

Variations in the Functional Properties of Soybean Flour Fermented with Lactic Acid Bacteria

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Abstract—The functional properties of soybean flour fermented with lactic acid (LAB)-consortium was evaluated. Soybean was processed into flour, fermented spontaneously and with LAB-consortium previously isolated from maize (*Lactobacillus plantarum* WCFS1+*Lactobacillus rhamnosus* GG, ATCC 53/03+*Lactobacillus nantensis* LP33+*Lactobacillus fermentum* CIP 102980+*Lactobacillus reuteri* DSM 20016) and sorghum (*Pediococcus acidilactici* DSM 20284+*Lactobacillus fermentum* CIP 102980+*Lactobacillus brevis* ATCC 14869+*Lactobacillus nantensis* LP33+*Lactobacillus plantarum* WCFS1) to evaluate their effects on the functional properties of the flour at 12 h intervals using standard techniques. The result shows gradual decrease in bulk density with increasing fermentation period ranging from 0.74 ± 0.03 g/mL to 0.72 ± 0.03 g/mL (natural), from 0.74 ± 0.03 g/mL to 0.70 ± 0.02 g/mL (LAB-consortium from maize) and from 0.74 ± 0.03 g/mL to 0.70 ± 0.02 g/mL (LAB-consortium from sorghum) fermentation. The swelling capacity decreased from 0.77 ± 0.03 g/mL to 0.64 ± 0.01 g/mL, from 0.77 ± 0.03 g/mL to 0.59 ± 0.01 g/mL and from 0.77 ± 0.03 g/mL to 0.61 ± 0.03 g/mL in natural, LAB-consortium from maize and sorghum fermentations respectively. Water holding capacity decreased from 2.4 ± 0.03 mL/g to 1.9 ± 0.03 mL/g, from 2.4 ± 0.03 mL/g to 2.0 ± 0.03 mL/g and from 2.4 ± 0.03 mL/g to 1.9 ± 0.03 mL/g in natural, LAB-consortium from maize and sorghum fermentation respectively. Oil holding capacity increased significantly ($p<0.05$) with increasing fermentation time, ranging from 8.92 ± 0.02 mL/g to 9.30 ± 0.03 mL/g (natural), 8.92 ± 0.01 mL/g to 9.63 ± 0.03 mL/g (LAB-consortium from maize) and from 8.92 ± 0.03 mL/g to 9.69 ± 0.03 mL/g (LAB-consortium from sorghum) fermentations. The least gelation concentration ranged from 3.0% (unfermented) to 6.0% (other fermentation products). Emulsion capacity (EC) increased from $35.88\pm 3.12\%$ to $44.33\pm 1.33\%$, from $35.88\pm 3.12\%$ to $46.83\pm 3.18\%$ and from $35.88\pm 3.12\%$ to $45.99\pm 2.21\%$ in natural, LAB-consortium from maize and sorghum fermentations respectively. This suggests the potentials of LAB-consortia fermentation in improving nutritional and functional properties of soybean flour.

Keywords— Fermentation, Functional Properties, LAB-consortium, Soybean flour.

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I. INTRODUCTION

IN developing countries of Africa like Nigeria, malnutrition in terms of protein is prevalent due to inadequate or high cost of meat and milk, hence the need for alternative sources of protein [1]. Legumes such as Bambara groundnut pigeon pea, soybean are nutritious foods which can serve as an alternative to animal proteins owing to their reported nutritional attributes [2-3]. Therefore, legume seeds flours can be employed in food formulations to supplement proteins and reduce over dependent on animal protein if they contain the desired functional properties. However, the utilization of legumes has been greatly affected by the presence of antinutritional factors such as phytate, tannins, oxalates, polyphenols and enzyme inhibitors. Although treatments such as heat, germination, soaking and fermentation have been reported to reduce the antinutritional factors in food products [4-8].

Soybean (*Glycine max*) was first grown in Eastern Asia. It has long been used as important sources of protein, complementing grain proteins, in Asian countries [9]. Fermented soybean products has been reported to contain functional components such as peptides, isoflavonoids and consumption of soybeans and soy foods is associated with lowered risks for several cancers including breast, prostate, and colon, and cardiovascular diseases and improves bone health [10]. [9] reported that the protein content of soybean is 32% to 42%, depending on the variety and growth conditions, of which approximately 80% is composed of 2 storage globulins, 7S globulin (β -conglycinin) and globulin (glycinin), having various functional and physicochemical properties.

