 3 The effect of open and controlled fermentation on the mineral content in mg/100 g dry mass of *Vigna racemosa* seeds

Processing methods	Fe	Ca	Na	Zn	Mg	K
Raw	2.39±0.0023	468.75±0.0088	14.35±0.0058	0.78±0.00058	23.64±0.0058	119.60±0.015
Open fermentation	1.43±0.0018	9.55±0.0028	4.23±0.015	0.56±0.0012	15.67±0.012	104.65±0.012
Controlled fermentation	0.33±0.00088	8.62±0.0017	3.40±0.0012	0.33±0.0021	13.58±0.0012	15.80±0.015
Relative Δ due to OF (%)	40.19*±0.048 [↓]	97.96*±0.00064 [↓]	70.55*±0.089 [↓]	27.64*±0.130 [↓]	33.70*±0.036 [↓]	12.50±0.021 [↓]
Relative Δ due to CF (%)	86.20*±0.045 [↓]	98.16*±0.00039 [↓]	76.31*±0.018 [↓]	57.07*±0.26 [↓]	42.55*±0.0091 [↓]	86.79*±0.011 [↓]

OF Open fermentation, CF Controlled fermentation, Δ represents change, [↓] represents percentage decrease, [↑] represents percentage increase, Values are means±SD for triplicate readings. Means with asterisks '*' are significantly different from the raw sample at P<0.05. 'ppm' means parts per million; Relative change=OF(CF)—Raw/Raw×100

growing species and consumes the fermentation media components but does not give the desired product. The fermentation processes in this study reduced the trypsin inhibitors greatly in contrast with the report of Fadahunsi (2009) in which case fermentation increased the trypsin inhibitors in their flours.

Effects of fermentations on mineral contents

The effect of open and controlled fermentation on the mineral content of *Vigna racemosa* flour is presented in Table 3. There was a significant (P<0.05) reduction in the levels of all minerals analysed in both open and controlled fermentation. They both reduced the level of calcium and sodium by over 90 and 70 % respectively. However, controlled fermentation reduced the levels of Fe and K two times and seven times, respectively, the amount of reduction shown by open fermentation. There was a general reduction in the level of mineral contents in the *Vigna racemosa* flour, which could be due to mineral utilization by the microorganisms (Zamora and Fields 1979) as well as leaching of the minerals into the fermentation water (Kazanas and Fields 1981). The reduction in the mineral content during fermentation could also be attributed to the effect of concentration due to the increase in biomass.

Table 4 Effect of open and controlled fermentation on some flatulence factors in g/100 g dry mass of *Vigna racemosa* seeds

Processing methods	Raffinose	Stachyose
Raw	1.85±0.02	12.33±0.80
Open fermentation	1.24±0.07	4.30±0.09
Controlled fermentation	0.75±0.04	1.52±0.03
Relative Δ due to OF (%)	33.11* ±2.96 [↓]	64.90* ±1.70 [↓]
Relative Δ due to CF (%)	59.47* ±2.20 [↓]	87.59* ±0.62 [↓]

OF Open fermentation, CF Controlled fermentation, Δ represents change, [↓] represents percentage decrease, [↑] represents percentage increase, Values are means±SD for triplicate readings and means with asterisks '*' are significantly different from the raw sample at P<0.05. Relative change=OF(CF)—Raw/Raw×100

Effects of fermentations on flatulence factors

Table 4 shows the effect of open and controlled fermentation on some flatulence factors in the *Vigna racemosa* flour. Open and controlled fermentation reduced the levels of both raffinose and stachyose significantly (P<0.05). However, the percentage reduction on the flatulence factors subjected to controlled fermentation is much more than that of open fermentation. The fermentation processes reduced greatly the levels of stachyose and raffinose, which could be attributed to the utilization of these oligosaccharides for energy by the microorganisms. Since these oligosaccharides are fermented by intestinal bacteria (Granito et al. 2001), the present finding is of great interest as it suggests that a simple method like open fermentation can be used to reduce flatulence-causing oligosaccharides.

Conclusion

In conclusion, fermentation is an efficient method for detoxifying tannins, phytates, alkaloids, saponins, hydrogen cyanide, trypsin inhibitors, lectins and oxalate in wild beans. 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 wild beans studied here compared to open fermentation.

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