JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

EFFECTS OF MICROBIAL STRAINS IN A SOLID-STATE FERMENTATION ON QUALITY ATTRIBUTES OF SOYBEAN-HULL

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https://doi.org/10.55251/jmbfs.9876

ARTICLE INFO

Received 8. 2. 2023
Revised 27. 6. 2023
Accepted 29. 6. 2023
Published 1. 10. 2023

Regular article

OPEN ACCESS

ABSTRACT

The by-product of soybean processing, soybean-hull, can have its fibre broken down by solid-state fermentation to improve digestibility and nutrient absorption. This research investigated the impacts of microbial strains in a solid-state fermentation on quality characteristics of soybean-hull. Soybean-hull was inoculated with a mono-culture of fungal (*Aspergillus oryzae*, *Saccharomyces cerevisiae)* and bacterial species (*Bacillus subtilis*, *Lactobacillus plantarum)* while unfermented soybean-hull served as control at (27±2ºC) for 0hour, 24hours, 48hours and 72hours**.** At 24hours of fermentation, pH ranged from 6.17-6.42. TSS decreased significantly ($p < 0.05$) while TTA gradually increased in all samples. Soybean-hull with *L plantarum* at 24hours of fermentation had the highest value of iron (3.18 mg/l). Ca:P interactions were influenced, as there was an increase from 0.15 in the control to 3.45 in *L. plantarum* at 72hours. The protein (4.98-22.42%), lipid (3.58-21.04%), moisture (7.07-8.23%) increased significantly ($P < 0.05$) while carbohydrate (36.29-26.04%) and fibre (60.32-15.97%) decreased as fermentation progresses. Phytate and trypsin inhibitors reduced significantly. Fibre fractions of the fermented substrate decreased except NDS which increased. This study revealed that fermented soybean-hulls innoculated with *Bacillus substilis* and *Aspergillus oryzae* at 72hours offers better nutritional values and could be adopted as a new nutrient source.

Keywords: Soybean-hull, Fibre, Solid-state fermentation, Fungal species, Bacterial species

INTRODUCTION

In the late 1970s, there was intense interest in the health benefits of increasing dietary fibre consumption, which sparked research on soybean-hulls. Dietary fibre is particularly important for the regulation of human bodies since it helps to relieve constipation and lower blood cholesterol by promoting gastrointestinal peristalsis **(Ayo and Kajo, 2016; Surampudi *et al*., 2016**). One of the most widely grown crops in the world is soybean (*Glycine max L.* Merrill). The crop is widely farmed for consumption as non-fermented food products including *beske*, soymilk, tofu, and soy nuts as well as fermented food products like *miso*, *sufu*, and *natto*. In addition to producing edible products, soy processing produces by-products including soy germ, hull, and steamed soybean effluent. In order to add value to these by products, which are the least expensive and contain minerals and phytochemicals that can reduce environmental pollution, the soybean-hull is removed and discarded during the production of this product. The by-product of processing soybeans for soybean oil and soybean meal is the soybean seed coat, sometimes referred to as soy hulls. It accounts for roughly 8–10% of the weight of the soya bean grain (**Van and Ruiz, 2021).** The effectiveness of the dehulling process determines the chemical composition of soybean-hulls, which can vary in their content of cellulose (29–51%), hemicelluloses (10–25%), lignin (1–4%), pectins (4–8%), proteins (11–15%), and minor extractives (**Karp *et al*., 2020**). Dry soybean-hulls are mostly composed of 85.7% carbohydrates, 9% protein, 4.3% ash, and 1% lipids **(Caballero *et al*., 2016**). Alphacellulose and hemicelluloses make up the majority of the carbohydrates found in soybean-hulls; because they contain less lignin, ruminant animals can digest these carbs with ease. They are practically similar to grains in terms of digestible energy content because they are so highly digested. Soybean-hulls also reduce the incidence of acidosis when added to diets high in fodder **(Salgado-Bautista *et al*., 2020**). However, only a limited number of bacteria can perform Solid-state fermentation, which happens when microbes grow on solid substrates without the availability of free water. Because their hyphae may grow on particle surfaces and pierce into inter particle voids, fungi are well adapted to SSF and can colonize solid substrate **Salgado-Bautista *et al.* (2020)**. The ability of *Aspergillus oryzae* to manufacture several enzymes, primarily xylanase and cellulase, has been investigated. Although SSF has demonstrated a great potential for generating valuable products, various challenges still need to be resolved, such as the substrate's lack of homogeneity and the need to regulate process variables (**Bahry *et al*., 2017**). If these issues are sufficiently addressed and resolved, SSF can play a significant role in emerging biotechnologies. To improve its digestibility, delay stomach emptying, and increase nutritional absorption in the body, SSF may have a significant potential to produce enzymes to break down the fibre in soybean-hulls (**Shahab *et al*., 2022**). The objective of this study was to use biotechnological knowledge to introduce some microbial strains into a solid state fermentation to utilize soybean-hulls. This was done by looking into the possibility of using the hulls' flour, which would reduce waste, a major problem in the soybean processing industry, and further break down the hull's fiber content to make it more easily digestible and utilized by the body.

MATERIAL AND METHODS

Sources of raw materials / ingredients and preparation of soybean-hull flour

Soybean-hulls from soybeans seeds *(Glycine max)* were obtained as a waste during the processing of beske, soy sauce and soymilk etc which was purchased from Ilorin Metropolis, Kwara State, Nigeria. Starter cultures (*Bacillus subtilis* (ATCC 6633), *Lactobacillus plantarum* (ATCC 8014), *Aspergillus oryzae* (ATCC 17891), *Saccharomyces cerevisae* (ATCC 3455) used to ferment the soybean-hulls were obtained from the analytical laboratory of Federal Institute of Industrial Research, Oshodi (FIIRO-ANALAB), Lagos state, Nigeria.

Inoculation of soybean-hull for mono-culture fermentation

Fungal and bacterial suspension of actively growing mid-log phase culture of each starter were prepared following the modified method adopted by **(Kayode and Sani, 2008).** A 1 kg of autoclaved soybean-hull was mixed with 1 litre of sterile distilled water in 13 different fermenters and stirred properly to obtain a uniform mash. Twenty millilitres from each of the mono-culture suspension (5×10^4 spore/ml) and (1×10^8 CFU/ml) were used as fermentation starter to inoculate each of the soybeans-hull in the fermenters before the commencement of fermentation at ambient temperature (27 ± 2 ºC) for 24 hours, 48 hours,72 hours and unfermented soybean-hull (0 day) was served as control.
Soybean-hull flour was produced as described by **Olaoye *et al*. (2006).**

Experimental Design

A total of thirteen samples consisting of four starter organisms were used for fermentation, three fermentation period and and unfermented soybean-hull (0 day)

as control. The microbial strains were inoculated for the fermentation of soybean-hull in this study. Data obtained were analyzed using Factorial design.

Physicochemical properties of fermented soybean-hull

The pH, titratable acidity and Total soluble solids (TSS) analysis were carried out using standard methods **(Omogie and Ogunsakin, 2013; Ashogbon and Akintayo, 2014; Khairul, 2014).** The L * a * b *(colour) parameters of the fermented soybean-hull samples at 72hours were determined using a Chroma meter **Oyeyinka *et al*. (2020)**

Determination of nutrient composition of fermented soybean-hull flour

The proximate compositions of fermented soybean-hull flour samples were analyzed using the standard methods of **(AOAC, 2012).** Mineral composition of fermented soybean-hull flour was determined as samples were first digested with nitric acid and Hydrochloric acid and then the aliquots were used for the determination of phosphorus, sodium, potassium, calcium, iron and magnesium content. Phosphorous was determined by spectrophotometer while Sodium and potassium were determined by flame photometer **(Khalil and Manan, 1990)**. Iron, calcium and magnesium were determined by atomic absorption spectrophotometer **(AOAC, 1990).** Standard stock solutions were prepared for each metal using suitable metal salts of each metal to prepare a standard curve, **(AOAC, 2012).**

Determination of functional properties of fermented soybean-hull

Bulk density, water absorption capacity (WAC), oil absorption capacity (OAC), swelling capacity (SC), and least gelation (LG) were done as described by **Ogodo *et al.* (2018).** Water solubility index (WSI) was determined according to the method described by (**Feyera, 2021).**

Total microbiological count of the fermented soybean-hull

Sample and media preparation was prepared according to manufacturer's specifications and sterilized at 121 ºC, 15psi for 15 minutes **Carraturo *et al*. (2018).**
Total bacteria counts was determined using the method of **(Jideani and Jideani, 2006)**. The total fungal count was analyzed as described by **Abdel *et al*. (2011)**. Visible fungal colonies were counted and expressed as colony forming units per gramme (cfu/g). Total coliform count was carried out according to the method described by (**Lawal, 2018).**

Determination of anti-nutrient composition and fibre fractions of the fermented Soybean-hull flour

The phytate content was determined using the method described by **Omoboyowa *et al.* (2015).** The trypsin inhibitor was determined according to the method descried by **(Prokopet and Unlenbruck, 2002).** The method of forage evaluation was used to determine the fibre fractions of fermented substrate **(Van Soest, 1982**)

Determination of particle size of fermented Soybean-hull flour

Particle size distributions of each of the fermented soybean-hull flour samples were determined as described by **(ASAE, 2003)** at mesh sizes approximately $\leq 20\mu$m and $> 20\mu$m

Statistical analysis

Results are expressed as the mean of two replícates ± standard deviation (SD) and analyzed using One-way ANOVA. Significant differences between means were assessed by the Duncan Post Hoc test, and $P \leq 0.05$ was considered statistically significant. Statistical analysis was done using statistical package for the social sciences (SPSS) software, version 20 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Effect of microbial species and period on the physicochemical quality of fermented soybean-hull flour