Fermentation is a major process used in the production of foods from soybeans [10]. Fermentation leads to changes the physico-chemical, organoleptic properties (colour, flavour) and active components of soybean products [11]. Changes to phenolic composition of legumes brought about by fermentation have been reported to significantly increase free radical scavenging capacity of legumes [12-13]. Major microorganisms implicated in soybean fermentation are filamentous fungi, *Bacillus* species and lactic acid bacteria [11]. It has been reported that soybeans fermented with filamentous fungi enhanced the phenolic content, radical scavenging activity and antioxidant activity in fermented

product [14-15].

Lactic acid fermentation is a common way of preparing food such as maize porridge, alcoholic beverages and dairy products traditionally in many African countries [16-17]. Fermentation by lactic acid bacteria increases food palatability, proteins, vitamins and as well confer preservative and detoxifying effect on food. Also, lactic acid bacteria fermented foods boost immunity against bacterial infections [18-20]. Beta glycosides are known to be hydrolyzed by Some Lactobacilli and *Bifidobacteria* thereby enhancing the bioavailability of isoflavones contained in soybean by fermentation [21]. Therefore, the present study evaluates the effect of lactic acid bacteria consortium fermentation on the functional properties of soybean flour.

II. MATERIALS AND METHODS

A. Source of materials

Cream coloured soybean (*Glycine max* (L) Merr.) was bought from Yaba market of Lagos, Lagos State, Nigeria and transported to Federal Institute of Industrial Research Oshodi (FIRO) where it was identified and analyzed. Lactic acid bacteria (*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) used were stock previously isolated from fermenting maize and sorghum.

B. Sample preparation

The raw seeds of soybeans were freed of foreign materials, washed, rinsed with distilled water and then dried with hot air oven (GL, England) at 60°C for 8 h. The dried samples were milled into powder using milling machine (CNC, Germany) disinfected with 70% ethanol. The flour was stored in sterile air tight containers at 40°C for further use [22].

C. Choice of Starter

The Lactic acid bacteria consortium 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.

D. Inoculum preparation

The starter culture was prepared following the method of [22] as described by [17] with slight modification. Each of the lactic acid bacteria obtained as stock previously isolated from maize and sorghum was grown at 37°C for 24 h on autoclaved MRS broth in test-tubes of 10 ml aliquots. Then five lactic acid bacteria were combined as follows, *Lactobacillus plantarum* WCFS1 + *Lactobacillus rhamnosus* GG, ATCC 53/03 + *Lactobacillus nantensis* LP33 + *Lactobacillus fermentum* CIP 102980 + *Lactobacillus reuteri* DSM 20016, for consortium from maize; and *Pediococcus acidilactici* DSM 20284 + *Lactobacillus fermentum* CIP 102980 + *Lactobacillus brevis* ATCC 14869 + *Lactobacillus nantensis* LP33 +

Lactobacillus plantarum WCFS1, for consortium from sorghum respectively. These were grown in a 250 ml Erlenmeyer flasks containing 210 ml MRS broth respectively, and incubated for 48 h at 37°C for the inoculum to build-up. The inocula were harvested by centrifugation and washed with sterile distilled water at 5000 rpm for 10 min. The washed cells were diluted using sterile distilled water to obtain 0.5 McFarland, standard of 1.0×10^8 cells/mL.

E. Fermentation Procedure of Soybean flour

Fermentation was carried out following a modification of the method described by [17] with slight modification. 500 g of the raw flour was mixed with sterile distilled water in the ratio of 1:2 w/v in sterile fermentation containers. 0.5 g/L potassium sorbate was added (to inhibit fungal growth and other contaminating organisms) except the set up for spontaneous fermentation. The mixture was inoculated with 10 ml of 1.0×10^8 cells/mL of the lactic acid bacteria consortium suspensions and allowed to ferment. Starter culture was not added to one of the set-ups to allow for spontaneous fermentation. Samples were taken at 12 h intervals for analysis of the functional properties.

