



Original Article

***In-vitro* starch and protein digestibility and proximate composition of soybean flour fermented with lactic acid bacteria (LAB) consortia**Alloysius Chibuike Ogo^{a,*}, Ositadinma Chinyere Ugbo^b, Reginald Azu Onyeagba^b, Hope Chukwuemeka Okereke^b^a Department of Microbiology, Faculty of Pure and Applied Sciences, Federal University Wukari, P.M.B. 1020, Wukari, Taraba State, Nigeria^b Department of Microbiology, Faculty of Biological and Physical Sciences, Abia State University Uturu, P.M.B. 2000, Uturu, Abia State, Nigeria

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ABSTRACT

The changes in the proximate composition, *in-vitro* starch/protein digestibility and microbiological quality of lactic acid bacteria (LAB)-fermented soybean flour were evaluated at 12 h intervals for 48 h. Soybean was processed into flour and fermented with LAB-consortium previously isolated from maize (*Lactobacillus plantarum* WCFS1+*Lactobacillus rhamnosus* GG ATCC53/03 + *Lactobacillus nantensis* LP33 + *Lactobacillus fermentum* CIP102980 + *Lactobacillus reuteri* DSM 20016), and sorghum (*Pediococcus acidilactici* DSM 20284 + *Lactobacillus fermentum* CIP102980 + *Lactobacillus brevis* ATCC14869 + *Lactobacillus nantensis* LP33 + *Lactobacillus plantarum* WCFS1). The flour was also fermented naturally for comparison. There was significant ($p < 0.05$) increase in the moisture content as the fermentation time increased and ranged from $10.32 \pm 0.05\%$ to $10.98 \pm 0.04\%$. The ash and protein contents also increased significantly ($p < 0.05$) with the highest increase occurring in the samples fermented with lactic acid bacteria consortia. The lipid, crude fibre and carbohydrate contents decreased with increasing fermentation time. *In-vitro* starch digestibility increased significantly ($p < 0.05$) with increasing fermentation time from $71.36 \pm 0.20\%$ to $80.11 \pm 0.02\%$. *In-vitro* protein digestibility increased significantly ($p < 0.05$) from $85.40 \pm 2.46\%$ to $93.56 \pm 2.50\%$. Bacteria load increased as fermentation time increased. Fungal growth was only observed in natural fermentation. The occurrence of isolates showed that only starter organisms were isolated from the consortia fermentation set-ups. This study has shown that combination of LAB in fermentation of soybean flour can improve their nutritional value and digestibility.

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Introduction

Soybean has been used for long in Asian countries as important source of protein which complements grain proteins, and can also be used as a substitute for meat and meat products proteins (Adelakun et al., 2013). Soybean products, especially fermented soybean products, contain various functional components such as peptides and isoflavonoids in addition to various nutrients (Kwon et al., 2011). A number of epidemiological studies have suggested that consumption of soybeans and soy foods is associated with lowered risks for breast, prostate, and colon, cancers as well as cardiovascular diseases (Kwon et al., 2011; Larsson et al., 2005).

Fermented soybean is also associated with reduction in menopausal syndrome frequency, osteoporosis protection as well as coronary heart disease prevention as a result of antiatherogenic properties (Yee, 2000; Babalola and Giwa, 2012).

According to Maidala et al. (2011) and Maidala et al. (2013), full fat soybean are rich in protein but contains anti-nutritional factors that reduce the digestibility and bioavailability of nutrients and utilization of amino acids in monogastric and immature ruminants. These antinutritional factors such as trypsin inhibitors, phytic acids etc. could be associated with fibre and reduces mineral bioavailability as well as protein digestibility which contribute to their hindrances for effective utilization (Dueñas et al., 2012; Olanipekun et al., 2015). However, fermentation has been reported as one of the processes that decreases the level of anti-nutrients in food grains and increases the starch/protein digestibility (Singh et al., 2012; Ogo et al., 2016). Fermentation also improves the protein content of food, enhances carbohydrate accessibility and amino acid

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balance (Singh et al., 2012). Lactic acid bacteria (LAB) fermentation is one of the processes of food preparation in Africa and other parts of the world and have been reported to have numerous benefits (Kohajdová et al., 2007; Ramos et al., 2013; Owusu-Kwarteng et al., 2015). Lactic acid bacteria fermentation increases food palatability, availability of proteins and vitamins, confers preservative as well as detoxifying effect on food (Masood et al., 2011; Onyango et al., 2013). Reports indicated that regular use of lactic acid bacteria fermented foods boost the immune system against bacterial pathogens (Masood et al., 2011; Onyango et al., 2013). Hence, the objective of this present study was to ascertain the effect of lactic acid bacteria (LAB) consortium fermentation on the proximate composition and *in-vitro* starch/protein digestibility of soybean flours and compare them with natural fermentation.