The pH of fermented soybean-hull varied significantly ($p \leq 0.05$) as fermentation progresses and greatly influenced by the activities of different microbial species, results are shown in figure 1. During the first 24 hours of fermentation, pH ranged from 6.17 to 6.42. *A. oryzae* and *L. plantarum* resulted into reduced pH when compared to the pH obtained for the 0 hour (control) of fermentation. At 48 hours of fermentation, only slight decrease was observed in *S. cerevisiae* and *L. plantarum*, while pH of *B. subtilis* fermented soybean-hull remain the same. However, increase in pH was recorded in all samples after 72 hours of fermentation except for *L. plantarum* fermented hull that had slightly reduced pH. The variation in the pH values at different fermentation time by different microbial species may be attributed to the effect of pH on the activities of different microbial strains and production of organic acids at different concentrations. Authors reported a reduction in pH for solid-state fermentation with different yeast strains **(Kasprowicz-Potocka and Zaworska-Zakrzewska, 2016)** and *L. plantarum* **Canibe *et al*. (2008).** The pH of a flour product is of great important since some functional properties such as solubility, emulsifying activity and foaming properties are affected by it **Osungbade *et al*. (2016).** The pH is also a good quality indicator for the values obtained in this study, it is devoid of characteristic sour aroma and taste due to fermentation, which makes the soybean-hull flour fermented by different microbial species desirable for use in bakery products **(Edwards, 2007).**
The total soluble solids (TSS) of fermented soybean-hull were found to decrease significantly ($p < 0.05$) from the initial value of 6.77 % brix to 6.13, 6.21, 5.90 and 6.07 % brix in *A. oryzae, S. cerevisiae, B. subtilis and L. plantarum* fermented soybean-hull respectively. Thereby it shows that this organisms may use the sugar for their growth during fermentation. After 72 hours of fermentation, *B. subtilis* fermented soybean-hull had the least total dissolved solids, while *S. cerevisae* fermented soybean-hull resulted in the highest total dissolves solids (Table 1). The high value obtained with *S. cerevisiae* may be attributed to its fermentative and enzymatic ability of *S. cerevisiae* to convert complex organic substances into simpler substances. Fungi strains have been studied for their ability to produce different enzymes mostly xylanase and cellulase which is capable of breaking down xylose and cellulose respectively, present in fibre of soybean-hull. This is in line with the findings of **(Lubis, 2019)** who attributed increase TSS of fermented soybean residue to the degradation and biological hydrolysis of dissolved solids.
Total titrable acidity (TTA) gradually increased ($p < 0.05$) from the initial value of 110 to 305, 267, 255 and 325 mg/l in *A. oryzae, S. cerevisiae, B. subtilis and L. plantarum* fermented soybean-hull (Figure 2) respectively. During the first 24 hours of fermentation, The *L. plantarum* fermented soybean-hull had the highest TTA while *S. cerevisiae* fermented soybean-hull recorded the lowest TTA. It can be observed that the TTA increased as fermentation progresses, resulting into higher TTA at the end of fermentation period. However, the TTA obtained in this study is still relatively high compared to the value obtained by other researchers in solid-state fermentation using similar substrate. Higher TTA is important to inhibit the proliferation of undesirable microorganisms that may cause poor fermentation **Mbata *et al*. (2009).** This is also in accordance with the findings of Olukomaiya ***et al*. (2020a)** who suggested that the increase in TTA values can discourage the rapid multiplication of unwanted microorganisms that may induce poor fermentation which may lead to unacceptable products. This suggests that the fermented soybean-hull obtained in this study can be used for wide variety of food applications due to good fermentation as a result of TTA.

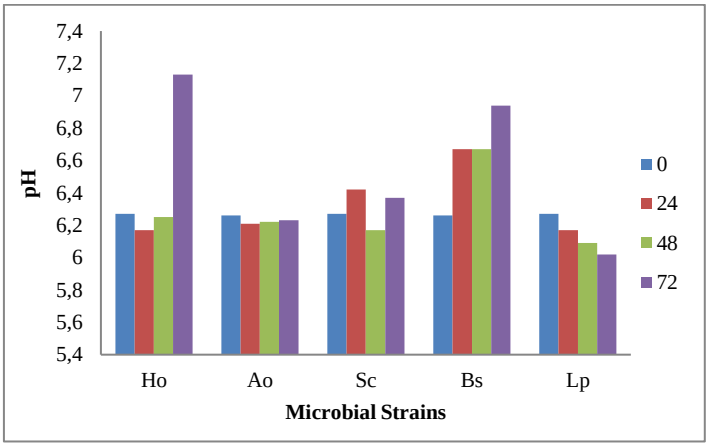

Figure 1 Effect of microbial species and period on the pH of fermented soybean-hull flour
Values are expressed as mean of two replicates ± SD Means with the different superscripts across a row are significantly ($P \leq 0.05$) different. $H_{0\,(control)}$ = unfermented hull; A_o = *Aspergillus oryzae*; S_c = *Saccharomyces cerevisiae*; B_s = *Bacillus subtilis*; L_p = *Lactobacillus plantarum*

Table 1 Effect microbial species and period on TSS (%brix) of fermented soybean-hull

Microbial Isolates	Fermentation period (Hours)			
	0	24	48	72
Unfermented soybean-hull	6.77^{a}±0.01	6.70^{a}±0.00	6.40^{a}±0.00	6.33^{a}±0.06
Aspergillus oryzae	6.77^{a}±0.01	6.47^{c}±0.06	6.22^{b}±0.01	6.13^{bc}±0.06
Saccharomyces cerevisiae	6.76^{a}±0.01	6.57^{b}±0.06	6.43^{a}±0.06	6.21^{b}±0.01
Bacillus subtilis	6.77^{a}±0.01	6.40^{d}±0.00	5.90^{c}±0.10	5.90^{d}±0.10
Lactobacillus plantarum	6.77^{a}±0.01	6.50^{c}±0.00	6.13^{b}±0.06	6.07^{c}±0.06

Values are expressed as mean of three replicates ± SD Means with the different superscripts across a row are significantly (P≤0.05) different

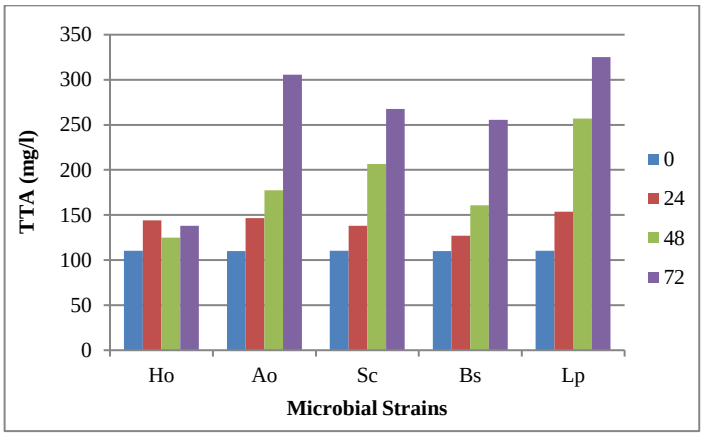

Figure 2 Effect of microbial species and fermentation period on titratable acidity (mg/l) of soybean-hull

Values are expressed as mean of two replicates ± SD Means with the different superscripts across a row are significantly (P≤0.05) different. $H_{0\ (control)}$ = unfermented hull; A_o = *Aspergillus oryzae*; S_c = *Saccharomyces cerevisiae*; B_s = *Bacillus subtilis*; L_p = *Lactobacillus plantarum*

Effect of microbial species and period on the colour determination of fermented soybean-hull flour

Colour determination shows that the L * (Lightness) values of the samples varied between 30.70 ± 0.83 and 50.28 ± 0.06, lowest and highest values were recorded for samples $H_{0\ (control)}$ and *A. oryzae* at 72 hours, respectively. a * (-ve = green, +ve = red) values ranged from 3.77 ± 0.30 to 7.48 ± 0.06 with the lowest and highest values recorded for samples $H_{0\ (control)}$ and *A. oryzae* at 72 hours, respectively, as shown in table 2. The values were positive indicating that the a * values tended towards the red axis. b * (-ve = blue, +ve = yellow) values ranged from 8.23 ± 0.02 to 14.44 ± 0.06 with samples $H_{0\ (control)}$ and *A. oryzae* at 72 hours having the lowest and highest values, respectively. The b * values were positive indicating that values tended towards the yellow axis. Furthermore, L *, a * and b * the redness (a*) values for fermented substrate flour samples were significantly (P≤0.05) higher than the control. Variation in colour values may be attributed to the various microbial strains used in the fermentation of the hull.

