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Functional properties of bambara groundnut flour fermented with lactic acid bacteria consortium

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Abstract. *Ogodo AC, Agwaranze DI, Opara JI, Iheanacho CC. 2023. Functional properties of bambara groundnut flour fermented with lactic acid bacteria consortium. Asian J Trop Biotechnol 20: 79-84.* The objective of this study was to evaluate the functional properties of bambara groundnut flour fermented with Lactic Acid Bacteria (LAB) consortium isolated from fermenting maize and sorghum. Sorghum was processed into flour and fermented with LAB-consortium previously isolated from maize (*Lactobacillus plantarum* WCFS1 + *Lactobacillus rhamnosus* GG, ATCC 53/03 + *Lactobacillus fermentum* CIP 102980 + *Lactobacillus nantensis* LP33 + *Lactobacillus reuteri* DSM 20016) and sorghum (*Pediococcus acidilactici* DSM 20284 + *Lactobacillus nantensis* LP33 + *Lactobacillus fermentum* CIP 102980 + *Lactobacillus brevis* ATCC 14869 + *Lactobacillus plantarum* WCFS1), respectively and then naturally to evaluate their effects on the functional properties of the bambara groundnut flour at 12 h intervals. Results showed that there was a gradual decrease in Bulk Density (BD), Swelling Capacity (SC), and Water Holding Capacity (WHC) with increasing fermentation period. Oil Holding Capacity (OHC) increased significantly ($p < 0.05$) with increase in the fermentation periods from 8.40±0.00 mL/g to 8.90±0.02 mL/g (spontaneous fermentation), 8.40±0.00 mL/g to 9.20±0.03 mL/g (LAB-consortium from maize fermentation) and from 8.40±0.00 mL/g to 9.70±0.03 mL/g (LAB-consortium from sorghum fermentation). The lowest gelation concentration ranged from 3.0% in the unfermented sample to 7.0% in the various fermentation products. The variations differ significantly ($p < 0.05$) between the unfermented, spontaneously fermented, and LAB consortium fermented samples. Emulsion Capacity (EC) increased with increasing fermentation period from 72.36±2.01% to 87.54±0.36%, from 72.36±2.01% to 87.22±1.44% and from 72.36±2.01 % to 88.56±0.14% in natural, LAB-consortium from maize and LAB-consortium from sorghum fermentation respectively. This result indicates that lactic acid bacteria consortia can potentially improve the functional properties of bambara groundnut flour.

Keywords: Bambara groundnut, consortium, fermentation, functional properties, lactic acid bacteria, nutritional quality

INTRODUCTION

Bambara groundnut (*Vigna subterranea* L. Verdc.) is a crop that is native to Africa and is grown in various countries across the African continent, especially Senegal, Kenya, and South Africa (Atiku et al. 2004; Goudoum et al. 2015) as well as Nigeria. It is regarded as one of the most neglected and underutilized crops. However, it can be used as a complete food (Ogodo et al. 2018a). The report of Aremu et al. (2006) revealed that bambara groundnut contains 64.9% carbohydrate, 9.7% moisture, 16% protein, 5.9% fat, and 2.9% ash. Similarly, Olanipekun et al. (2012) reported that bambara groundnut contains an appreciable amount of some amino acids, such as lysine, trypsin, and chymotrypsin.

In sub-Saharan Africa, leguminous crops indigenous to the region can reduce the vulnerability of rural households to food insecurity and malnutrition, as revealed through impact assessment (Matsa and Mukoni 2013; Mubaiwa et al. 2018). Moreover, efforts are geared towards processing legumes to make proteins available due to the high cost of animal protein, thereby combating malnutrition (Goudoum et al. 2015). Bambara groundnut is an important legume that is a source of cheap and affordable protein, especially in areas where animal proteins are expensive (Mubaiwa et al. 2018). The report of Plahar and Yawson (2004) showed that bambara groundnut is rich in essential amino acids and has a protein score of 80%, which is higher than other legumes such as soybean (74%) and cowpea (64%). This indicates that bambara groundnut contains more available human proteins than other legumes in Africa, although it does not contain sulfur-rich amino acids like other legumes (Brough and Azam-Ali 1992; Schaafsma 2012). However, it can be mixed with cereals like maize, which is rich in methionine and cysteine, and can form good nutritional fortification strategy (Akpapunam and Darbe 1994; Mubaiwa et al. 2018). Fermentation of food substrates leads to improvement in their nutritional composition, such as increase in protein and amino acids as well as improvement in the protein and starch digestibility with concomitant decrease in the antinutritional factors, leading to bioavailability of minerals (Singh et al. 2012). Fermentation contributes to the valuable properties of food products as well as in the production of beneficial products, including biomass protein, amino acids, minerals, vitamins, aroma, and flavor compounds as well as products of biosynthetic pathway (lactic acid, ethanol, pyruvate, acetaldehyde etc.) which contributes to the reduction of food pH leading to the control of the growth of pathogens, which enhances the shelf-life of food and food safety and preservation (Onyango et al. 2013; Ojokoh and Bello 2014).