Materials and methods

Source of materials

Soybean (*Glycine max* (L) Merr.) was procured from Mushin markets, Lagos, Nigeria and transported to the laboratory in a clean polythene bags for identification and analysis at Federal Institute of Industrial Research Oshodi (FIRO). Lactic acid bacteria (LAB) used for fermentation (*Lactobacillus plantarum* WCFS1, *Lactobacillus rhamnosus* GG, ATCC 53/03, *Lactobacillus nantensis* LP33, *Lactobacillus fermentum* CIP 102980, *Lactobacillus reuteri* DSM 20016, *Pediococcus acidilactici* DSM 20284, *Lactobacillus fermentum* CIP 102980 and *Lactobacillus brevis* ATCC 14869) were previously isolated from fermenting maize and sorghum.

Choice of inoculum

Lactic acid bacteria consortium from maize and from sorghum were selected based on their tolerance to acid, salt, lowering of pH, level of acid production and growth on nutrient depleted medium after a pre-fermentation studies as well as their benefits in food fermentation as reported (Kohajdová et al., 2007; Ramos et al., 2013; Owusu-Kwarteng et al., 2015).

Sample preparation

The raw grains of cream-coloured soybean was freed of foreign materials, washed with clean tap water and rinsed with distilled water. The sample was dried with hot air oven (GL, England) at 60 °C for 8 h. This was milled into powder using laboratory disc mill and then stored in clean air tight containers at 4 °C for further use (Singh et al., 2012).

Inoculum preparation

The inoculum (starter culture) was prepared according to the method described by Ogo et al. (2016) with slight modification. Each of the lactic acid bacteria was grown at 37 °C for 24 h on autoclaved de-Man Rogosa and Sharpe (MRS) broth (Oxoid, UK; containing peptone 20 g/L, lactose 10 g/L, bile salt 5.0 g/L, neutral red 0.075 g/L, agar 12 g/L and pH of 7.4 ± 0.2), deMan Rogosa & Sharpe agar (MRS) (Oxoid, UK; consisting of peptone 10 g/L, Lab-lemco powder 8.0 g/L, yeast extract 4 g/L, glucose 20 g/L, Sorbitan monoleate 1 ml, Dipotassium hydrogen phosphate 20 g/L, sodium acetate 3H₂O 5 g/L, triammonium citrate 2.0 g/L, magnesium sulphate 7H₂O 0.2 g/L, manganese sulphate 4H₂O 0.05 g/L, agar 10 g/L and pH of 6.2 ± 0.2) distributed in test-tubes in 10 ml aliquots. After 24 h of incubation, the contents of five (5) test-tubes, containing individual growth of lactic acid bacteria were transferred to 250 ml Erlenmeyer flasks containing fresh 160 ml aliquot of

MRS broth. The growth were combined to obtain LAB-consortium from organisms isolated from maize and LAB-consortium from organisms isolated from sorghum respectively. The combinations were as follows; *Lactobacillus plantarum* WCFS1 + *Lactobacillus rhamnosus* GG ATCC 53/03 + *Lactobacillus nantensis* LP33 + *Lactobacillus fermentum* CIP 102980 + *Lactobacillus reuteri* DSM 20016 (consortium from maize); and *Pediococcus acidilactici* DSM 20284 + *Lactobacillus fermentum* CIP 102980 + *Lactobacillus brevis* ATCC 14869 + *Lactobacillus nantensis* LP33 + *Lactobacillus plantarum* WCFS1 (consortium from sorghum). The combined organisms were respectively incubated for 48 h in an orbital shaker incubator (REMI/396LAG) (100 rpm) at 37 °C for the inoculum to build-up. The inocula were harvested by centrifugation at 5000 g for 10 min and maintained in fresh MRS broth before fermentation. The washed cells were diluted using sterile distilled water to obtain 0.5 McFarland, standard (Dajanta et al., 2009).

Fermentation of soybean flour

Fermentation of the soybean flour was carried out following a slight modification of the method of Ogo et al. (2016). Exactly 500 g of the uncooked flour was mixed with 1000 mL of distilled water in sterile fermentation container. 0.5 g/L of potassium sorbate was added to the mixture to inhibit fungi and other contaminating microorganisms (verified by no observable growth on plate count agar after 18–24 h of incubation). The mixture was inoculated with 10 ml of 1.0×10^8 cells/mL of the mixture of the lactic acid bacteria suspension and allowed to ferment. The same preparation was made without addition of potassium sorbate and the starter organisms to ferment naturally as control. Samples were withdrawn at 0, 12, 24, 36 and 48 h for analysis.

