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Effect of Open and Controlled Fermentation on the Proximate and Antinutrient Compositions of *Glycine max* (Soya Bean)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The effect of open and controlled fermentation on the proximate and anti-nutrients compositions of *Glycine max* were investigated.
Study Design: Ability of fermentation to improving the proximate composition and lowering the antinutrients in *G. max* was studied.
Place and Duration of Study: Department of Biochemistry, Ahmadu Bello University Zaria-Nigeria, between August 2013 and March 2014.
Methodology: *Glycine max* seeds were processed and subjected open and controlled fermentation in the presence of *Aspergillus niger.* Analysis on the proximate and anti-nutritional parameters were conducted in both fermented products.
Results: This study revealed the effects of open and controlled fermentation on physiochemical, the proximate compositions, microbial load and anti-nutritional factors in *Glycine max.* Range from moisture: (2.173 – 10.033)%, temperature: (25±3ºC), color of spores: (black), microbial spore suspension: (1.06 × 10^7 spores/25 g), crude protein (25.247 – 36.502)%, crude fiber: (7.061 – 1.253)%, crude lipid: (13.549 – 21.474)%, ash: (7.061 – 3.520)%, nitrogen free extract: (44.176 – 27.217)%, odour: (pleasant chocking), phytates: (26.140 – 9.138mg/100g), cyanide: (0.258 – 0.06mg/100g). The effects of open fermentation were very pronounced compared to the

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controlled fermentation.
Conclusion: It could be concluded that *A. niger* is not effective as the open in *G. max* fermentation.

Keywords: Fermentation; Aspergillus niger; antinutrient; proximate.

1. INTRODUCTION

Traditional diets in West Africa often lack variety and consist of large quantities of the staple foods (cassava, yam, maize) with supplement of Plantain, Coco yam, Rice and Beans depending on availability and season [1]. Soups eaten with the staples are an essential component of the diet and may contain a variety of seeds, nuts, pulses and leaves [2]. The staple foods provide the calories but are poor in other nutrient. Soups are the main sources of proteins and minerals, and one of the ways to improving the diet is to improve the nutrient quality of the soups. There is interest not only in increasing the quality and concentration of seed proteins but also in developing cultivars with low levels of undesirable compounds, while maintaining or improving the resistance to pests and diseases [3].

Locally available protein containing plant food stuffs have been screened and are still undergoing more screening for their nutritional potential in Nigeria with the aim of avoiding increased dependence and competition between man and livestock for conventional sources of the protein. Proteins of plant origin are found to be of higher nutritional value than the conventional animal protein sources [4]. However the presence of insoluble and/indigestible carbohydrates, toxic factors or inhibitors are sometimes responsible for their low nutritional utility. These anti-nutrients are removed or reduced by various ways during processing, such as fermentation, heating, threshing, and germination among others.

Legumes belong to the family, *Leguminosae* the second most important source of food and fodder next only to the family *Gramineae* (the cereal grains). The family *Leguminosae* is a very important crop in terms of production system. *Glycine max* [5] is a member of the family *Leguminosae.* It is an important source of inexpensive and high quality protein and oil [5]. *G. max* constitutes the staple food in some parts of the world. It is a rich, cheap and a good source of vegetable protein available with high polyunsaturated fat content and absence of cholesterol. *G. max* is an excellent source of the essential amino acids vital for body growth, maintenance and reproduction. It is also a good source of minerals and vitamins. *G. max* has remained the main vegetable protein source that has exerted significant influence in the diet of both animal and humans on account of bioavailability of complete protein (eight amino acids) needed for human and animal health [6]. Unprocessed *G. max* is not suitable for food and its use for animal feed remains limited because it contain anti-nutrients such as phytates, tannins and trypsin inhibitors among others.

In this study, the effects of open and controlled fermentation using *Aspergillus niger* on the proximate and some antinutrient compositions of *G. max* were investigated.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

Glycine max seeds were obtained from Samaru central-market, and identified and given a voucher no. 394 at the department of Biological Sciences, Ahmadu Bello University, Zaria. The sample was kept under room temperature until analysis.

2.2 Preparation of Inoculum

The *Aspergillus niger* spore Inoculum was prepared by adding 10 ml of sterile distilled water containing 2.5% Tween 80 (polyoxyethylene sorbitan monoolate) to a fully sporulated slant culture. The spores were dislodged by vigorous shaking and spores number estimated by direct microscopic enumeration using cell-counting hemocytometer (Neubauer chamber; Merck, S.A., Madrid, Spain). The volume of spores' suspension was adjusted to 1.064×10^7 spores/ml and the harvested *A. niger* was washed in sterile distilled water and re-centrifuged. The *A. niger* was used as inoculum in the fermentation of the soy beans [7].