F. Determination of functional properties of fermented soybean flour

The bulk density of the fermented soybean flour was determined using the method of [23]. The water and oil holding capacities were determined according to the method described by [6]. The method of [24] was used to determine the swelling capacity of the soybean flour. The least gelation concentration of the flour was determined with the method described by [25] while the emulsion capacity was determined using the method described by [26].

III. RESULTS

The bulk density decreased gradually as fermentation time increased and ranged from 0.74 ± 0.03 g/mL to 0.72 ± 0.03 g/mL (natural fermentation), 0.74 ± 0.03 g/mL to 0.70 ± 0.02 g/mL (LAB-consortium from maize fermentation) and from 0.74 ± 0.03 g/mL to 0.70 ± 0.02 g/mL (LAB-consortium from sorghum) fermentation (Figure 1).

Figure 2 presents the swelling capacity of the fermented soybean flour which showed a gradual decrease with increasing time of fermentation. In natural fermentation, it ranged from $0.77 \pm 0.03\%$ to $0.64 \pm 0.01\%$, from $0.77 \pm 0.03\%$ to $0.59 \pm 0.01\%$ and from $0.77 \pm 0.03\%$ to $0.61 \pm 0.03\%$ in natural, LAB-consortium from maize and sorghum fermentations respectively.

The result of water holding capacity of the fermented flour decreased with increasing time of fermentation from 2.4 ± 0.03 mL/g to 1.9 ± 0.03 mL/g, from 2.4 ± 0.03 mL/g to 2.0 ± 0.03 mL/g and from 2.4 ± 0.03 mL/g to 1.9 ± 0.03 mL/g in natural, LAB-consortium from maize and sorghum fermentation respectively (Figure 3).

Oil holding capacity increased significantly ($p < 0.05$) with increasing fermentation time ranging from 8.92 ± 0.02 mL/g to

9.30±0.03 mL/g (natural), 8.92±0.01 mL/g to 9.63±0.03 mL/g (LAB-consortium from maize) and from 8.92±0.03 mL/g to 9.69±0.03 mL/g (LAB-consortium from sorghum) fermentations (Figure 4).

The result of the least gelation concentration of the fermented flour is presented in Table 1 and ranged from 3.0% (unfermented) to 6.0% (other fermentation products). The lowest was observed in the unfermented sample while the highest was observed at the 36 and 48 h of the fermented products.

Figure 5 presents the emulsion capacity (EC) of the fermented soybean flour. The result showed a significant increase ($P < 0.05$) in the EC as fermentation time progressed. The increase ranged from 35.88 ± 3.12% to 44.33 ± 1.33%, from 35.88 ± 3.12% to 46.83 ± 3.18% and from 35.88 ± 3.12% to 45.99 ± 2.21% in natural, LAB-consortium from maize and sorghum fermentations respectively.

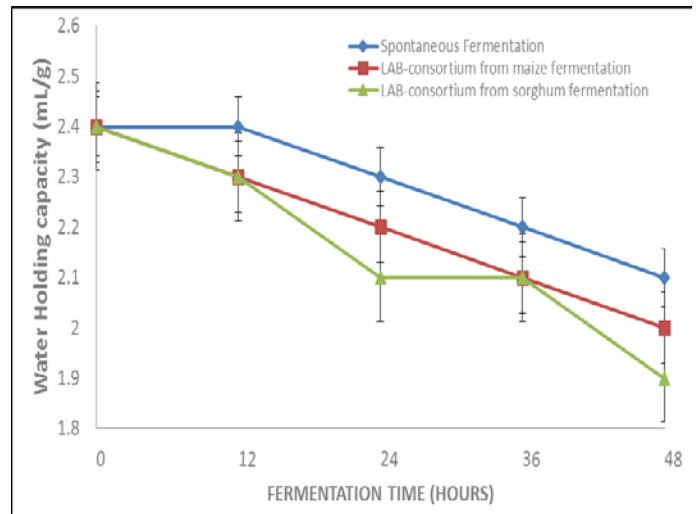


Fig. 3 Water Holding Capacity of LAB-consortium fermented soybean flour at different time interval