Proximate analysis

Moisture, ash and protein contents were determined according to AOAC (2005) methods. The lipid composition was determined using Soxhlet extraction method as described by Ojiako and Akubugwo (1997). The fibre content of the flour samples was determined using the methods described by Akubugwo and Arukwe (2006). The carbohydrate contents of the samples was determined using the Lane and Eynon method as described by Akubugwo and Arukwe (2006).

In vitro starch and protein digestibility (IVSD)

In vitro starch digestibility (IVSD) activity was determined according to the method described by Singh et al. (2012). The *in vitro* protein digestibility activity of the sample was determined by enzymatic method of Monjula and John (1991).

Enumeration, isolation and identification of microorganisms

Enumeration and isolation of microorganisms were carried out according to the method of Ogo et al. (2015) with slight modification. Exactly 1.0 g (wet weight) of the fermenting flour sample were homogenized in 9.0 ml sterile peptone water for 30 s at 0, 8, 24 and 48 h. A ten-fold serial dilution of the mixtures was made. Exactly 0.1 ml from 10^{-6} dilution was transferred to plates of plate count agar (PCA) (Oxoid, UK; consisting of tryptone 5 g/L, yeast extract 2.5 g/L, glucose 1.0 g/L, agar 9.0 g/L and pH of 7.0 ± 0.2), MacConkey agar (MA) (Oxoid, UK; consisting of peptone 20 g/L, lactose 10 g/L, bile salt 5.0 g/L, neutral red 0.075 g/L, agar 12 g/L and pH of 7.4 ± 0.2), deMan Rogosa & Sharpe agar (MRS) (Oxoid, UK; consisting of peptone 10 g/L, Lab-lemco powder 8.0 g/L, yeast extract 4 g/L, glucose 20 g/L, Sorbitan monoleate 1 ml, Dipotassium

hydrogen phosphate 20 g/L, sodium acetate 3H₂O 5 g/L, triammonium citrate 2.0 g/L, magnesium sulphate 7H₂O 0.2 g/L, manganese sulphate 4H₂O 0.05 g/L, agar 10 g/L and pH of 6.2 ± 0.2) and Sabouraud dextrose agar (SDA) (Oxoid, UK; consisting, 10 g/L mycological peptone, 40 g/L Dextrose, 15 g/L agar and pH 5.6 ± 0.2) for enumeration of total viable count (TVB), total coliform count (TCC), total lactic acid bacteria count (TLBC) and total fungal counts (TFC) respectively. The cultured plates were incubated at room temperature (27 ± 2 °C) for 24 h and then counted. After 24 h of incubation, each cultured plate was examined closely for distinct colonies from which inocula were taken and sub-cultured in a fresh sterile, nutrient medium and incubated at room temperature (27 ± 2 °C) for 24 h to obtain pure cultures which were subsequently identified (Fawole and Oso, 1988; Okereke and Kanu, 2004; Holt et al., 1994). Further characterization was done using the molecular technique. DNA extraction was carried out using Jena Bioscience bacteria DNA preparation kit (PP-206S-Jena Bioscience GmbH, Germany). PCR amplification of the 16S rRNA gene was carried out using the primer pair 27F (5' aga gtt tga tcc tgg ctc ag) and 1541R (aag gag gtg atccag ccg ca). PCR products were sequenced using Sanger sequencing technique and strains were identified by partial sequences of 16S rRNA gene at <http://blast.ncbi.nlm.nih.gov/Blast.cgi>. The fungi were identified on the basis of their phenotypic characteristics including morphology, biochemistry and physiology and with reference to the methods described by Barnett et al. (2000), de Hoog et al. (2000) and Navis et al. (1999).

Statistical analysis

All the analyses were conducted in triplicate and the mean data $\pm$ SD (standard deviation) were reported. Data were subjected to analysis of variance (ANOVA) for repeated measurements. Each of the parameters analyzed were compared between natural fermentation, LAB consortium from maize and LAB consortium from sorghum fermentations respectively using statistical package for the social sciences (SPSS) software (version 20; IBM, Armonk, NY, USA). Significance was accepted at $p \leq 0.05$.

Results and discussion

Proximate analysis

Fig. 1 presents the effect of fermentation on the moisture content of soybean which increased with increasing fermentation time. It ranged from $10.32 \pm 0.05\%$ to $10.98 \pm 0.04\%$. The variations in the moisture content of soybean differ significantly ($p < 0.05$) at each successive time interval when compared between natural and LAB consortia fermentation. No significant difference ($p > 0.05$) between LAB consortium from maize and LAB consortium from sorghum fermentations at 36 h and 48 h respectively. However, the highest moisture content was observed in sample fermented with LAB-consortium from maize (10.96%) and LAB-consortium from sorghum (10.98%). The result of the present study is higher than the range (5.15–5.94%) reported by Olanipekun and Adekun (2015) but lower than the report of Jeff-Agboola and Oguatuase (2006) who reported 59.16% and 59.40% for unfermented and fermented soybean, respectively. The present study also corresponds with the reports from other researchers for cowpea, $9.2 \pm 0.10\%$ (Olalekun and Bosede, 2010) and pigeon pea, 10.20–13.24% (Adebowale and Maliki, 2011). Moreover, the moisture content reported in the present study is considerably low. This is desirable as low moisture discourages the growth of spoilage microorganisms and hence, prolong the shelf-life of the final product (Adegbehingbe, 2014).