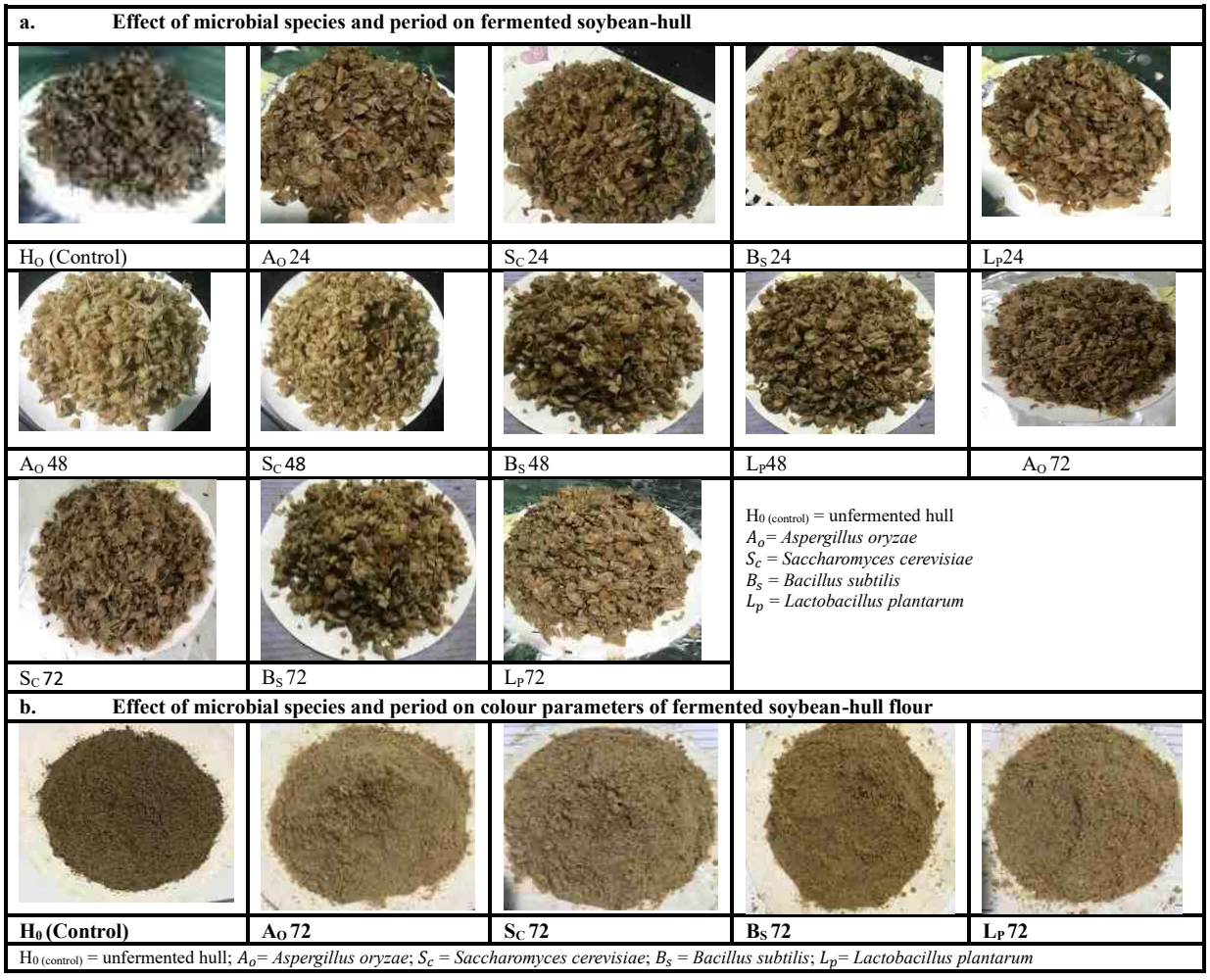

Figure 3 Effect of microbial species and period on the colour parameters of fermented soybean-hull flour

The brightness with high L * value in fermented substrate plays a crucial role in food development due to consumer preferences, especially in bakery products that claim to be good or excellent sources of dietary fibre. In this study, the colour in fermented soybean-hull using different microbial strains showed brighter than that of the unfermented hull, obviously revealing that fermentation process significantly affects the pigment change. Therefore, it is likely that the yellow colour of the hull can be primarily attributed to phenolic pigments in the seed coats, particularly flavonoids and partially anthocyanins. Differences in color parameters could be attributed to nonenzymatic maillard browning which occurred under the conditions prevailing during the drying process, which would favor color change **(Kolawole and Oyeyinka, 2014)**. This investigation showed that the colour was produced by the fungus *saccharomyces* itself either in the spores or the mycelium as a result of microbial activity. This shows that when the fungus consumes nutrients, it starts producing spores and mycelium; at the same time, it also produces colour, protein and other primary and secondary products, which are related to the growth of the fungus. From a theoretical point of view, it is clearly demonstrated that the colour produced increased with fermentation time in fungal submerged fermentation (SmF); this can be explained by changes in the chemical composition (colour in this case) of the fungus itself and by microbial activity as a result of solid substrate degradation by fungi to acquire nutrients for growth. Changes in the colour of the fermented substrates during SmF can be related to changes in the cell population **(Musaalbakri and Colin, 2018)**.

Table 2 Effect of microbial species and period on colour parameters of fermented soybean-hull

Microbial Isolates	L*	a*	b*
$H_{0(control)}$	30.70^{e} ±0.83	3.77^{d}±0.30	8.23^{c}±0.02
A_{O72}	50.28^{a} ±0.06	7.48^{a}±0.06	14.44^{a}±0.06
S_{C72}	48.82^{b}±0.42	7.10^{b}±0.03	14.17^{a}±0.04
B_{S72}	43.53^{d}±0.49	6.62^{c}±0.01	11.87^{b}±0.32
L_{P72}	46.38^{c}±1.20	6.95^{bc}±0.04	12.23^{b}±0.02

Values are expressed as mean of two replicates ± SD Means with the different superscripts across a row are significantly (P≤0.05) different. $H_{0\,(control)}$ = unfermented hull; A_o = *Aspergillus oryzae*; S_c = *Saccharomyces cerevisiae*; B_s = *Bacillus subtilis*; L_p = *Lactobacillus plantarum*; L*: Lightness; a*: Redness; b*: yellowness

Effect of microbial species and period on proximate composition of fermented soybean-hull flour

There were significant variations in the proximate composition of soybean-hull flour fermented for 72 hours using *A. oryzae* (A_o), *S. cerevisae* (S_c), *B. subtilis* (B_s) and *L. plantarum* (L_p). Result are shown in the table 3.

Crude protein of fermented soybean-hull flour varied significantly with values ranging from 4.98% to 22.42% for unfermented (control) soybean-hull and *A. oryzae* at 72 hours fermentation respectively. **Olukomaiya *et al*. (2020b)**, reported a similar increase in protein for camelina meal fermented in solid-state using food grade *aspergillus* fungi. The increase in protein content after fermentation may be due to a decrease of carbon ratio in the total mass, resulting in redistribution of nutrient percentages **Ojokoh *et al*. (2011).** A fermentation involving *A. oryzae* and *B. subtilis* showed consistent increase in crude protein during fermentation which occurs due to microbial growth **Teng *et al*. (2012)**. However, the proportion of soluble protein increased from 19.4% to 63.11%, respectively, which may occur due to stronger hydrolysis by *B. subtilis* under fermentation conditions. The increase in crude protein may be explained by two processes. Firstly, the increased crude protein observed could be due in part to the decrease in carbohydrate. Some bacteria can break down cellulose, polysaccharides, and oligosaccharides and utilize its sugar subunits for their metabolism and respiration processes.

During the first 24 hours of fermentation the activity of *S. cerevisae* resulted into the highest crude protein (15.75%) of soybean flour, while *B. subtilis* and *L. plantarum* showed no significant differences. However, during 48 and 72 hours of fermentation the activity of *B. subtilis* and *A. oryzae* resulted into the highest protein content 19.58% to 22.42% respectively during these hours of fermentation. Also, it can be observed that there was no significant difference in the protein content of *S. cerevisae*, *B. subtilis* and *L. plantarum* after 72 hours of fermentation. It can be observed that the protein content of soybean-hull increased with increasing fermentation time resulting in higher protein content during the last hours of fermentation period (72 hours). The increase could be as a result of the enzymatic hydrolysis of some protein inhibitors during fermentation. It may also be due to the higher amounts of structural proteins, which are an integral part of the microbial cell, as fermentation progresses (**Achi, 2005)**.

Fermentation time and microbial species had significant effect on the carbohydrate content of soybean-hull. The values obtained for carbohydrate varied significantly ($p>0.05$) from 16.95 to 36.29 %. The peak carbohydrate was recorded for *A. oryzae* during 24 hours while the control sample had the least carbohydrate content. During the first 24 hours of fermentation, the carbohydrate content of all sample were at their peak, which was found to decrease as fermentation progresses. This is in line with the findings of **Granito *et al*. (2002)** and **Kasprowicz-Potocka *et al.* (2018)**, who reported lower carbohydrate content of fermented common beans and lupin flour. The decrease in carbohydrate content of fermented soybean-hull may be attributed to breakdown of carbohydrate and use of by-product (glucose) as a source of energy by microorganism. It can therefore be suggested that the microbial species employed for fermentation in this study resulted into reduction carbohydrate content of soybean-hull at different fermentation time via hydrolysis of polysaccharides into glucose. According to **(Kaczmarska *et al*., 2017)** fermentation increased the glucose content of lupin and soy flours, which was attributed to break down of starch by microbial species. *S. cerevisae* at 72 hours had the least carbohydrate content which is not significantly different from value obtained with *A. oryzae* at the same fermentation time which differs significantly with the values obtained during 24 and 48 hours of fermentation. This may be attributed to fermentative ability of the fungi which might have activated the release of bound sugar during solid-state fermentation, thus, the observed decrease in the carbohydrate.

The crude lipid of fermented soybean-hull ranged from 3.58% to 21.04% for control at 0 hr and *A. oryzae* at 48 hours of fermentation. The low-level crude lipid is not surprising, as soybean-hull contain low amount of lipid compared to the whole soybean seed. However, increase in crude lipid was recorded as fermentation progresses. This may be attributed to continuous fermentation activities of selected microbial strains used in this study. Also, the increase in crude lipid may also be attributed to reduction in carbohydrate content during SSF. The result of this study is in line with the findings of **(Kasprowicz-Potocka and Zaworska-Zakrzewska, 2016)** who reported increase in crude fat content fermented legumes using various yeast strains.