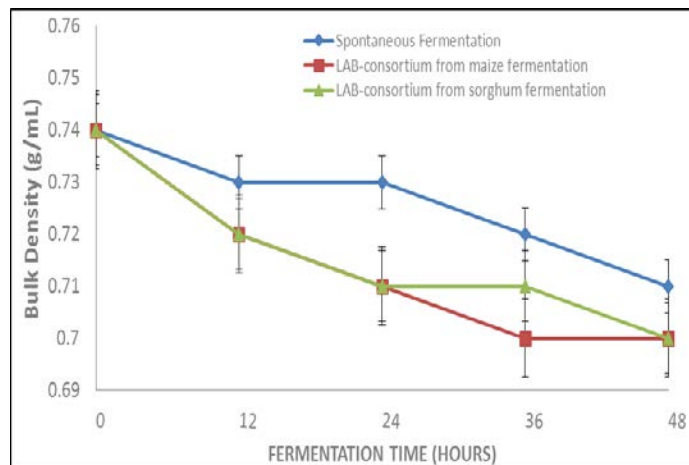


Fig. 1 Bulk density of LAB-consortium fermented soybean flour at different time interval

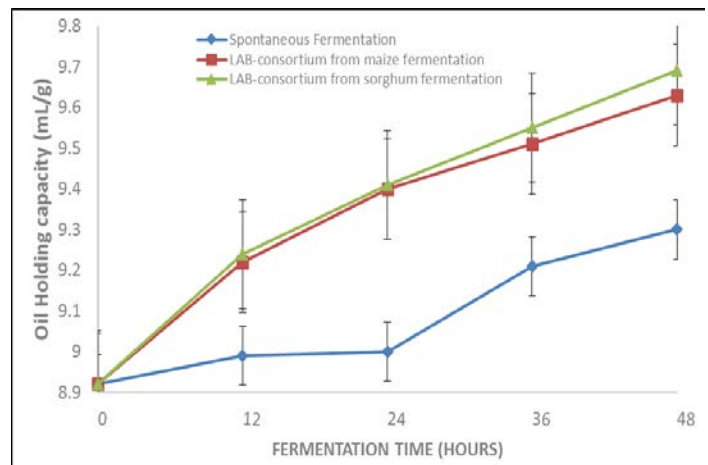


Fig. 4 Oil Holding Capacity of LAB-consortium fermented soybean flour at different time interval

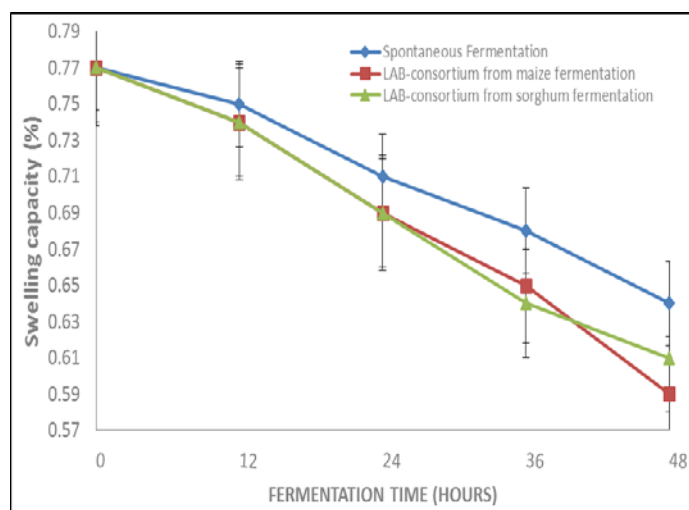


Fig. 2 Swelling Capacity of LAB-consortium fermented soybean flour at different time interval