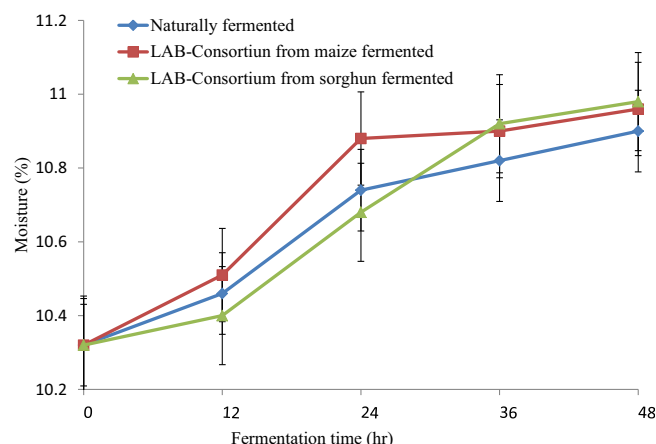


Fig. 1. Effect of fermentation on the moisture content of soy bean flours. Each point represent the mean of three independent experiments and error bars indicate $\pm$ SE. There were significant differences ($P < 0.05$) at each successive time interval when compared between natural and LAB consortia fermentation. No significant difference ($P > 0.05$) between LAB consortium from maize and LAB consortium from sorghum fermentations at 36 and 48 h respectively.

Fig. 2 presents the effect of fermentation on the ash content of the soybean flours which showed a progressive increase as the fermentation time increased. It increased from $4.09 \pm 0.11\%$ to $5.18 \pm 0.02\%$. There were significant differences ($p < 0.05$) at each successive time interval when compared between natural and LAB consortia fermentations. However, no significant difference ($p > 0.05$) between LAB consortium from maize and LAB consortium from sorghum fermentations at all time intervals. The highest ash content was found in LAB-consortium from maize fermented sample ($5.18 \pm 0.02\%$) while the naturally fermented sample was the least ($4.09 \pm 0.11\%$). These results are lower than the report of Maidala et al. (2013) who reported ash content of 10.40% in raw soybean. Adebowale and Maliki (2011) reported increase in ash content with increasing fermentation period in the range of 4.61–5.52% for pigeon pea which is consistent with the present study. Reports have shown that ash is an indication of the level of mineral composition of the substrates (Ntuli et al., 2013). This demonstrated the effectiveness of the consortia in the present

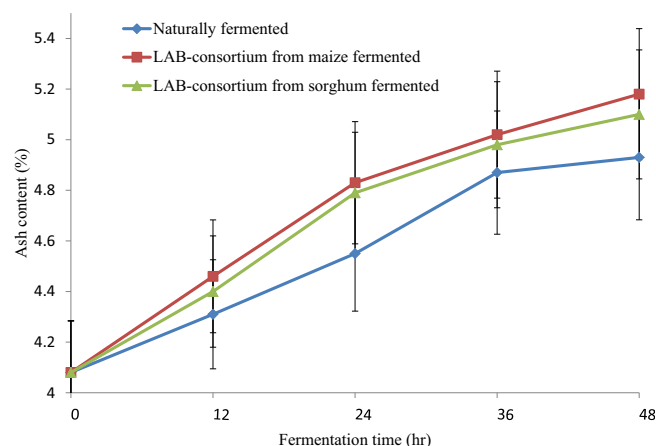


Fig. 2. Effect of fermentation on the ash content for soy bean flours. Each point represent the mean of three independent experiments and error bars indicate $\pm$ SE. There were significant differences ($P < 0.05$) at each successive time interval when compared between natural and LAB consortia fermentations. No significant difference ($P > 0.05$) between LAB consortium from maize and LAB consortium from sorghum fermentations at all time interval.

study to improve mineral composition of the soybean flour more than natural fermentation.