There were some significant ($p < 0.05$) differences in the moisture content of fermented soybean-hull ranging from 7.07% to 8.23%. *S. cerevisae* at 24 hours and 72 hours had the lowest and highest moisture content. This differs slightly with the findings of **(Ayo and Kajo, 2016)** were he discussed on the pre-treatment of soybean-hull for the production of acha biscuit. The variation in moisture content may not be reliably attributed to varying activities of microbial species but may also be attributed to the different drying methods and packaging used by different researchers. However, the moisture obtained is relatively low and within the recommended value for composite flour. The relatively low moisture content obtained in this study can be considered as an added advantage as it could improve keeping quality of the flour and reduce cost in terms of packaging preservation of transportation **Elleuch *et al.* (2011)**

Similar to moisture, total ash values of fermented soybean-hull flour were relatively low and varied significantly ($p < 0.05$) with values ranging from 5.13% in $H_{o\,(control)}$ to 8.40 % for soybean-hull flour fermented using *S. cerevisae*. The total ash values obtained in this study was observed to increase after the first 24 hours of fermentation. The increase in ash content is an indication of increase in mineral constituents/ component in soybean-hull flour and could make the flour a good source of minerals. This is similar to the findings of **(Alabi and Anuonye, 2007)**, who suggests that ash content is an indication of the presence of mineral content in foods, which is a non-organic compound containing the mineral content of food. Values in the range from 15.97 to 60.32 % were recorded as crude fibre content of fermented soybean-hull flour (Table 3). The high level of fibre is expected particularly at the initial time of fermentation and the unfermented soybean-hull flour, since soybean-hull is known to be rich source of both soluble and insoluble dietary fibre **Dust *et al*. (2004).** Soybean-hull samples from $H_{o\,(control)}$ and *B. subtilis* had the highest and least crude fibre content respectively. During the first 24 hours of fermentation, the activities of *B. subtilis* $_{resulted}$ into the least crude fibre content (27.49 %) when compared to the activities of other microbial species at the same fermentation time. However, as fermentation progresses crude fibre content soybean-hull continue to decrease, while some samples increased slightly. It can be observed that the crude fibre content obtained from *B. subtilis* at 72 hours is not significantly different from the values obtained with *A. oryzae* and *B. subtilis* at 48 hours. This suggests that different microorganism's ferment and reduce fibre at different fermentation times. The reduction fibre content, particular by the activities of fungi, may be attributed to the capacity of fungi to produce enzymes such as hemicellulases and pectinase which is capable digesting the dietary fibre and degrading crude fibre and complex polysaccharides of soybean-hull **Mathivanan *et al*. (2006)**. **Song *et al*. (2008)**, reported in their studies conducted using *S. cereviseae* and bacterial strains *L. plantarum, that* both fermentations resulted in breakdown of larger molecule of soybean meal.

Table 3 Effect of microbial species and fermentation period on proximate composition of soybean-hull flour

Microbial Isolates	Crude protein (%)	Crude lipid (%)	Moisture content (%)	Total ash (%)	Crude fibre (%)	Carbohydrate (%)
$H_{0(control)}$	4.98^{g}±0.08	3.58^{f}±0.30	7.82^{b}±0.23	5.13^{g}±0.49	60.32^{a}±1.20	16.95^{f}±0.15
A_{O24}	6.13^{f}±0.93	13.59^{e}±0.14	6.51^{e}±0.10	8.21^{ab}±0.30	30.38^{bc}±1.17	36.36^{a}±0.54
S_{C24}	15.75^{e}±0.93	13.96^{e}±1.11	7.07^{d}±0.10	6.92^{ef}±0.02	30.05^{bc}±0.54	26.27^{bcde}±0.30
$\boldsymbol{B_{S24}}$	14.55^{e}±0.16	14.13^{e}±0.26	7.41^{cd}±0.04	6.74^{f}±0.33	27.49^{cd}±4.34	29.70^{bc}±3.56
$\boldsymbol{L_{P24}}$	14.55^{e}±0.47	13.95^{e}±0.25	7.39^{cd}±0.34	7.48^{cde}±0.03	33.84^{b}±1.05	22.80^{e}±0.02
$\boldsymbol{A_{O48}}$	14.66^{e}±0.31	21.04^{a}±0.23	7.82^{b}±0.06	7.22^{def}±0.35	18.99^{ef}±0.13	30.29^{b}±0.08
$\boldsymbol{S_{C48}}$	17.50^{d}±0.31	16.95^{d}±0.16	7.35^{cd}±0.13	7.70^{bcd}±0.37	22.04^{e}±0.11	28.47^{bcd}±0.08
$\boldsymbol{B_{S48}}$	19.58^{bc}±0.47	18.94^{c}±0.76	8.05^{ab}±0.10	8.19^{ab}±0.26	18.80^{ef}±0.24	26.45^{bcde}±0.23
$\boldsymbol{L_{P48}}$	18.60^{cd}±0.62	17.38^{d}±0.11	7.98^{ab}±0.13	8.25^{ab}±0.35	23.17^{de}±2.27	24.73^{de}±1.05
$\boldsymbol{A_{O72}}$	22.42^{a}±0.47	19.96^{b}±0.34	7.43^{c}±0.03	7.63^{bcd}±0.24	19.22^{ef}±1.11	23.35^{e}±0.04
$\boldsymbol{S_{C72}}$	20.46^{b}±0.77	17.83^{d}±0.14	8.23^{a}±0.05	8.40^{a}±0.09	22.72^{de}±2.18	22.38^{e}±1.13
$\boldsymbol{B_{S72}}$	20.24^{b}±0.15	19.90^{b}±0.48	8.07^{ab}±0.09	7.48^{cde}±0.03	18.29^{ef}±3.29	26.04^{cde}±2.54
$\boldsymbol{L_{P72}}$	20.02^{b}±0.16	19.98^{b}±0.08	7.47^{c}±0.18	7.96^{abc}±0.06	15.97^{f}±4.43	26.61^{bcd}±3.95

Values are expressed as mean of two replicates ± SD Means with the different superscripts across a row are significantly (P≤0.05) different.$H_{(control)}$ = unfermented hull; A_o= *Aspergillus oryzae*; S_c = *Saccharomyces cerevisae*; B_s = *Bacillus subtilis*; L_p= *Lactobacillus plantarum*

Effect of microbial species and period on mineral composition of fermented soybean-hull flour

The values obtained for calcium (Ca) content of fermented soybean-hull (5.20 to 12.20 mg/ml) varied significantly (p < 0.05). The highest value was recorded for unfermented soybean-hull, while *L. plantarum* at 72 hours had the least value. It can be observed as shown in table 4 that the calcium content of fermented soybean-hull decreased significantly as fermentation progresses irrespective of microbial strains. This suggests that the activities of yeast and bacteria resulted into the reduction of calcium. This may be as a result of the breakdown of starch and protein structure of soybean-hull, which results into loss of calcium.

Generally, fermentation of soybean-hull improved the value of magnesium (Mg), potassium (K) and phosphorus (P). *A. oryzae* and *S. cerevisiae* had significant influence on Mg with values ranging from 53.00 to 60.00 mg/l, while *B. subtilis* fermented hull had the most significant influence of the value of K and P. **Ugwuona *et al*. (2021)** reported a similar trend, these authors stated clearly that fermentation of soybean resulted in improve mineral concentration of soybean flour. This was also in accordance with the findings of **Darwish *et al*. (2012)**, who observed increased mineral content in maize stalks after SSF by *S. cerevisiae*.

Fermentation of soybean-hull was observed to increase phosphorus content of fermented soybean-hull. This is in accordance to the findings **Kim *et al*. (2009)** for fermented soybeans meal. The high concentration of Mg, K and P recorded in this study suggests that fermented soybean-hull could serve as rich source of Mg, K and P which will be useful in the fortification of food products low in these minerals. It can also serve as innovative product for tackling malnutrition resulting deficiency of these minerals.

Iron appears to be the least concentrated and available nutrients in unfermented soybean-hull, which was not significantly improved by fermentation by the activities of any of the selected microbial species except *L. plantarum* during the first 24 hours of fermentation. This simply suggest that fermented soybean-hull flour might require fortification to improve its iron concentration. Iron is known to play important role in human body because it is a component of haemoglobin. It helps in oxygen transport and, together with haemoglobin and ferrodixin, it plays a vital role in man's metabolism **(Okwu, 2001)**. **Martino *et al*. (2007)** reported that heat-treated soybean-hull flour had a high content of iron and low content of phytate, which favors iron bioavailability.

Values ranging from 9.35 to 16.40 mg/l were recorded for sodium (Na), *L. plantarum* at 48 hours had the highest value while the lowest value was recorded for *S. cerevisiae* at 72 hours of fermentation. The concentration of Na was also observed to be influenced by fermentation. All fermented samples had an increased Na concentration when compared with the unfermented sample except S_c at 72 hours. Asides from magnesium, potassium, phosphorus which were improved by fermentation, the other minerals reduce with increasing fermentation time. This decrease may be attributed to possible leaching of the soluble mineral elements into the fermenting medium, or due to the utilization of the minerals by the fermenting micro-flora for their biochemical activities, while the increasing amount of Mg, K, P and Na may be attributed to the reduction of the anti-nutritional factors binding them. This suggested fact agrees with the findings of by **Thomas *et al*. (2017)**. The fermentative activities of selected microbial species used in this study seem not to favour sodium (Na) to potassium (K) ratio interaction. However, *L. plantarum* and *A. oryzae* resulted into signification interaction between Na and K with values of 17.30 and 6.15 during 48 and 72 hours of fermentation respectively, Also, calcium to phosphorus ratio interactions was badly influenced,), *L. Plantarum* resulted into a favourable interaction, with values increase from 0.15 in control to 3.45 in *L. plantarum* during 72 hours of fermentation. This is similar to the findings of **Darwish *et al.* (2012)** for maize husk fermented using *S. cerevisiae* in SSF. Sodium is an important mineral from medicinal point of view; although too much consumption of sodium rich foods or substances used as food additives should be discouraged for people prone to have high blood pressure. Potassium is important for reducing blood pressure and also increasing blood circulation, as well as preventive aid on general health of the heart, calcium helps in transporting of long chain fatty acid which helps in prevention of heart diseases, high blood pressure and other cardiovascular diseases. It was reported that magnesium works with calcium to transmit nerve impulse in the brain. Magnesium also has calming effect and works on the nervous system of the people with depression **Aslam *et al*. (2005)**.