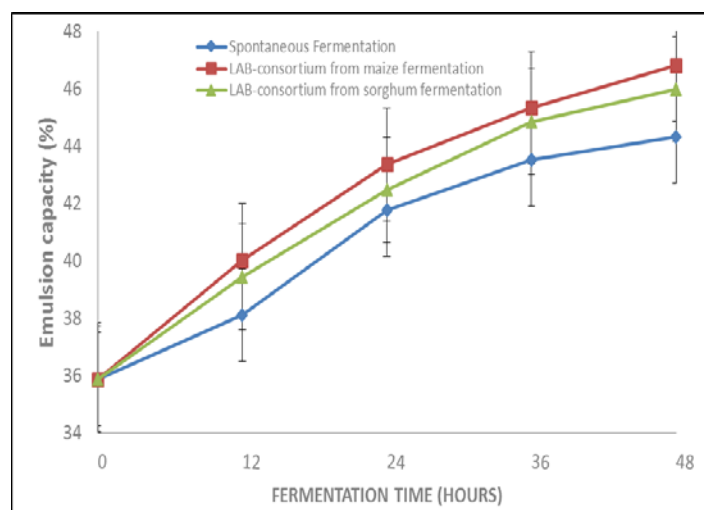


Fig. 5 Emulsion Capacity of LAB-consortium fermented soybean flour at different time interval.

Table 1: Least gelation concentration (LGC) (%) of LAB-consortium fermented soybean flour

Conc. (%)	0hr		12hr		24hr		36hr		48hr				
	F*	NF	MF	SF	NF	MF	SF	NF	MF	SF	NF	MF	SF
1.0	V	V	V	V	V	V	V	V	V	V	V	V	V
2.0	V	V	V	V	V	V	V	V	V	V	V	V	V
3.0	G	V	V	V	V	V	V	V	V	V	V	V	V
4.0	G	G	G	G	V	V	V	V	V	V	V	V	V
5.0	G	G	G	G	G	G	G	V	G	G	V	V	V
6.0	G	G	G	G	G	G	G	G	G	G	G	G	G
7.0	G	G	G	G	G	G	G	G	G	G	G	G	G
8.0	G	G	G	G	G	G	G	G	G	G	G	G	G
9.0	G	G	G	G	G	G	G	G	G	G	G	G	G
10.0	G	G	G	G	G	G	G	G	G	G	G	G	G
LGC	3.0 ^a	4.0 ^{a,b}	4.0 ^{a,b}	4.0 ^{a,b}	5.0 ^{b,c}	5.0 ^{b,c}	5.0 ^{b,c}	6.0 ^{c,d}	5.0 ^{b,c}	5.0 ^{b,c}	6.0 ^{c,d}	6.0 ^{c,d}	6.0 ^{c,d}

F* = Unfermented sample; NF = naturally fermented; MF = fermented with LAB consortium from maize; SF = fermented with LAB consortium from sorghum; V = viscous; G = gel; LGC = Least gelation concentration in percentage. Values with the same superscript are not significantly difference (P>0.05).

IV. DISCUSSION

The bulk density of the fermented flour in the present study decreased with increasing fermentation period. It ranged from 0.74 ± 0.03 g/mL (raw sample) to 0.70 ± 0.02 g/mL (48 h LAB consortium from maize and sorghum fermentations). This observation is in agreement with the report of other researchers on similar legumes. For instance, [2] reported decrease in bulk density of fermented pigeon pea flour in the range of 0.80 to 0.63 g/mL. [27], reported a decrease in the bulk density of quality protein maize (QPM)-soybean blend after 48 h fermentation which was observed to be consistent with the report of [28] on a soybean based weaning food. The values in the present study is also comparable with 0.68 reported by [29] on the effect of soybean/cassava on the proximate composition of Ethiopian traditional bread and 0.62-0.69 g/mL reported by [30] on fermented mahogany beans (*Afzelia africana*) flour. The reduction in the density as a result of fermentation is an advantage when the flour are to be used for the formulation of infant foods since fermentation has been reported by [6] to be a traditional means of preparing low density foods.

In the present study, the swelling capacity (SC) decreased with increasing fermentation time. It ranged from 0.77 ± 0.03 g/mL (raw sample) to 0.59 ± 0.01 g/mL (LAB consortium from maize fermentation). The lowest reduction was found in LAB consortium from maize fermentation (0.59 ± 0.01 g/mL), followed by LAB consortium from sorghum fermentation (0.61 ± 0.01 g/mL) and then natural fermentation (0.64 ± 0.01 g/mL). The variations in the SC of the sample differ significantly ($p < 0.05$) when compared between unfermented and fermented samples. The result of this study is lower than the report of [30] on fermented *Afzelia Africana* flour which

ranged from 2.50 - 2.87 g/mL. However, the decrease in the SC during fermentation agreed with the reports of [2] and [6] who reported decrease in SC with increasing fermentation in sorghum, millet, sorghum and pigeon pea respectively. Swelling capacity have been reported to be due to the quantity of amylopectin which are subject to microbial degradation during fermentation [31].