Fig. 3 presents the effect of fermentation on the percentage lipid content of soybean which showed a significant ($p < 0.05$) decrease with increasing fermentation time. It ranged from $16.51 \pm 0.12\%$ to $19.78 \pm 0.02\%$. No significant difference ($p > 0.05$) at each time interval when compared between the three fermentation set-ups. The highest reduction was observed in LAB-consortium from sorghum fermented sample ($16.51 \pm 0.12\%$) while the naturally fermented sample (17.08 ± 0.09) was the least. This report is consistent with the work of Babalola and Giwa (2012) who reported decrease in lipid content of soybean during fermentation. The reduction could be attributed to hydrolysis of fat into fatty acids and glycerol (Chinma et al., 2009). It could also be due to biochemical and physiological changes that occurs as fermentation progresses and part of the lipids are used as a source of energy (El-Beltagi and Mohamed, 2010; El-Beltagi et al., 2011; Afify et al., 2011). It could be asserted here that fermentation with LAB consortium could serve as a means of reducing rancidity in soybean flour as fat content triggers rancidity during storage, giving rise to off flavor as reported by Shobha et al. (2011) and Ntuli et al. (2013).

The protein content increased with increasing fermentation period from $38.41 \pm 1.01\%$ to $47.15 \pm 0.75\%$. The variations differ significantly ($p < 0.05$) with the naturally fermented sample (Fig. 4). There were significant differences ($p < 0.05$) at each successive time interval when natural fermentation is compared to LAB consortia fermentations except at 48 h where LAB consortium from maize fermentation did not differ with natural fermentation. The highest increase in protein was observed in LAB-consortium from sorghum ($47.15 \pm 0.75\%$) and LAB consortium from maize ($46.35 \pm 0.30\%$) and then naturally fermented sample ($45.86 \pm 0.05\%$). This demonstrated that LAB consortia are more effective in improving the protein content of final the product. Babalola and Giwa (2012), reported increase in protein content of soybean during fermentation in the range of 48.19 ± 0.02 – $56.53 \pm 2.16\%$ which are similar with the values obtained in the present study. The increase in protein contents could be attributed to the increase in the number of the fermenting microorganisms (Ojokoh et al., 2013, 2014) or due to proteolytic enzymes produced by the fermenting organisms (Amankwah et al., 2009; Ojokoh et al., 2015) or as a result of synthesis of protein by fermenting substrates which could have resulted to production of amino acids (Babalola and Giwa, 2012).

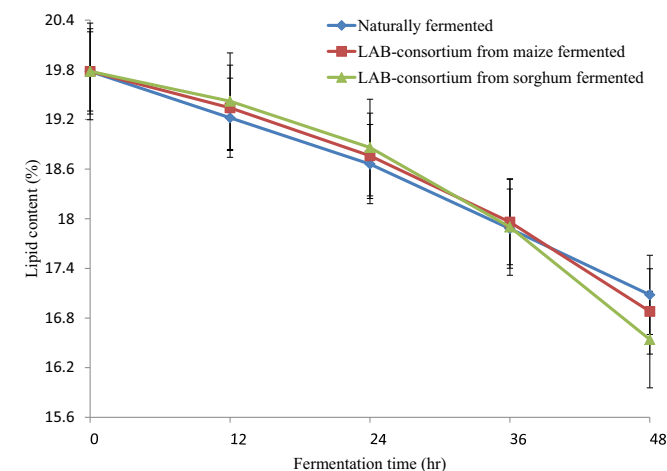


Fig. 3. Effect of fermentation on the lipid content for soy bean flours. Each point represent the mean of three independent experiments and error bars indicate $\pm$ SE. No significant difference ($P > 0.05$) at each time interval when compared between the three fermentation set-ups.

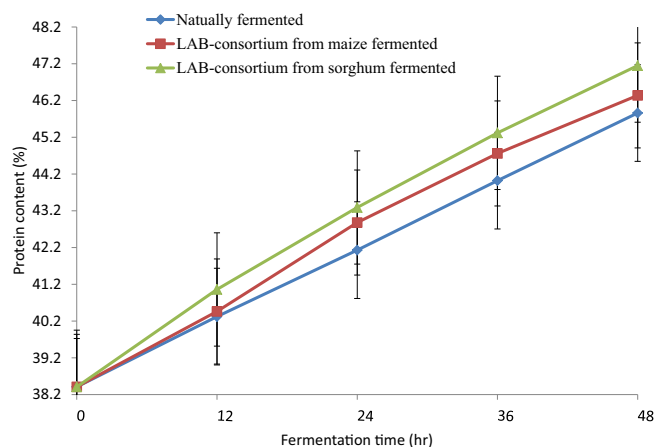


Fig. 4. Effect of fermentation on the protein content of soy bean flours. Each point represent the mean of three independent experiments and error bars indicate $\pm$ SE. There were significant differences ($P < 0.05$) at each successive time interval when natural fermentation is compared to LAB consortia fermentations except at 48 h where LAB consortium from maize fermentation did not differ with natural fermentation.