2.3 Fermentation of the Samples

2.3.1 Controlled fermentation

The fermentation procedures were carried out under aseptic conditions according to the methods of Dapiya et al. [7], Bhat et al. [8] and Fadahunsi [9]. The ground *G. max* seeds were weighed (250 g) into 500 ml flat bottom flask and autoclaved at 121ºC for 15 min. Moisture content of the samples were adjusted to 25 % before aseptic inoculation with spore suspension of *Aspergillus niger* containing 4.256 x 10^5 spores/ g of flour and incubated at room temperature (29±3ºC) for 48 hours. The fungal growth was terminated by drying at 55ºC in oven for 24 hours and re-ground using blender.

2.3.2 Open fermentation

The traditional method of "dawadawa" preparation was used. The raw seeds were boiled for 2 hours. This was then drained through a sieve and the residue wrapped and fermented for 36 hours at room temperature. The end product, a sticky dark-brown, grayish outer layer with a pungent smell is known locally in Nigeria as; "Iru", "dawadawa" or "Nune".

2.4 Proximate Analysis of Raw and Fermented *Glycine max*

The proximate analysis - crude protein, crude lipid, crude fiber, moisture content, total minerals were carried out according to the AOAC [10], except otherwise stated.

2.5 Anti-nutrient Compositions

Tannins, saponins, phytates, flavonoids, and cyanogenic glycosides levels in the raw and fermented *Glycine max* were quantified according to AOAC [10].

2.6 Statistical Analysis

The results were expressed as mean ± standard error of mean (SEM). The data were analysed using Analysis of Variance (ANOVA). Microsoft excels and SPSS version 20 was used for the analysis. The difference between the various fermentation methods were compared using the Duncan range test. P values less than 0.05 ($P<0.05$) were taken as significant.

3. RESULTS AND DISCUSSION

3.1 Proximate Compositions of Raw and Fermented *Glycine max*

The proximate compositions of the *Glycine max* (Table 1) indicates that % crude protein, % moisture, and % crude lipid (ether extract) significantly ($P<0.05$) increased in the open and controlled fermented samples respectively, compared to that in the raw sample. However the % crude fiber, % NFE and % ash significantly ($P<0.05$) decreased in the open and controlled fermented samples respectively, compared to that in the raw sample.

The rise in crude protein may be due to the activity of microorganisms, some of which produces amino acids as secondary metabolites. The dead micro-organisms involved in the fermentation process also could contribute to the increased crude protein content. The ether extract was found to be 13.549% in the raw *Glycine max.* This is in agreement with the ILSI reference standards [11]; and the report by Babalola and Giwa [12].

Moisture content (amount of water vapor) determines the shelf life of food samples. The increased moisture fermentation is an indication of increased susceptibility of food sample to spoilage by microbes. This is in agreement with the observation as reported by Thingom and Chety [13]. The % moisture was lower (2.173) compared the [14] reference standards.

The crude lipid (oil) content increase may be attributed to the low lipase activity of the fermented samples. Low lipase activity in some fermented foods is considered desirable due to the problems of objectionable taste and development of rancidity. Crude fiber increase is in contrast to report by Ganiyu [15].

Table 1. Proximate composition of raw, open and controlled fermented *Glycine max* seeds (%)

Sample	Moisture	Crude fibre	Crude protein	Ether extract	NFE	Ash
RSB	$2.173±.064^a$	$7.061±.079^c$	$25.247±.069^a$	$13.549±.034^a$	$44.176±.123^c$	$7.061±.080^c$
OFSB	$10.033±.064^c$	$1.253±.044^a$	$36.502±.157^c$	$21.474±.283^c$	$27.217±.479^a$	$3.520±.066^a$
CFSB	$9.493±.077^b$	$2.674±.116^b$	$33.208±.339^b$	$15.141±.069^b$	$35.096±.461^b$	$4.392±.003^b$

- *Values are means ± standard error of triplicate determinations*
- *Values in the same column with different superscripts are significantly (P<0.05) different*

Meaning of abbreviations; RSB = raw Soya bean, OFSB = open fermented Soya bean, CFSB = controlled fermented Soya bean, NFE = nitrogen free extract (carbohydrate)

The crude fiber of the unfermented *Glycine max* is higher compared to the fermented samples. This suggests that it contains higher levels of indigestible materials. Indigestible carbohydrates and non-carbohydrates (lignin, cellulose, gums, hemicellulose, pectins) are associated with abdominal extension and flatulence in humans. Crude fiber enhances bowel movement and excretion. But crude fiber corresponded with the ILSI [11]. This however is in contrast to the work of Ganiyu [15].