Table 4 Effect of microbial species and period on mineral concentration of fermented soybean-hull flour

Microbial Isolates	Calcium (mg/l)	Magnesium (mg/l)	Potassium (mg/l)	Phosphorous (mg/kg)	Iron (mg/l)	Sodium (mg/l)	Na:K	Ca:P
$\boldsymbol{H_{0(control)}}$	12.20^{a}±0.14	54.50^{bcde}±2.12	150.00^{de}±0.00	82.50^{f}±0.01	1.39^{c}±0.06	10.25^{j}±0.07	0.07^{f}±0.00	0.15^{e}±0.00
$\boldsymbol{A_{O24}}$	6.80^{f}±0.00	57.50^{abcd}±0.71	2.00^{h}±0.00	91.50^{e}±0.00	0.49^{ij}±0.00	12.30^{g}±0.28	6.15^{b}±0.14	0.07^{fg}±0.00
$\boldsymbol{S_{C24}}$	7.95^{c}±0.07	52.50^{cde}±2.12	140.00^{e}±0.00	91.00^{e}±1.41	0.74^{f}±0.01	14.10^{c}±0.14	0.10^{ef}±0.00	0.09^{f}±0.00
$\boldsymbol{B_{S24}}$	8.45^{b}±0.07	52.00^{de}±1.41	150.00^{de}±0.00	434.50^{a}±0.71	0.70^{f}±0.00	11.70^{h}±0.42	0.08^{f}±0.00	0.02^{h}±0.00
$\boldsymbol{L_{P24}}$	7.45^{d}±0.07	49.50^{e}±0.71	75.00^{f}±7.07	125.00^{b}±1.41	3.18^{a}±0.00	15.30^{b}±0.99	0.21^{e}±0.04	0.06^{g}±0.00
$\boldsymbol{A_{O48}}$	6.60^{gh}±0.14	59.50^{abc}±2.12	2.00^{h}±0.00	51.45^{h}±0.07	0.84^{e}±0.01	11.15^{i}±0.21	5.58^{c}±0.11	0.13^{e}±0.00
$\boldsymbol{S_{C48}}$	6.50^{h}±0.00	60.00^{ab}±0.00	190.00^{b}±14.14	77.49^{g}±0.14	0.53^{i}±0.01	12.65^{f}±0.07	0.07^{f}±0.01	0.08^{fg}±0.00
$\boldsymbol{B_{S48}}$	5.95^{i}±0.07	53.00^{bcde}±1.41	205.00^{a}±7.07	29.90^{i}±0.14	0.64^{g}±0.01	11.85^{h}±0.07	0.06^{f}±0.00	0.20^{d}±0.00
$\boldsymbol{L_{P48}}$	7.50^{d}±0.00	39.00^{f}±0.00	1.00^{h}±0.00	8.45^{k}±0.07	0.60^{h}±0.01	16.40^{a}±1.27	17.30^{a}±0.00	0.89^{b}±0.01
$\boldsymbol{A_{O72}}$	6.70^{fg}±0.14	58.00^{abcd}±8.49	170.00^{c}±0.00	97.48^{d}±0.03	1.85^{b}±0.01	11.15^{i}±0.07	0.07^{f}±0.00	0.07^{fg}±0.00
$\boldsymbol{S_{C72}}$	7.15^{e}±0.07	41.50^{f}±3.54	140.00^{e}±0.00	102.45^{c}±0.07	1.22^{d}±0.01	9.35^{k}±0.07	0.07^{f}±0.00	0.07^{fg}±0.00
$\boldsymbol{B_{S72}}$	6.65^{fgh}±0.07	64.00^{a}±4.24	18.00^{g}±0.00	25.49^{j}±0.02	0.47^{j}±0.01	12.95^{e}±0.07	0.72^{df}±0.00	0.26^{c}±0.00
$\boldsymbol{L_{P72}}$	5.20^{j}±0.00	53.00^{bcde}±1.41	155.00^{d}±7.07	1.510^{l}±0.01	0.46^{j}±0.01	13.20^{d}±0.71	0.09^{f}±0.01	3.45^{a}±0.04

Values are expressed as mean of two replicates ± SD Means with the different superscripts across a row are significantly (P≤0.05) different. $H_{0\ (control)}$ = unfermented hull; A_o= *Aspergillus oryzae*; S_c = *Saccharomyces cerevisae*; B_s = *Bacillus subtilis*; L_p= *Lactobacillus plantarum*.

Effect of microbial species and period on functional properties of fermented soybean-hull flour

The functional properties of fermented soybean-hull using selected microbial species are shown in table 5. The values obtained for oil absorption capacity (OAC) and bulk density of soybean hull flour hwere significantly (> 1%) low for all microbial species throughout the fermentation period. However, slight significant (p < 0.05) variations were recorded, the values obtained ranged from 0.44 g/100g to 60 g/100g. soybean-hull flour from *L. plantarum* had the least OAC while that of *S. cerevisiae* recorded the highest value. This is quite different from the findings of **(Ayo and Kajo, 2016)** for fermented soybean-hull flour supplemented with acha flour for biscuit. The low OAC obtained in this study may be attributed to low

exposure of the hydrophobic interaction sites of fermented soybean-hull samples irrespective of the microbial strains. However, the low value obtained suggest that the flour may be used in the production of food product requires only small amount of oil. According to **(Elkhalifa and Bernhardt, 2010)** the binding of oil is correlated with hydrophobic binding sites of amino acid present on the surface of product. The low oil absorption capacity of fermented Soybean-hull flour may serve as an innovative way to reduce the absorption of oil in certain food products such as biscuit.

Water absorption capacities (WAC) vary significantly ($p<0.05$) among the flour samples. The values obtained ranged from 2.63 g/100g to 2.83 g/100g for *B. subtilis* and *A. oryzae* respectively, both at 48 hours of fermentation. The values obtained in this study disagrees with the values obtained (1.50-1.92 g/ 100g) by **(Ayo and Kajo, 2016)** for soybean-hull supplemented acha composite flour. The variation in WAC may be attributed to variation in flour supplements, the processing or pretreatment applied by different researchers to the Soybean-hull flour. The relative increase in the water absorption could be linked to the hydroxyl and carboxyl groups of the cellulose and hemicelluloses present in the soybean-hulls which have strong affinity for water **(Ayo and Nkama, 2004)**. It can also be observed that WAC of fermented soybean-hull increase slightly after the first 24 hours of fermentation and decrease towards the last hour of fermentation. This may be attributed to activities of microbial strains on the hulls, leading to break down of starch, protein and fibre component of Soybean-hull. WAC explains the ability of flour product to associate with water under a condition where there is limiting water available for binding **Omueti *et al.* (2009)**. Higher WAC means higher starch, better water absorption and better swelling. The relatively high WAC obtained in this study may suggest lower water absorption and reduced swelling. Flour with low WAC has a very useful application in the production of low starch food products. According to **Mbofung *et al.* (2006)** the ability of flour to absorb water was reported to have a significant correlation with its starch content.

Values ranging from 0.69 % to 1.11 % were recorded for solubility index of soybean-hull flour. *B. subtilis* had the highest value while *L. plantarum* had the least values at 24 and 48 hours respectively. The values obtained are higher than the values (0.60-0.77 %) reported by **Ogodo *et al.* (2018).** All the microbial species used selected for the fermentation of soybean-hull resulted into better SI of the flour. For solubility index, the values obtained also varied significantly ($p < 0.05$). The least and the highest value were recorded for *A. oryzae* at 48 hours and *B. subtilis* at 24 hours respectively. However, there was no significant ($p > 0.05$) difference in the solubility index of the control sample and flour obtained from *B. subtilis* during the first 24 hours of fermentation. The obtained result is lower compare to the report of **(Adeleke and Odedeji, 2010)** who reported solubility of 8.63% for wheat flour which is much higher than the recorded values for soybean-hull fermented with selected of yeast and bacteria (Table 5) in this research. The relatively high solubility index of *B. subtilis* at 24 hours suggests that it will more digestible than other samples and therefore could be suitable for use as ingredient in infant snack or food formulations. According to **Ikegwu *et al*. (2010)** solubility is indicative of water penetration ability into starch granules.

Swelling index (SI) is the amount of water-soluble solids per unit weight of sample. According to **Lawal *et al.* (2005)** it is the index of protein functionality such as denaturation and its potential applications. Higher SI explains more soluble solid available for absorption; hence the fermented soybean-hull flour may be useful and fit for use in food product requiring good swelling index. **(Ashogbon and Akintayo, 2014)** explained indicated that S.I is evidence of non-covalent bonding between molecules within starch granules of flour and also a factor of the ratio of alpha amylose and amylopectin ratios.

The values obtained for bulk density varied significantly ($p < 0.05$) with value ranging from 0.63 to 0.70, which was observed to increase slightly in *A. oryzae* and *S. cerevisiae* and decrease slightly in *B. subtilis* and *L. plantarum*. The increase bulk density recorded with the activities of yeast strains (*Aspergillus oryzae* and *Saccharomyces cerevisae*) may be attributed to fermentative ability of the fungi to break down starch, cellulose and hemicelluloses which may improve the bulkiness of soybean-hull. This is in line with the findings of **Olukomaiya *et al.* (2020b)** who reported an increase in the bulk density of fermented camelina meal using *Aspergillus* fungi.

Bulk density is a very important parameter in the production flour particularly for extruded products and it is a measure of heaviness of food samples and gives an indication of relative volume and bulkiness of food products **(Butt and Batool, 2010).** Increased bulk density suggests better product packaging as higher quantity may be packed within a constant volume thus; this may be an advantage for the fermented soybean-hull flour **(Fagbemi, 1999)**.