The result on the effect of fermentation on the fibre content of the samples under study showed a significant ($p < 0.05$) decrease with increasing fermentation periods. It decreased from $4.35 \pm 0.07\%$ to $0.86 \pm 0.06\%$ (Fig. 5). There were significant differences ($p < 0.05$) at each successive time interval when natural fermentation is compared to LAB consortia fermentations. There was no significant difference when LAB consortium from maize is compared to LAB consortium from sorghum fermentation respectively at all time intervals. The highest decrease was observed in LAB-consortium from sorghum fermented sample ($0.86 \pm 0.06\%$). Babalola and Giwa (2012) reported decrease in fibre content of soybean as a result of fermentation. The decrease could be attributed to the ability of LAB to metabolize and breakdown fibre during fermentation (Ojokoh and Bello, 2014).

The effect of fermentation on the carbohydrate composition of soybean flour showed a decrease as the fermentation periods increased. It decreased from $22.99 \pm 0.01\%$ to $19.37 \pm 0.05\%$ (Fig. 6). There were significant differences ($p < 0.05$) at each successive time

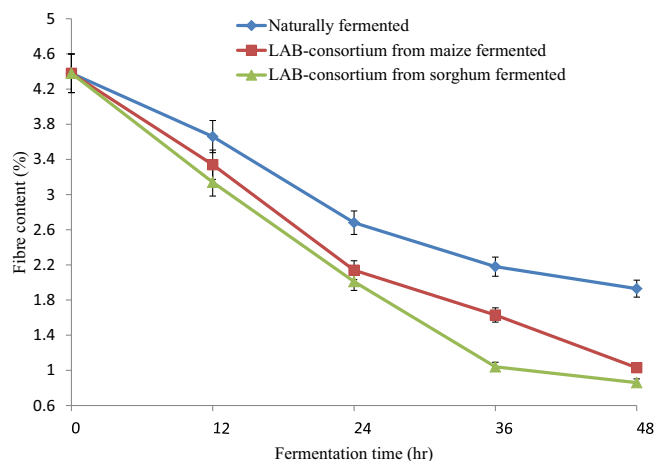


Fig. 5. Effect of fermentation on the fibre content of soy bean flours. Each point represent the mean of three independent experiments and error bars indicate $\pm$ SE. There were significant differences ($P < 0.05$) at each successive time interval when natural fermentation is compared to LAB consortia fermentations. No significant difference when LAB consortium from maize is compared to LAB consortium from sorghum fermentation respectively.

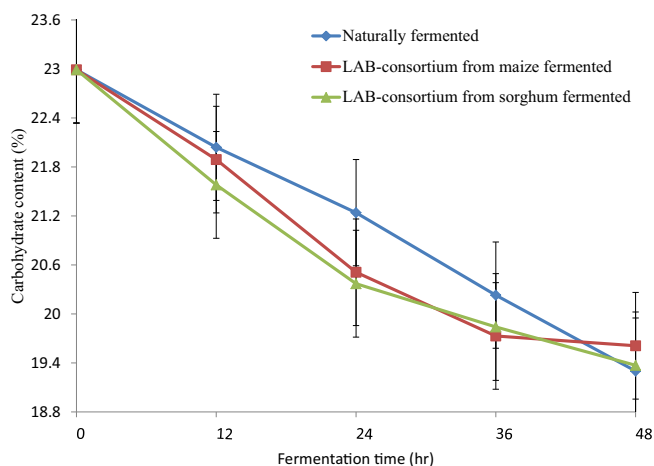


Fig. 6. Effect of fermentation on the carbohydrate content of soy bean flour. Each point represent the mean of three independent experiments and error bars indicate $\pm$ SE. There were significant differences ($P < 0.05$) at each successive time interval when natural fermentation is compared to LAB consortia fermentations at 24 h and 36 h only. No significant difference when LAB consortium from maize is compared to LAB consortium from sorghum fermentation respectively in all time interval.

interval when natural fermentation is compared to LAB consortia fermentations at 24 h and 36 h only. No significant difference showed when LAB consortium from maize was compared to LAB consortium from sorghum fermentations respectively in all time interval. The decrease could be due to hydrolyses of starch as a result of amylases produced by LAB into simple fermentable sugars. Roger et al. (2015) reported that lactic acid bacteria from maize have strong amylolytic activity which is consistent with the present observation.

In-vitro starch digestibility

The *in-vitro* starch digestibility (IVSD) of soybean samples under study showed a significant ($p < 0.05$) increase (Fig. 7). It increased from $71.36 \pm 0.20\%$ to $80.11 \pm 0.02\%$. There were significant differences ($p < 0.05$) at each successive time interval when natural fermentation is compared to LAB consortium from maize and LAB consortium from sorghum fermentation respectively. The increase