Lactic Acid Bacteria (LAB) are large groups of non-sporing, non-motile firmicutes, Gram-positive cocci, and rods. They are catalase and oxidase-negative and utilize carbohydrates to produce lactic acid during fermentation (Masood et al. 2011). LAB includes *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Leuconostoc* species, etc. Lactic Acid Bacteria (LAB) fermentation makes food more palatable and increases food's protein and vitamin contents (Masood et al. 2011; Pang et al. 2011; Ogodo et al. 2017). It is one of the major ways of preparing food locally in Africa. Cereals, such as maize and sorghum and legumes such as cowpea and bambara groundnut are fermented using LAB (Ogodo et al. 2016). In addition, lactic acid fermentation helps in food preservation. Moreover, the regular use or consumption of LAB-fermented foods strengthens the immune system in fighting bacterial infections, promoting health (Chelule et al. 2010).

Legumes, like soybean, bambara groundnut, lima beans, cowpea, pigeon pea, African yam beans, etc., serve as sources of nutrients, including proteins, calories, minerals, and vitamins (Olanipekun and Adelakun 2015). These legumes contain healthy proteins, carbohydrates, dietary fiber, and diverse vitamins and minerals. However, their content of antinutritional factors, such as phytic acid, tannins, polyphenols, are associated with fiber, reduces mineral bioavailability (Dueñas et al. 2012; Olanipekun and Adelakun 2015). Bambara groundnut and other legumes have been identified to have a major role in the fight against malnutrition in poor countries, especially in Africa (Yusufu and Ejeh 2018). Hence, it is very important to increase the consumption of legumes in developing countries, which serve as a source of affordable proteins for poor countries (Borget 1992). Therefore, the purpose of present study was to determine the LAB consortium's effect on bambara groundnut flour's functional properties.

MATERIALS AND METHODS

Sample collection

Bambara groundnut seeds (*V. subterranea*) were purchased from Lagos (Yaba market), Nigeria. The seeds were brought to the Federal Institute of Industrial Research Oshodi (FIIRO) for identification, processing and analysis. Lactic acid bacteria were obtained from stored cultures isolated from previously fermented maize and sorghum.

Sample preparation

The sample was prepared following the method described by Ogodo et al. (2018b). The bambara groundnut seeds were sorted, washed, and dried at 60°C for 8 hours in a hot air oven (GL, England). The dried seeds were milled into flour, and then stored in a clean, airtight container for further use.

Inoculum preparation

Inoculum was prepared according to the method described by Ogodo et al. (2017). A consortium of 5 lactic acid bacteria, each from the stock of bacteria isolated from fermented maize (*Lactobacillus plantarum* WCFS1, *Lactobacillus fermentum* CIP 102980, *Lactobacillus rhamnosus* GG, ATCC 53/03, *Lactobacillus nantensis* LP33, *Lactobacillus reuteri* DSM 20016) and fermented sorghum (*Pediococcus acidilactici* DSM 20284, *Lactobacillus nantensis* LP33, *Lactobacillus fermentum* CIP 102980, *Lactobacillus brevis* ATCC 14869, *Lactobacillus plantarum* WCFS1) were selected. The organisms were combined by growing them in a 250 mL conical flask and incubating them in an orbital shaker for 48 h in a co-culture for the inocula to build up. Afterward, the cells were harvested using the centrifuge at 5,000 rpm for 10 min and maintained (before being used for fermentation) in fresh de Manne Rogosa and Sharpe (MRS) broth. The bacteria cells were cleaned with sterile distilled water and standardized to 0.5 McFarland standard (Dajanta et al. 2009).

