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Detoxification of Common bean (*Phaseolus vulgaris*) Flour Through Open and Controlled Fermentation Methods

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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 (Phaseolus vulgaris) was studied. The open fermentation was carried out using the microorganisms present in the atmosphere, while the controlled fermentation was carried out using Aspergillusniger as a starter. The two types of fermentation brought about less than 20% increase in the protein content.The lipids, carbohydrates, crude fibre and ash content were all reduced by less than 75% during the two types of fermentation, except the moisture content which suffered from increase 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 suffered from 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. So, considering the common beans as the main source of proteins in developing countries,fermentation is an efficient processing method for considerable reduction of the above studiedantinutrients in the cultivated beans studied in this work with no much impact on the protein level.***

Keywords: *Phaseolusvulgaris*, antinutrients, detoxification, open fermentation, controlled 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*., 2012). 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). Fermentation process is a process which involves the conversion of large molecules to small molecules or molecular oxidation/ reduction mechanisms mediated by selected micro-organisms (Yadav*et al*., 2012). Thus fermentation in food processing is defined as the conversion of carbohydrates to alcohol and carbondioxide or organic acids using yeast and/or bacteria, under anaerobic conditions (William and Dennis, 2011). The fermentation technology depends on the microbial components and produces 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).

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 *Vignaunguiculata*, *Glycine max* and *Arachishypogaea* to the lesser known ones such as *Sphenostylisstenocarpa*, *Mucunaflagellipes* and *Vignaracemosa*(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. 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; Oboh and Lajide, 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 *Vignasavi*, (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 (Mbagwu and Edeoga, 2006).Common bean usually refers to food legumes of the genus *Phaseolus*, family *Leguminosae*, subfamily *Papilionoideae*, tribe Phaseoleae, and subtribePhaseolinae. The genus *Phaseolus* contains some 50 wild-growing species distributed only in the Americas (Asian *Phaseolus* have been reclassified as *Vigna*) (Gepts, 2001). The genus also contains five domesticated species: in decreasing order of importance, common bean (*Phaseolusvulgaris L.*), lima bean (*P. lunatus L.*), runner bean (*Phaseoluscoccineus L.*), tepary bean (*P. acutifolius*A. Gray), and year bean (*P. polyanthus* Greenman), with distinct adaptations and reproductive systems. Beans in general, are important sources of macronutrients, micronutrients and antioxidant compounds with a great potential for human and animal nutrition and even sometimesconsidered as the poor- man's meat because of their protein level (Gloria *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 *Phaseolus vulgaris* flour.

2. Literature Survey

Many developing countries in the world are living in abject poverty and are malnourished, which necessitates the sourcing for novel food sources and adequate processing techniques to reduce food intoxication issues due to consumption of edible plant food sources because of their production of secondary metabolites (including antinutrients).

Considering the level of poverty and malnutrition in the world, especially the developing world (FAO, 2011), there is need to process hitherto wild and domesticated beans which will make them available for safe consumption. Moreover, the consumption of antinutrients such as polyphenols(e.g. Tannins) has an impact on the metabolism of glucose (Kati et al., 2010).

Beans in general, are important sources of macronutrients, micronutrients and antioxidant compounds with a great potential for human and animal nutrition (Jesus *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).

3. Materials and Methods

3.1 Collection and Preparation of Samples

Matured *Phaseolus vulgaris* seeds (Fig.1) were purchased from local farmers in Ngaoundere, Adamaoua region, Cameroon. 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 1462 was assigned to *Phaseolus vulgaris*. The seeds were then stored in a plastic container at room temperature (27-30^{0}C) for subsequent analysis. Bambara nuts (*Vignasubterranea*) used in the isolation of *Aspergillusniger*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.

3.2 Open Fermentation

Raw beans were washed with distilled water and dried in an oven at 55^{o}C for 24h. 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 were collected. The bean flour was suspended in distilled water at 300 g/l concentration as found to be the optimal concentration for fermentation by Dabladoet *al*., (2002). The suspension was allowed to ferment naturally with the microorganisms present in the seeds and in the surrounding atmosphere for 48 hours. 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.

3.3 Controlled Fermentation

About 250 g of bean flour was weighed into 500ml flat bottom flask and autoclaved at 121^{o}C for 15min. Moisture content of the samples was adjusted to 25% before aseptic inoculation with spore suspension of *Aspergillusniger*, containing 1.064 x 10^7 spores/25 g of flour (Bhat*et al*., 1997), and incubated at room temperature (29 ± 3^{o}C) for 48 h. After the fermentation, the fungal growth was terminated by drying at 55^o C in oven for 24 h (Fadahunsi, 2009) and re-ground using laboratory bench mill.