Table 5 Effect of microbial species and period on functional properties of fermented soybean-hull flour

Microbial Isolates	OAC (g /100g)	WAC (g/100g)	Solubility Index (%)	Swelling Index (%)	Bulk density
$H_{0(control)}$	0.54^{b}±0.01	2.70^{d}±0.02	1.11^{a}±0.04	1.45^{b}±0.02	0.65^{d}±0.01
A_{O24}	0.47^{cde}±0.03	2.65^{e}±0.02	1.09^{ab}±0.02	1.54^{a}±0.02	0.63^{f}±0.01
S_{C24}	0.53^{b}±0.02	2.74^{bc}±0.01	0.81^{d}±0.03	1.11^{d}±0.04	0.66^{c}±0.01
B_{S24}	0.58^{a}±0.04	2.70^{d}±0.02	1.11^{a}±0.04	1.44^{b}±0.02	0.66^{c}±0.01
L_{P24}	0.61^{a}±0.03	2.82^{a}±0.01	0.74^{e}±0.02	1.04^{e}±0.02	0.70^{a}±0.01
A_{O48}	0.53^{b}±0.01	2.83^{a}±0.01	0.48^{g}±0.02	0.69^{g}±0.02	0.69^{a}±0.01
S_{C48}	0.60^{a}±0.03	2.72^{cd}±0.02	1.05^{b}±0.02	1.43^{b}±0.02	0.66^{c}±0.01
B_{S48}	0.46^{de}±0.02	2.63^{e}±0.02	0.78^{d}±0.03	1.14^{cd}±0.03	0.65^{d}±0.01
L_{P48}	0.44^{e}±0.01	2.65^{e}±0.02	0.56^{f}±0.02	0.73^{f}±0.01	0.64^{e}±0.01
A_{O72}	0.53^{b}±0.02	2.71^{cd}±0.02	1.08^{ab}±0.02	1.46^{b}±0.02	0.65^{de}±0.00
S_{C72}	0.53^{b}±0.02	2.77^{b}±0.02	0.49^{g}±0.02	0.69^{g}±0.02	0.68^{b}±0.00
B_{S72}	0.48^{cd}±0.02	2.55^{f}±0.03	0.86^{c}±0.02	1.16^{c}±0.02	0.63^{f}±0.00
L_{P72}	0.50^{bc}±0.01	2.76^{b}±0.02	0.78^{de}±0.01	1.05^{e}±0.01	0.68^{b}±0.01

Values are expressed as mean of three replicates ± SD Means with the different superscripts across a row are significantly ($P\leq0.05$) different. $H_{0\ (control)}$ = unfermented hull;
A_o= Aspergillus oryzae; *S_c = Saccharomyces cerevisae*; *B_s = Bacillus subtilis*; *L_p= Lactobacillus plantarum*

There was no effect of microbial species and fermentation time on the least gelation property of fermented soybean-hull at 0.5g/ml and 1 g/ml. However, at 1.5 g/ml and 2g/ ml, all samples irrespective of the microbial isolate and fermentation time, showed a positive response. Least gelation is explained as the lowest protein concentration at which gel remained in the inverted tube, which was used as index of gelation capacity. The result obtained for least gelation properties in this study is quite different from the values obtained by **Ugwuona *et al.* (2021)**, for fermented soybean powder, who suggested continuous fermentation was more effective and produced soybean powder of better least gelation quality. The absence of least gelation at 0.5g/ml and 1g/ml could be attributed to the relatively low ratios proteins and carbohydrates component that make up the flour which differs from whole legume flour, which may have a significant impact on the functional properties of the products **(Adebowale and Maliki, 2011)**.

The total viable count of the bacteria cultures ranged from 25×10^1 CFU/g to 265×10^1 CFU/g, with samples *B. Subtilis* at 48 hours, *A. oryzae* at 72 hours having the lowest counts and B. subtilis at 72 hours with the highest counts, Results are shown in table 7. Across the sample there is a statistical significant difference ($P\leq0.05$) in the colony forming units of bacteria. The result also showed increase in the number of bacteria count with increase in the number of days of fermentation, *B. subtilis* fermented for 72 hours had the highest growth. This could be due to prolonged fermentation which increased the presence of lactic acid bacteria as reported by **Sunny-Roberts *et al.* (2003**).

Total fungal count ranged from 11×10^1 CFU/g to 65×10^1 CFU/g, with samples *B. subtiilis* at 24 hours and *L.plantarum* at 48 hours having the lowest and highest counts, respectively. Across the sample there is a statistical significant difference ($P\leq0.05$). However, the values were within the safe limits stated by **(Ihekoronye and Ngoddy, 1985)**. The low pH which was due to increase in population of lactic acid bacteria favoured growth of yeast within the period of fermentation as also reported by **Sunny-Roberts *et al*. (2003)**. The populations of fungi in the sample *L. plantarum* at 48 hours was 65×10^1 CFU/g at 48 hours of fermentation. The reason is probably due to prolonged fermentation which increased the presence of viable spores of *Rhizopus oligosporus* as reported by **Sunny- Roberts *et al*. (2003)**. In comparison with the control (H_0), which yielded reduced counts of bacteria and fungi.

The total coliform count ranged from 15 ±7.07 CFU/100g to 60 ±14.14 Cfu/100g, with samples of *A. oryzae* at 24 hours and *L. plantarum* at 48 hours having the lowest and highest counts, respectively. Majorly all samples having coliform are negligible and no significant ($P\leq0.05$) difference was recorded among samples. This could be due to samples have been prepared aseptically under laminar flow to ensure samples and equipment used were sterilised to avoiding external contamination. No significant coliform bacteria were detected in any of the samples fermented within the period (0 - 72 hours). This is probably due to low pH which did not support the survival and growth of pathogen, this could be corroborated with the result obtained from microbiological analysis of fermented soybean **(Babalola and Giwa, 2012)**. These microorganisms therefore constitute hygienic and sanitary indicators of the manufacturing processes and of the microbiological quality of the final product (**FAO, 2007)**. The total microbial load of the products is lower than the acceptable level, hence the safety of food is guaranteed if packed hygienically. The microbiological quality of foods is a vital criterion in food safety, and this is due to the health risks posed by microbial contamination. Therefore, foods must be assessed to ensure that good hygiene

practices have been employed in their production and that they are safe for consumption (**WHO, 2001**).

Table 6 Effect of microbial species and period on least gelation properties of fermented soybean-hull flour

Microbial Isolates	Least gelation			
	0.5g/ml	1g/ml	1.5g/ml	2g/ml
$H_{0(control)}$	−	−	+	+
A_{O24}	−	−	+	+
S_{C24}	−	−	+	+
B_{S24}	−	−	+	+
L_{P24}	−	−	+	+
A_{O48}	−	−	+	+
S_{C48}	−	−	+	+
B_{S48}	−	−	+	+
L_{P48}	−	−	+	+
A_{O72}	−	−	−	+
S_{C72}	−	−	+	+
B_{S72}	−	−	−	+
L_{P72}	−	−	+	+

+ means present, + means absent; $H_{0\ (control)}$ = unfermented hull; A_o= *Aspergillus oryzae*; S_c = *Saccharomyces cerevisae*; B_s = *Bacillus subtilis*; L_p= *Lactobacillus plantarum*

Table 7 Effect of microbial species and period on microbiological quality of fermented soybean-hull flour

Microbial Isolates	TVC ($\times 10^1$cfu/g)	TFC ($\times 10^1$cfu/g)	TCC (cfu/100g)
$H_{0\ (control)}$	30^{ef}±14.14	0^{e}±0.00	0^{e}±0.00
A_{O24}	40^{e}±14.14	25^{bc}±7.07	15^{c}±7.07
S_{C24}	0^{f}±0.00	15^{cd}±7.07	20^{c}±0.00
B_{S24}	105^{cd}±21.21	11^{de}±0.71	35^{a}±7.07
L_{P24}	145^{b}±21.21	25^{bc}±7.07	0^{e}±0.00
A_{O48}	90^{d}±14.14	0^{e}±0.00	0^{e}±0.00
S_{C48}	130^{bc}±14.14	35^{b}±7.07	0^{e}±0.00
B_{S48}	25^{ef}±7.07	0^{e}±0.00	25^{bc}±7.07
L_{P48}	80^{d}±14.14	65^{a}±7.07	10^{d}±7.07
A_{O72}	25^{ef}±7.07	0^{e}±0.00	0^{e}±0.00
S_{C72}	130^{bc}±14.14	0^{e}±0.00	0^{e}±0.00
B_{S72}	265^{a}±35.36	0^{e}±0.00	25^{bc}±7.07
L_{P72}	115^{bcd}±7.07	25^{bc}±7.07	0^{e}±0.00

Values are expressed as mean of two replicates ± SD TVC = total viable count; TFC = total fungi count; TCC = total coliform count; $H_{0\ (control)}$ = unfermented hull; A_o= *Aspergillus oryzae*; S_c = *Saccharomyces cerevisae*; B_s = *Bacillus subtilis*; L_p= *Lactobacillus plantarum*.

Effect of microbial species and period on anti-nutritional properties of fermented soybean-hull flour

Anti-nutritional properties of soybean-hull flour is shown in table 8. All the antinutritional properties analyzed; phytate and trypsin inhibitor decreased with increase in fermentation time and there was significant difference between the samples. Phytate content of the flour samples reduced significantly ($p \leq 0.05$) from 3.01µg/100g to 0.72 µg/100g. $H_{0\ (control)}$ the unfermented hull and A_{O72} (*A. oryzae* at 72hours) having the highest and lowest values, respectively; all the samples are significantly ($P \leq 0.05$) different from one another. The result agrees with the earlier report of **Marfo *et al*. (1990)** on a decrease in phytate content of cocoyam tubers from 855mg/g to 13mg/g with increase in fermentation time. This finding is also consistent with the results of **Olanipekun *et al*. (2015)** who reported 96.3 and 54.77% reduction in phytic acid content of peanut and soyabean respectively. The reduction in phytate content may be attributed to the activity of the endogenous phytase enzyme from the raw ingredient and microorganisms which are capable of hydrolyzing the phytic acid in the fermented food preparations into inositol and orthophosphate **Sandberg and Andlid *et al*. (2002)**. The residual phytate content of the fermented soybean-hull flour falls within the FAO recommended safe level making the soybean-hull flour safe for human and animal consumption, Phytic acid makes phosphorus and zinc less available to the humans. In order to maximize the nutritional quality of soybean-hull flour and for a wider acceptability, these anti-nutritional factors need to be inactivated or minimized. Fermentation has been widely used to increase the bioavailability of nutrients (**Hotz and Gibson, 2007**) and reduce the levels of anti-nutritional factors (**Egounlety and Aworh, 2003**) of soybean. Several studies (**Kishida *et al*., 2000; Frias *et al*., 2008; Song *et al*., 2008**) have also confirmed the ability of fermentation process in degrading anti-nutritive and allergenic compounds of soybean meal, thereby increasing the possibilities of utilization of various processed products of soybean. A wide variety of microorganisms were used to ferment soybean-hull for nutritional enhancement. The fermentation process is facilitated by the use of fungi or bacteria. The fermentation conditions and nutritional quality of the fermented soybean-hull thus produced can varied depending on the type of microorganism used. *Aspergillus oryzae* after 72hours fermentation had the lowest value of 0.72 µg/100g. As reported by **Pinto *et al*. (2001) and Mathivanan *et al*. (2006)** *Aspergillus oryzae* is the most popular species due to its capacity to produce enzymes such as hemicellulases, hydrolases, pectinases, protease, amylase, lipases, and tannases. Fermentation with *Aspergilli* almost completely eliminates phytate, resulting in a protein source for feed with highly available phosphorus as well as zinc. Fermentation also successfully reduced the amount of stachyose and raffinose in soybean-hull. Apart from degrading the anti-nutritional factors, fermentation increases the nutritional value of the hull by increasing the crude fat, crude ash and crude protein contents **Hong *et al*. (2004).** Phytic acid also binds various other minerals such as calcium, iron, magnesium and zinc. According to the GRAS property of these both strains and various improvements of nutritional values, the fermented Soybean-hull proved to be a potential feed ingredient, especially for the monogastric animals. Therefore, phytate is naturally present in many foods especially cereals and legumes, when above a certain level phytates reduce the availability of minerals and functionality and digestibility of proteins (**Roboy, 2001**)