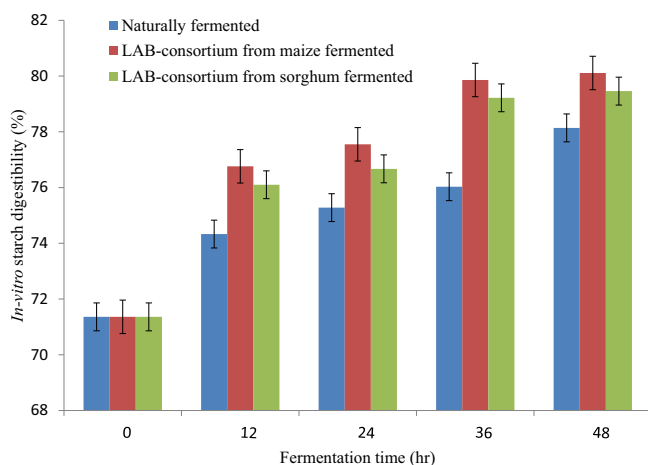


Fig. 7. Effect of fermentation on In-vitro starch digestibility of soybean flours under study at different time interval. Each point represent the mean of three independent experiments and error bars indicate $\pm$ SE. There were significant differences ($P < 0.05$) at each successive time interval when natural fermentation is compared to LAB consortium from maize and LAB consortium from sorghum fermentation respectively.

could be attributed to the role of fermentation in causing changes in the endosperm protein which makes starch more accessible to the digestive enzymes (Hassan et al., 1995; Singh et al., 2012).

In-vitro protein digestibility

The percentage *in-vitro* protein digestibility (IVPD) of the soybean flour showed a significant increase ($p < 0.05$) as fermentation time increased (Fig. 8). It increased from $85.40 \pm 2.46\%$ to $93.56 \pm 2.50\%$. There were significant differences ($p < 0.05$) at each successive time interval when natural fermentation is compared to LAB consortium from maize and LAB consortium from sorghum fermentation respectively. Chen et al. (2010) reported increase in IVPD of soybean meal fermented with a combination of *Aspergillus* and *Lactobacillus* from 87.50 to 92.23%. The present result also agreed with the assertion of other researchers who worked on other grains that fermentation improves protein digestibility (Mohiedeen et al., 2010; Singh et al., 2012). This could be as a result of the reduction in pH during fermentation which plays an important role in enhancing the proteolytic enzyme activity and in turn leads to the breakdown of proteins to smaller polypeptides which are easily digested (Singh et al., 2012; Olanipekun et al., 2015). The highest protein digestibility were observed in samples fermented with the LAB consortia which shows their effectiveness in improving the IVSD and IVPD of the sample more than the natural fermentation.

Microbial changes during soybean fermentation

Table 1 presents the effect of fermentation on the microbial load of soybean sample under study. The total viable count (TVC) increased with increasing fermentation and ranged from 3.80×10^8 CFU/g to 9.02×10^9 CFU/g. The total lactic acid bacteria count (TLBC) increased consistently to 36 h of fermentation and showed a slight decrease at 48 h of fermentation in all the fermentation set-ups and ranged from 3.42×10^6 CFU/g to 8.44×10^8 CFU/g. Coliform count (TCC) was only observed at 0 h of the naturally fermented sample (1.0×10^3 CFU/g). Fungi were only observed at the naturally fermented samples. This finding is consistent with the report of Olanipekun and Adekun (2015) who

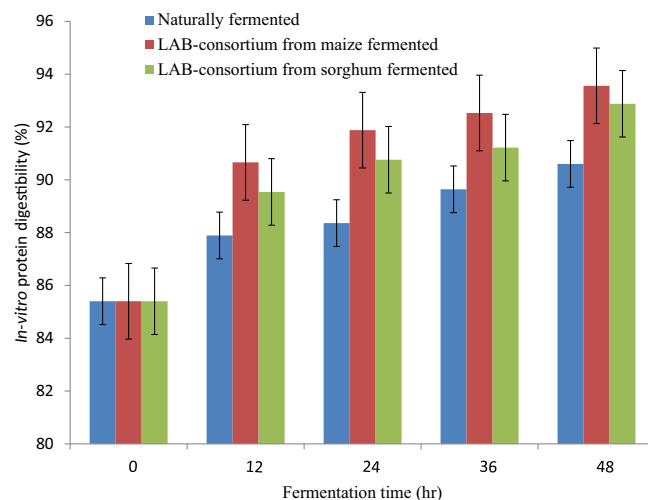


Fig. 8. Effect of fermentation on In-vitro protein digestibility of Soybean flours under study. Each point represent the mean of three independent experiments and error bars indicate $\pm$ SE. There were significant differences ($P < 0.05$) at each successive time interval when natural fermentation is compared to LAB consortium from maize and LAB consortium from sorghum fermentation respectively.

Table 1
Effect of fermentation on the microbial load (CFU/g) of the soybean flour after natural (N), LAB consortium from maize (M) and LAB-consortium from sorghum (S) fermentation on different media. Plate count agar (PCA) was used for total viable count (TVC), de Man Rogosa and Sharpe agar (MRS) used for lactic acid bacteria count (TLBC), MacConkey agar (MCA) used for total coliform count (TCC) while Sabouraud Dextrose agar (SDA) was used for total fungal count.