Nitrogen free extract (NFE) represents the total carbohydrate contents. NFE is higher in the raw beans compared to the fermented beans. This may be due to amylase and sucrase by the fermenting microorganisms which hydrolyses carbohydrates into sugars. This is in line with Giwa et al. [16].

The ash content is a measure of the total amount of minerals present within a sample. The lower amounts in the fermented samples may be due to loss of minerals during fermentation processes- owing to the simulator effects of cooking and leaching. The % ash content in the raw bean was slightly higher compared to the ILSI [11] and USDA-ARS [14].

3.2 Anti-nutrient Compositions

Anti-nutrients are those substances that prevent the availability of certain nutrients to the animal when fed. The anti-nutrients compositions of *Glycine max* (Table 2) significantly (P<0.05) decreased in the fermented samples, and varies between both fermentation methods. The variation in the nutrient contents can be attributable to the differences in fermentation microbes associated with the different methods. The different microbes might have used different nutrient to different extents as sources of energy and protein for their growth and survival the noticeable reduction in the entire anti-nutrients in the fermented samples could be attributed to the activities of the indigenous microbes as well as processing, and the activities of some indigenous enzymes that degrade these anti-nutrients [17].

Phytates are salts of phytic acid. Phytates inhibits non-heme iron absorption, probably by binding to it. Phytate contents were substantially reduced in the fermented samples. This may be due to the specific enzyme activity (phytase).

Saponins significantly reduced from 15.087 mg/100 g in the raw *Glycine max* to 7.861 mg/100 g after open fermentation. Unlike other plant saponins, Soya saponins have only a weak effect on intestinal permeability and therefore have little impact on active nutrient transport [18]. Consequently, Soya bean saponins are not considered to be true anti-nutrients.

Flavonoid is a family of compounds that contributes the blues, reds and purples in plants. Both fermentation processes significantly reduced the total flavonoids composition in the raw sample.

The significant decrease in cyanogenic glycosides (hydrogen cyanide) may be due to the volatility of the toxicant, and this is in agreement with Ekop and Eddy, [19] who reported that total hydrogen cyanide which is often present in food items both as free oxygen cyanide and bound cyanogenic glycosides from enzymatic hydrolysis of decarboxylated amino acids can be greatly reduced by fermentation, and boiling for more than 30 minutes.

Tannins are significantly (P<0.05) reduced in the fermented samples. Tannins bind to proteins, carbohydrates and minerals. To reduce these negative effects; decortication, fermentation, germination, and chemical treatments (HCL, formaldehyde, and alkali) are used [20].

Table 2. Effects of open and controlled fermentation on some antinutrients in *Glycine max*

Sample	Cyanide	Tannins	Phytates	Flavonoids	Saponins
RSB	$0.258\pm.0007^{C}$	$1.431\pm.0065^{C}$	26.140 ± 0.45^{C}	$10.819\pm.0167^{C}$	$15.087\pm.0381^{C}$
OFSB	$0.069\pm.0014^{a}$	$0.774\pm.0214^{a}$	$9.138\pm.0192^{a}$	$8.412\pm.1264^{a}$	$7.8610\pm.1087^{a}$
CFSB	$0.161\pm.0005^{b}$	$1.126\pm.0065^{b}$	$11.554\pm.1905^{b}$	$9.109\pm.0273^{b}$	$10.805\pm.0329^{b}$

- *Values are in mg/100g of sample*
- *Values are means± standard error of triplicate determinations*
- *Values in the same column with different superscripts are significantly (P<0.05) different*

Meaning of abbreviations; RSB = raw Soya bean, OFSB = open fermented Soya bean, CFSB = controlled fermented Soya bean, NFE = nitrogen free extract (carbohydrate)

4. CONCLUSION

Glycine max was observed to be protein and oil rich. *Glycine max* also contains anti-nutrients, the highest was observed to be phytates. These anti-nutrients were significantly reduced by both open and controlled fermentation using *Aspergillus niger*. However a significant variation existed between the two fermentation methods, in terms of anti-nutrients reduction and nutrient increase. These differences are due to different microbes associated with the fermentation methods. There was also significant decrease in the carbohydrate, ash, and fiber contents by fermentation and milling.

From experimental observations, it will therefore be more efficient to create an optimized condition of growth for *Aspergillus niger*, if it is to be used for fermentation in industries or experiments for anti-nutrients reduction and / or nutrient addition. Also the leached water during fermentation should be analysed to determine the total nutrient lost.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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