Trypsin inhibitor (TI) content of the flour samples reduced significantly ($p \leq 0.05$) from 6.04mg/kg to 0.02 mg/kg. $H_{0\ (control)}$ the unfermented hull and A_{O72} (*Aspergillus oryzae* at 72hours) having the highest lowest and lowest values, respectively; all the samples are significantly ($P \leq 0.05$) different from one another, there was a decrease in trypsin inhibitor content of fermented soybean-hull with increase in fermentation time. It was shown that after fermentation with the microbial strains, the trypsin inhibitor in soybean-hull was reduced. SSF also resulted in an increase of *in vitro* trypsin digestibility and nitrogen solubility under alkaline conditions and improvement of the nutritional quality of soybean meal. The efficiency of SSF in improving nutritional quality and reducing the anti-nutritional factors were ascertained by the works of **Amadou *et al*. (2010)**. Fermentation using *A. oryzae* eliminated trypsin inhibitor content from 2.6 mg/g to zero, as reported by **Liu *et al*. (2007)**. The level of degradation of trypsin inhibitor for both fermentations were of comparable values; while fermentation with fungi using *Aspergillius oryzae* after 72 hours fermentation reduced by 0.02mg/kg, bacterial fermentation using *B. subtillus* at 72 hours fermented resulted in 1.14 mg/kg reduction. Trypsin inhibitors (TI) are the most important group of anti-nutritional factors (ANF) present in soybean-hull. An excess of heat, however, increases the incidence of maillard reactions, reducing the digestibility and nutritive value of the soybean meal **(Fontaine *et al*., 2007; González-Vega *et al*., 2011)**. Raw soybeans and untreated soybean-hulls can contain more than 25 mg of trypsin inhibitors per gram of product, fermentation deactivates most of the trypsin inhibitors. Antinutrients are compounds which affect the nutritive value of food products such as hydrogen cyanide, oxalate and phytate, before fermentation the composition of these antinutrients content were higher in all the samples but after fermentation, the antinutrients content were greatly reduced.

Table 8 Effect of microbial species and period on anti-nutritional properties of fermented soybean-hull flour

Microbial Isolates	Phytate (µg/100g)	Trypsin Inhibitor (mg/kg)
$H_{0(control)}$	3.01^{a}±0.02	6.04^{a}±0.08
A_{O24}	2.51^{d}±0.85	4.90^{c}±0.22
S_{C24}	2.07^{ef}±0.03	5.80^{ab}±0.03
B_{S24}	2.80^{b}±0.05	4.82^{c}±0.00
L_{P24}	2.95^{a}±0.01	5.55^{b}±0.12
A_{O48}	1.96^{f}±0.01	1.22^{g}±0.01
S_{C48}	2.67^{c}±0.05	2.50^{e}±0.03
B_{S48}	2.01^{f}±0.06	1.88^{f}±0.03
L_{P48}	2.16^{e}±0.01	3.85^{d}±0.10
A_{O72}	0.72^{i}±0.04	0.02^{h}±0.00
S_{C72}	1.28^{g}±0.08	1.03^{g}±0.10
B_{S72}	0.98^{h}±0.08	1.14^{g}±0.01
L_{P72}	1.01^{h}±0.08	2.64^{e}±0.59

Values are expressed as mean of two replicates ± SD Means with the different superscripts across a row are significantly ($P \leq 0.05$) different. $H_{0\ (control)}$ = unfermented hull; A_o= *Aspergillus oryzae*; S_c = *Saccharomyces cerevisae*; B_s = *Bacillus subtilis*; L_p= *Lactobacillus plantarum*

Effect of microbial species and fermentation period on fibre fraction properties of soybean-hull flour

The effect of microbial species on the fibre fraction of soybean-hull showed significant variation as shown in table 9. Neutral detergent soluble (NDS) fraction varied significantly ($P < 0.05$) with values ranging from 27.91 to 35.93 %. The highest value was recorded for *A. oryzae* fermented hull at 72 hours of fermentation while the control had the lowest value. The NDS fraction was observed to increase with increase in fermentation time.

This correlates with findings of Olukomaiya ***et al*. (2020a)** for fermented lupin flour. The increase in NDF fibre fraction can be attributed to the breakdown of soybean-hull fibre due to microbial action. This also shows the microbial species used for fermentation in this study were able to break down the fibre fraction of

soybean-hull leading to increased NDS when compared to the control sample. This follows a similar trend with the result of **Ramli *et al.* (2005)** for SSF of sugarcane bagass using *Aspergillus sojae* and *Aspergillus awamori*. During the last hour of fermentation, all fermented samples recorded the highest NDS value. This may imply that increase in fermented time irrespective of microbial species will result into higher NDS of soybean-hull.

The values obtained for neutral detergent fibre (NDF) ranged from 13.77 to 17.57 for *Aspergillus oryzae* at 72 hours and control at 0 hour respectively. NDF was observed to decrease as fermentation progresses resulting into reduced NDF towards the last hour of fermentation. NDF is the in-soluble part of a neutral detergent solution. The reduction in NDF may be attributed to the fermentative activities of microbial strains which led to the breakdown of NDF fraction of soybean-hull. This is lower compared to the findings of **Olukomaiya *et al.* (2020b)** for SSF using *Aspergilus* species. However, during the first 24 hours fermentation NDF values showed only slight reduction compared to the control, and as fermentation progresses, further reduction in NDF was observed. This may explain the fact that fermentation had significant effect to break down NDF of soybean-hull.

Values ranging from 6.91 to 12.04% were obtained for acid detergent fibre (ADF). The fermented samples had reduced ADF compared to the control. During fermentation acid detergent fibre values were observed to also decrease as fermentation time increases. This is similar to the findings of **Ogodo *et al*. (2018)** for fermented undehulled soybean flour. This may be attributed to the breakdown of lignin by activities of the microbial strains used and the enzymes they produced. Increase in acid detergent fibre may be an indication of continuous microbial and enzymatic activities during Solid-state fermentation and it is the fraction of insoluble components in the acid detergent solution. ADF is important in fibre analysis to determine the percentage lignin and silica present in food.

There were significant ($P < 0.05$) differences in the Acid detergent lignin (ADL) of fermented soybean-hull at different fermentation times by the activity of different microbial species. ADL values ranged from 3.23 to 4.36 % for *A. oryzae* after 72 hours of fermentation and the control respectively. ADL values showed significant decrease as fermentation time increases resulting into reduced ADL at the last hour of fermentation (72 hours) when compared to the control sample. However, only slight decrease was observed during 24 hours of fermentation when compared to the decrease recorded during 48 hours and 72 hours. The decrease in ADL may be attributed to the enzymatic breakdown of soluble fibre of soybean-hull. This is in line with the report of **Olukomaiya *et al*. (2020b)** for solid-state fermentation of canola meal. These authors attributed it to buildup of acid of alkaline and/or neutral detergent insoluble substances during SSF. Lignin is a component of ADF which is explained as the insoluble lignin fraction in 72% sulphuric acid **Mastutik *et al*. (2004)**

Silica appears to be present in only minute amount when compared to other fibre fraction. However, fermentative activities of the selected microbial strains resulted into increase silica fraction of fermented soybean-hull when compared to the control. The values obtained varied significantly ($p < 0.05$) with *L. plantarum* at 72 hours having the highest value (1.92%). It was observed that among the four microbial strains used in this study, activities of *L. plantarum* resulted into the least amount of silica. The result obtained in this study is higher than the value reported by **(Liu *et al*., 2015)** for soybean straw and who attributed the reduce silica value to microbial activities during fermentation. Silica fraction of fibre can be related to ash content of fibre of food products **Liao *et al.* (2004)**. Both lignin and silica are essentially indigestible even by microorganisms.

The values obtained for cellulose fraction varied significantly ($p < 0.05$). The control sample had the highest cellulose fraction while the *B.substilis* fermented soybean-hull had the least cellulose value. However, among the fermented samples *S.cerevisiae* fermented soybean-hull had the highest cellulose value. It can be observed that the cellulose value also decrease as fermentation progresses, and the most significant reduction was noted during the last hour of fermentation. This is in line with the findings of **Martín-Cabrejas *et al.* (2004)**, who reported a decrease in cellulose content of fermented common bean flour. Decrease in cellulose may be attributed to breakdown polysaccharide of soybean-hull by enzymes. The value obtained in this study is in line with the findings of **Martín-Cabrejas *et al*. (2004)** for common bean flour. Reduction in cellulose implies that the fermented soybean-hull flour obtained can be used for production food product that the flour can be easily digestible by human for prevention of colorectal cancer **Mastutik *et al*. (2004)**.