Water holding capacity (WHC) in this study decreased with increasing fermentation time in the range of 2.4 ± 0.03 mL/g to 1.9 ± 0.03 mL/g. There were significant differences ($p < 0.05$) when the 48 h fermented products is compared to the raw sample (0 h). This observation is comparable to the work of [2] who reported decrease in WHC of pigeon pea from 142.0 g/100g to 113.0 g/100g after a 5-day fermentation. Similar observation has been reported by [22] on lactic acid bacteria fermented maize flour and [27] on QPM-soybean blend. The values of the present report is higher than 1.12 g/100 g reported by [32] on soybean. Also, the present observation disagrees with the report of [30] who reported increase in WHC of fermented *Afzelia Africana*. WHC is an indication of the amount of water available for gelatinization and according to [6], low absorption capacity is desirable for making thinner gruels.

In the present study, the oil holding capacity (OHC) of the flour increased significantly ($p < 0.05$) with increasing fermentation time. The highest increase was observed in 48 h sample fermented with LAB consortium from sorghum (9.69 ± 0.03 mL/g), followed by LAB consortium from maize fermented sample (9.63 ± 0.03 mL/g) while the spontaneous fermentation was the least (9.30 ± 0.03 mL/g). This observation agreed with the report of [6] that fermentation

increased the OHC in the range of 8.0 to 9.7 for sorghum, millet and maize. Also, [22] who reported increase in OHC of maize flour fermented with LAB consortium which is in agreement with the present investigation. However, the result of this study is higher than 1.43 ml/g reported by [1] for soybean. The increase in the OHC of the present study suggests that the flour could be useful in food formulation where an oil holding capacity is considered [6]. Moreover, where optimum oil absorption is desired in food system, the ability of the flours to bind with oil makes it very useful, hence potential for use in foods such as sausages [26].

The least gelation concentration (LGC) concentration in the present study ranged from 3.0% to 6.0%. [2], also reported decrease in gelation power with increasing fermentation time in pigeon pea which agreed with the present investigation. The values obtained in the present study are lower than the report of other researchers on similar legumes such as cowpea (16%), soybean flour (10%), pigeon pea flour (4%), and African bread fruit flour (6 to 12%) [32-35]. The variations in the gelation capacities could be attributed to the relative ratios proteins, carbohydrates and lipids that make up the flour which may have a significant impact on the functional properties of the products [2, 36].

Emulsion capacity (EC) of soybean flour in the present study increased significantly ($p < 0.05$) with increasing fermentation time. It ranged from $44.33 \pm 1.22\%$ (spontaneous fermentation) to $46.83 \pm 3.18\%$ (LAB consortium from maize fermentation). The highest increase was observed in LAB consortium from maize fermentation ($46.83 \pm 3.18\%$) followed by LAB consortium from sorghum fermentation ($45.99 \pm 1.18\%$) while the spontaneous fermentation was the least ($44.33 \pm 1.22\%$) after 48 h fermentation. This result is consistent with the reports of [26] who reported EC during fermentation of wheat and rice as 43 and 41% respectively. Also, [22] reported increase in EC of maize with increasing fermentation time. However, the present observation did not agree with the report of [37] who reported decrease in EC of brown hamburger beans (*Mucuna sloanei*) and sweet detar seeds (*Detarium microcarpum*) during fermentation. Difference in the EC of the various samples may be related to solubility and hydrophobicity of proteins [26, 38].

V. CONCLUSION

The functional properties of sorghum flours in the present study after natural fermentation, LAB-consortium from maize and LAB-consortium from sorghum fermentations showed improvement. The improvements suggest the potential use of the flour for formulation of infant foods and making of thinner gruels. Hence, the flour can be used to blend foods for improved nutritional qualities. These suggest the possible use LAB-consortium fermentation in improving the nutritional qualities of local staple legume products.

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