3.4 Selection of Simultaneous Tannin and Phytate Degrading Aspergillusniger Isolate

The tannin and phytate degrading *Aspergillusniger*isolate used for the controlled fermentation was obtained from red color seed coat Bambara nuts as reported earlier by Difo et al. (2013). *Aspergillusniger*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 *Aspergillusniger*. 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 *Vignaunguiculata*

3.5 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).

3.6 Anti-nutritional Factors Analysis

TrypsinInhibitor was analyzed by using the spectrophotometric method, 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). Phytatic acid was determined using the procedure described by Lucas and Markakas (1975). Oxalate was determined by using the method of Oke (1969), Saponins and alkaloids were determined by the gravimetric method of AOAC (1984) and Lectins by the method describe by Onwuka, (2005).

3.7 Mineral content Analysis

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

3.8 Flatulent Factors Anlysis

Flatulence causing oligosaccharides (mainly stachyose and raffinose) were extracted by the method used by 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 Stahl and Kaltenbach (1992).

4. Results and Discussion

The table 1 shows the proximate composition of raw and fermented *Vignaungulculata* flour. It was observed that the protein content increased by more than 20% due to the two types of fermentation, while the lipid, ash, moisture, fiber and carbohydrate were decreasing.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 content suffered from decrease during the open and controlled fermentation which is consistent with earlier workers (Martin-cabreja*set al*, 2004, Granito*et al*, 2002). The reduction in these parameters is due to the metabolism of the microorganisms in the fermentation medium.

The Table 2 shows that phytate, tannin, lectins, saponins, hydrogen cyanide, trypsin inhibitors and oxalate were all reduced by the two types of fermentation. 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 was 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 elements evaluated in the present study (Fe, Ca, Na, Zn, K, Mg) were all reduced by the two types of fermentation (table 3). Irrespective of leaching in fermentation water, mineral utilization could be taken place by microorganisms responsible of the 48 hours fermentation (Zamora and Fields, 1979) as well as reduction in ash content and minerals by leaching in soaking or cooking water (Kazamas 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.

The level of raffanose and stachyose was reduced by the two types of fermentation. Open fermentation reduced raffinose more than controlled fermentation did, while stachyose level was more reduced by controlled fermentation than open fermentation (Table 4). 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.

5. Conclusion

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 *Aspergillusniger*as a starter is more efficient in detoxifying the above mentioned antinutrients in the domesticated beans studied here compared to open fermentation.

6. Future Scope

Although the fermentation techniques used in this study have shown considerable reduction on the antinutrient levels, there is still need for further studies on the effect of fermentation on other antinutrients not studied. Moreover other processing as well as other types of fermentation methods need to be employed on other domesticated and wild beans (such as *Vignaunguiculata* and *Vignaracemosa* species) in order to further study the effect of processing on the level of reduction of antinutrients. Also the researcher encourages other researchers in the field of agriculture to carry out investigations on the cultivation of these species of bean in arid regions of the world and most especially in the savannah part of Africa since he discovered that the plant does not produce seeds when planted in the northern part of Nigeria and Cameroon.

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Table 1: The Effect of Open and Controlled Fermentation on Proximate Composition of*Phaseolus vulgaris**

Processing methods	*%Protein*	*%Lipid*	*%Moisture*	*%Ash*	*%Fibre*	*%Carbohydrate*
Raw	27.75±0.30	7.63±0.16	3.29±0.067	3.38±0.055	3.2±0.058	54.75±0.43
Open Fermented (OF)	33.05± 0.97	6.003±0.28	2.58±0.034	3.08±0.049	2.9±0.029	52.3±1.17
%Change due to OF**	19.07± 2.37$^{\uparrow}$	21.40±2.02$^{\downarrow}$	21.66±0.65$^{\downarrow}$	9.06±0.50$^{\downarrow}$	9.33±1.44$^{\downarrow}$	4.49±1.44$^{\downarrow}$
Controlled Fermented (CF)	30.48±0.22	2.53±0.018	5.66±0.047	2.65±0.018	3.08±0.044	55.59±0.13
%Change due to CF**	9.86±0.81$^{\uparrow}$	66.77±0.78$^{\downarrow}$	72.12±2.07$^{\uparrow}$	21.73±1.54$^{\downarrow}$	3.63±0.46$^{\downarrow}$	1.55±0.55$^{\uparrow}$