Hemicellulose fraction of soybean-hull also vary significantly ($p< 0.05$), which decreased gradually as fermentation progresses. Among the fermented samples at different fermentation times, *B. subtilis* and *L. plantarum* had the highest hemicellulose fraction at 24 hours while the lowest value was recorded for *A. oryzae* after 72 hour of fermentation. Generally, all microbial strains resulted into lower hemicelluloses at the final hour of fermentation. This may be as a result of degradation of polysaccharides carried out by the enzymatic activities of the microbial strains used in SSF. According to **Dordevic *et al*. (2010)** fermentation is a very important process in the breakdown of high fibre food products. The low cellulose and hemicelluose content of fermented soybean-hull flour suggest its application in the production of low fibre products for consumption by kids and people with difficulties of digestion of high fibre products. The cellulose and hemicellulose are content of the cell wall and contribute to the cell structure.

Table 9 Effect of microbial species and fermentation period on fibre fraction properties of soybean-hull flour

Microbial Isolates	NDS (%)	NDF (%)	ADF (%)	ADL (%)	Silica (%)	Cellulose (%)	Hemicellulose (%)
$H_{0(control)}$	27.91^{f}±0.17	17.57^{a}±0.06	12.04^{a}±0.09	4.36^{a}±0.01	1.01^{fg}±0.06	8.69^{a}±0.18	3.52^{a}±0.05
A_{O24}	33.63^{b}±0.57	15.26^{de}±0.01	10.92^{c}±0.04	3.78^{gh}±0.11	1.23^{cde}±0.27	7.76^{bcd}±0.26	2.50^{f}±0.09
S_{C24}	31.48^{d}±0.04	16.05^{c}±0.03	11.92^{ab}±0.04	4.18^{bc}±0.04	0.99^{g}±0.08	8.10^{abc}±0.16	2.79^{e}±0.01
B_{S24}	30.37^{e}±0.75	15.75^{cd}±0.04	11.85^{b}±0.03	4.10^{cd}±0.01	1.07^{efg}±0.00	7.43^{cde}±0.00	3.15^{b}±0.03
L_{P24}	30.74^{de}±0.02	17.19^{a}±0.38	11.05^{c}±0.03	4.26^{ab}±0.04	1.81^{a}±0.05	7.98^{bc}±0.50	3.15^{b}±0.05
A_{O48}	35.21^{a}±0.111	15.08^{e}±0.06	9.68^{e}±0.02	3.48^{i}±0.10	1.16^{defg}±0.05	8.15^{ab}±0.06	2.29^{g}±0.06
S_{C48}	32.33^{c}±0.34	15.96^{c}±0.03	9.79^{de}±0.03	3.93^{ef}±0.04	1.40^{bc}±0.03	8.08^{abc}±0.04	2.56^{f}±0.06
B_{S48}	31.38^{d}±0.11	15.17^{e}±0.09	9.86^{d}±0.04	3.98^{e}±0.01	1.20^{def}±0.01	6.92^{ef}±0.07	3.07^{bc}±0.02
L_{P48}	31.24^{d}±0.08	16.63^{b}±0.11	9.95^{d}±0.03	4.03^{de}±0.03	1.85^{a}±0.01	7.69^{bcd}±0.08	3.06^{bcd}±0.05
A_{O72}	35.93^{a}±0.63	13.77^{g}±0.75	6.91^{h}±0.22	3.23^{j}±0.06	1.24^{cde}±0.02	7.21^{de}±0.80	2.10^{h}±0.03
S_{C72}	33.25^{b}±0.55	14.78^{ef}±0.02	8.68^{g}±0.00	3.74^{h}±0.02	1.47^{b}±0.02	7.19^{de}±0.07	2.39^{g}±0.09
B_{S72}	33.36^{b}±0.44	14.36^{f}±0.03	8.36^{g}±0.09	3.72^{h}±0.03	1.34^{bcd}±0.03	6.35^{f}±0.01	2.95^{d}±0.03
L_{P72}	32.46^{c}±0.24	15.23^{de}±0.16	8.86^{f}±0.01	3.86^{fg}±0.02	1.92^{a}±0.02	6.46^{f}±0.08	3.01^{cd}±0.04

Values are expressed as mean of two replicates ± SD Means with the different superscripts across a row are significantly ($P\leq0.05$) different.$H_{0\ (control)}$ = unfermented hull; A_o= *Aspergillus oryzae*; S_c = *Saccharomyces cerevisae*; B_s = *Bacillus subtilis*; L_p= *Lactobacillus plantarum.* NDS = Neutral detergent soluble; NDF = Neutral detergent fibre; ADF = Acid detergent fibre; ADL = Acid detergent lignin

Effects of microbial species and period on particle size distribution of fermented soybean-hull flour

The fermented soybean-hull flour samples were determined by a Ro tap apparatus with sieves arranged in order of decreasing mesh size. The results are shown in table 10, the values of the fermented substrate ranged from 95.64 to 96.74 ($\leq 20\mu$m), with unfermented substrate having the lowest and B_{S72} (*B. substillis* at 72 hours) having the highest. The samples were significantly ($P\leq0.05$) different from each other, while the flour particle size ($>20\mu$m) had values between 3.27μm to 4.36 μm with B_{S72} (*Bacillus substillis* at 72hours) having the lowest values compared with the unfermented substrate having value of 4.36 ±0.01 ($>20\mu$m). Particle size that pass through the size ($>20\mu$m) had lesser surface area. The particle size of the fermented substrate ($\leq20\mu$m) were higher than the control (unfermented substrate), this implies that the higher values of the fermented substrate might be as a result of fermentation process, since this organisms might breakdown some of the lignocellulose and fibre component, thereby creating higher surface area on the substrate, during fermentation the organisms breakdown the hull and enabling easy processing of the hull during grinding so that they can be easily chipped up at the same processing regime leading to greater surface area, It is well established that the degree of flour fineness in a milling operation depends on the type and efficiency of the applied machine **Oladunmoye *et al.* (2010)**. Grinding of soybean-hull improved the digestible nutrient values probably the hyphae had broken down the substrate thereby enhancing the grinding ability of the fermented product compared to the control, as such leading to higher surface area of the final product. Particle size is one of the most important characteristics of a flour, which may influence other physicochemical properties such as swelling, water binding capacity and pasting properties **Vouris *et al.* (2018).** Particle size distribution was within the range of values expected for flour that can be used for bakery products. As particle size decreases, digestibility of a feed ingredient increases because of an increase in surface area and an improvement in feed efficiency **(Fastinger and Mahan, 2003).**

Table 10 Effect of microbial species and fermentation period on particle size distribution of soybean-hull flour

Microbial Isolates	(≤ 20μm)	(> 20μm)
$H_{0(control)}$	95.64^{e}±0.01	4.36^{e}±0.01
A_{O24}	95.45^{f}±0.04	4.55^{d}±0.04
S_{C24}	94.90^{h}±0.04	5.10^{b}±0.04
B_{S24}	95.99^{c}±0.08	4.01^{g}±0.00
L_{P24}	94.81^{h}±0.02	5.20^{a}±0.02
A_{O48}	96.01^{c}±0.05	4.00^{g}±0.05
$\boldsymbol{S_{C48}}$	95.76^{d}±0.01	4.25^{f}±0.01
$\boldsymbol{B_{S48}}$	96.67^{a}±0.05	3.34^{i}±0.05
$\boldsymbol{L_{P48}}$	95.12^{g}±0.02	4.89^{c}±0.02
$\boldsymbol{A_{O72}}$	96.29^{b}±0.06	3.71^{h}±0.06
$\boldsymbol{S_{C72}}$	95.99^{c}±0.06	4.01^{g}±0.05
$\boldsymbol{B_{S72}}$	96.74^{a}±0.02	3.27^{i}±0.02
$\boldsymbol{L_{P72}}$	95.82^{d}±0.04	4.19^{f}±0.04

Values are expressed as mean of two replicates ± SD Means with the different superscripts across a row are significantly (P≤0.05) different. $H_{0\,(control)}$ = unfermented hull; A_o = *Aspergillus oryzae*; S_c = *Saccharomyces cerevisae*; B_s = *Bacillus subtilis*; L_p= *Lactobacillus plantarum*

CONCLUSIONS

This study investigated the effects of solid-state fermentation on quality attributes of soybean-hull. Processing of soybean-hull flour by fermentation using bacterial (*B. subtilis* and *L. plantarum*) and fungal (*A. oryzae* and *S. cerevisiae*) strains enhanced the physicochemical, proximate, functional and mineral compositions of the soybean-hull. Also, the anti-nutrients (trypsin and phytate) decreased with increase in fermentation period.

The result of proximate composition shows increment in fat, protein, ash and moisture while fibre and carbohydrate were found to decrease after fermentation as well as a decrease in the total soluble sugar as a result of these microbial species feeding on the sugar for their growth. There was an improvement in the mineral composition and breakdown of fibre content in the fermented substrate, therefore soybean hull fermented with *B. subtilis at* 72 hours *and A. oryzae* at 72 hours fermentation improved the nutritive value of soybean-hull. The nutritional value of a fermented soybean-hull could be regarded as a new protein resource, which has been improved after fermentation with fungi and bacteria. This study has clearly demonstrated the possibility of beske producers in utilizing soybean-hull flour in baked and value-added food product due to its digestibility and nutrient absorption. This would support industrial utilization, low-cost product and the consumption of under-utilized hull. Therefore, government interventions especially to the producers of beske on the possibility of using the hull for baked products, this will in turn discourage environmental pollution and provide new value-added nutritious products for consumers. The level of fibre content and anti-nutrients in each of the fungal and bacterial monoculture fermented soybean-hull may be safe human consumption as it can improve nutrients bioavailability and digestibility over the unfermented soybean-hull. Further studies could focus on *in vivo* assay for true protein digestibility of fermented soybean-hull using an animal model.

Acknowledgment: We gratefully acknowledge the assistance of Mr Emmanuel Olonye for providing the various microbial stains needed in this research

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