Time (hr)	PCA (TVC) (CFU/g)			MRS (TLBC) (CFU/g)			MCA (TCC) (CFU/g)			SDA (TFC) (CFU/g)		
	N	M	S	N	M	S	N	M	S	N	M	S
0	3.50×10^9	4.22×10^8	3.80×10^8	5.40×10^6	3.64×10^8	3.42×10^8	1.00×10^3	<10	<10	1.30×10^3	<10	<10
12	4.46×10^9	6.70×10^8	6.50×10^8	5.81×10^6	5.88×10^8	5.88×10^8	<10	<10	<10	1.52×10^3	<10	<10
24	6.70×10^9	7.64×10^8	7.42×10^8	6.10×10^6	7.22×10^8	7.22×10^8	<10	<10	<10	2.20×10^3	<10	<10
36	7.98×10^9	8.33×10^8	8.62×10^8	6.80×10^6	8.14×10^8	8.44×10^8	<10	<10	<10	2.80×10^3	<10	<10
48	9.02×10^9	8.20×10^8	8.31×10^8	6.44×10^6	8.00×10^8	8.13×10^8	<10	<10	<10	2.60×10^3	<10	<10

There was no significant difference ($p > 0.05$) on the microbial load for each media.

reported increase in total viable count during soybean fermentation. Absence of coliform in the present study could probably be due to low pH and increased population of (LAB) within the period of fermentation which produced antimicrobial substances that inhibited coliform (Olanipekun and Adelekan, 2015). Similar observation has been noted by Babalola and Giwa (2012) on the microbiology of fermented soybean.

Table 2 presents the occurrence of various bacteria and fungi in the fermented product. The predominant organism isolated from the present study belong to lactic acid bacteria (LAB) group including *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus natensis*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus brevis*, *Lactococcus lactis*, *Lactobacillus bulgaricus*, *Lactobacillus brevis*, *Pediococcus acidilactici* and *Leuconostoc* species. This showed the ability of the starter organisms to thrive in the substrate and created environment that was conducive for spoilage organisms to grow. Similar organisms have been reported by the other researchers during fermentation of millet and soybean blend flours (Ojokoh and Bello, 2014). *L. fermentum*, and *L. plantarum* were isolated from all the fermentation set ups. This is an indication of codominance of the facultative heterofermenter with the obligate heterofermenters (Ventimiglia et al., 2015). *L. lactis*, *Leuconostoc* species, *Candida utilis* and *Rhizopus* species were isolated from the naturally fermented sample only. This indicated that the normal microbial flora were dominated by the starter organisms in the LAB consortia fermentation.

In conclusion, the proximate composition of soybean flour in the present study improved significantly ($p < 0.05$) during natural and LAB-consortia fermentations. Also, the *in-vitro* protein/starch digestibility improved significantly ($p < 0.05$). The highest improvements were observed in the samples fermented with the LAB-consortia in most of the parameters analyzed. The LAB-consortium from maize and LAB-consortium from sorghum fermented samples

did not differ significantly in most of the parameters analyzed. This suggests that LAB-consortium fermentation is an effective means of improving the nutritional qualities as well as IVSD and IVPD of the sample more than natural fermentation. Moreover, the microorganisms encountered in this study are mostly lactic acid bacteria which also confer preservative property on the final product.

Conflicts of interest

The authors have declared no conflict of interest.

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Table 2
Occurrence of bacteria and fungi isolates in the fermented flour after natural (N), LAB consortium from maize (M) and LAB-consortium from sorghum (S) fermentation.

S/N	Organisms isolated	Soybean			Accession Number
		N	M	S	
1.	<i>Lactobacillus fermentum</i>	+	+	+	NR_104927.1
2.	<i>Lactobacillus plantarum</i>	+	+	+	NR_075041.1
3.	<i>Lactobacillus nantensis</i>	–	+	+	NR_043114.1
4.	<i>Lactobacillus reuteri</i>	–	+	–	NR_075036.1
5.	<i>Lactobacillus rhamnosus</i>	–	+	–	NR_102778.1
6.	<i>Lactobacillus brevis</i>	–	–	+	NR_044704.1
7.	<i>Lactococcus lactis</i>	+	–	–	–
8.	<i>Lactobacillus bulgaricus</i>	+	–	–	–
9.	<i>Pediococcus acidilactici</i>	–	–	+	NR_042057.1
10.	<i>Leuconostoc</i> species	+	–	–	–
11.	<i>Candida utilis</i>	+	–	–	–
12.	<i>Rhizopus species</i>	+	–	–	–

+ = present; – = absent.

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