Table 2: The Effect of Open and Controlled Fermentation on Antinutritional factors of *Phaseolus vulgaris**

Processing Methods	*%Phytate*	*%Tannin*	*%Alkaloids*	*%Saponin*	*Hydrogen Cyanide (mg/100g)*	*%Lectines*	*Trypsin inhibitors (TIU/g)*	*%Oxalate*
Raw	0.233± 0.0033	0.124± 8.82E -05	1.31± 0.026	0.433± 0.033	0.04± 0.00058	2.33± 0.033	1.07± 0.0059	5.75E -08± 5.77E -11
Open Fermented (OF)	0.139± 0.001	0.104± 0.00056	1.023± 0.0088	0.223± 0.023	0.029± 0.00058	1.83± 0.037	0.077± 0.0050	1.58E -08± 3.33E -11
%Change due to OF**	40.39± 1.26$^{\downarrow}$	16.15± 0.50$^{\downarrow}$	21.84± 1.13$^{\downarrow}$	48.67± 1.33$^{\downarrow}$	27.49± 1.28$^{\downarrow}$	21.73± 0.51$^{\downarrow}$	92.77± 0.46$^{\downarrow}$	72.58± 0.083$^{\downarrow}$
Controlled Fermented (CF)	0.0603± 0.00012	0.0044± 0.0001	0.85± 0.029	0.000187± 8.82E -06	0.0024± 0.00012	0.00012± 8.82E -06	0.0024± 0.00015	5.8E -09± 0.000
%Change due to CF**	74.16± 0.38$^{\downarrow}$	96.46± 0.083$^{\downarrow}$	35.09± 2.17$^{\downarrow}$	99.96± 0.0050$^{\downarrow}$	93.99± 0.38$^{\downarrow}$	99.99± 0.00038$^{\downarrow}$	99.78± 0.013$^{\downarrow}$	89.91± 0.010$^{\downarrow}$

Table 3: The Effect of Open and Controlled Fermentation on the Mineral content of *Phaseolus vulgaris**

Processing Methods	*Fe (ppm)*	*Ca (ppm)*	*Na (ppm)*	*Zn (ppm)*	*Mg (ppm)*	*K (ppm)*
Raw	2.488± 0.0018	370.407± 0.012	8.99± 0.0058	0.819± 0.00058	22.36± 0.0088	132.32± 0.012
Open Fermented (OF)	1.856± 0.0037	10.647± 0.0015	4.85± 0.023	0.435± 0.0012	17.68± 0.0058	66.08± 0.012
%Change due to OF**	25.398± 0.160$^{\downarrow}$	97.126± 0.00043$^{\downarrow}$	46.05± 0.24$^{\downarrow}$	46.89± 0.18$^{\downarrow}$	20.94± 0.057$^{\downarrow}$	50.06± 0.012$^{\downarrow}$
Controlled Fermented (CF)	1.31± 0.021	16.960± 0.0018	3.60± 0.0012	0.424± 0.0023	21.61± 0.0015	19.53± 0.017
%Change due to CF**	47.35± 0.840$^{\downarrow}$	95.421± 0.00033$^{\downarrow}$	59.95± 0.019$^{\downarrow}$	48.19± 0.32$^{\downarrow}$	3.38± 0.045$^{\downarrow}$	85.24± 0.014$^{\downarrow}$

*: Values are means ± standard error of the mean for triplicate samples; E: means time ten raised to power.
**: $^{\downarrow}$ means percentage decrease; $^{\uparrow}$ means percentage increase

Table 4: Effect of Open and Controlled Fermentation on Some flatulent factors of *Phaseolus vulgaris**

Processing methods	*Raffinose (g/100g)*	*Stachyose (g/100g)*
Raw	5.74± 0.36	9.692± 0.68
Open Fermented (OF)	0.152± 0.014	4.21± 0.24
%Change due to OF**	97.32± 0.38$^{\downarrow}$	56.45± 1.44$^{\downarrow}$
Controlled Fermented (CF)	1.062± 0.030	0.410± 0.017
%Change due to CF**	81.30±1.61$^{\downarrow}$	95.71± 0.43$^{\downarrow}$

*: Values are means ± standard error of the mean for triplicate samples; E: means time ten raised to power.
**: $^{\downarrow}$ means percentage decrease; $^{\uparrow}$ means percentage increase

Figure 1: Phaseolus vulgaris seeds