Fermentation of bambara groundnut flour

The method described by Ogodo et al. (2017) was conducted to carry out the fermentation process. The prepared bambara flour was combined with sterile distilled water in a ratio of 1:2. Exactly 500 g of the bambara flour sample was transferred to a sterile fermentation container and mixed with 1,000 mL of sterile distilled water followed by the addition of 0.5 g/L potassium sorbate to help eliminate microbial contaminants. The mixture was inoculated with 10 mL of 10^8 cells/mL of the consortium of lactic acid bacteria suspension and allowed to ferment. The potassium sorbate and starter organisms were not added to one of the set-ups, which was allowed to ferment spontaneously. The analysis of the functional properties of the flour was carried out at 12 h intervals.

Determination of functional properties

The method of Chau and Huang (2003) was used to determine the bulk density of the flour. Water Holding Capacity (WHC) and Oil Holding Capacity (OHC) were respectively determined following the method of Singh et al. (2012), while the swelling capacity of the flour was determined using the Robertson et al. (2000) method. The gelation properties of the bambara flour were determined following the method given by Aremu et al. (2008). The method of Suresh and Samsher (2013) was used to determine the various flours' Emulsion Capacity (EC).

RESULTS AND DISCUSSION

The results of Bulk Density (BD) of all the fermentation set-ups at different time intervals is presented in Figure 1. The bulk density decreased from 0.78±0.02 g/mL (0 h) to 0.72±0.02 g/mL (48 h) (spontaneous fermentation), 0.78±0.02 g/mL (0 h) to 0.71±0.03 g/mL (48 h) (LAB-consortium from maize fermented), and from 0.78±0.02

g/mL (0 h) to 0.71±0.03 g/mL (0 h) in LAB-consortium from sorghum fermented samples.

The Swelling Capacity (SC) of the LAB-consortium and spontaneous fermented bambara flour, which decreased with increasing fermentation time, is presented in Figure 2. The decreased ranged were 0.46±0.01% (0 h)-0.30±0.02% (48 h), 0.46±0.01% (0 h)-0.29±0.03% (48 h), and 0.46±0.01% (0 h)-0.29±0.03% (48 h) for spontaneous, LAB-consortium from maize and LAB-consortium from sorghum fermented samples, respectively.

Figure 3 shows the fermentation products' Water Holding Capacity (WHC) at different intervals. There was a decrease in the WHC ranging from 1.7±0.02 mL/g (0 h)-1.3±0.03 mL/g (48 h) (spontaneous fermentation), 1.7±0.02 mL/g (0 h)-1.3±0.03 mL/g (48 h) (LAB-consortium from maize fermentation) and 1.7±0.02 mL/g (0 h)-1.2±0.01 mL/g (48 h) (LAB-consortium from sorghum fermentation).

The Oil Holding Capacity (OHC) of all the fermentation products at different time intervals is presented in Figure 4. Results revealed that there was an increase in the OHC ranging from 8.40±0.00 mL/g (0 h)-8.9±0.02 mL/g (48 h), 8.40±0.00 mL/g (0 h)-9. 20±0.03 mL/g(48 h) and 8.40±0.00 mL/g (0 h)-9.70±0.03 mL/g (48 h) for spontaneous, LAB-consortium from maize and LAB-consortium from sorghum fermentations respectively.

The gelation concentration ranged from 3.0% (unfermented sample) to 6.0% in 48 h spontaneous and LAB-consortia fermentations, respectively (Table 1).

The fermented samples' Emulsion Capacity (EC) increased with fermentation time. It ranged from 72.36±2.01% (0 h)-87.54±0.36% (48 h) during spontaneous fermentation, 72.36±2.01% (0 h)-89.22±1.44% (48 h) and 72.36±2.01% (0 h)-88.56±0.14% (48 h) for LAB-consortium from maize and LAB-consortium from sorghum fermentations, respectively (Figure 5).

Table 1. Least gelation concentration (LGC) (%) of LAB-consortium fermented bambara groundnut flour

Concentrations	**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	G	G	G	V	V	V	V	V	V	V	V	V
4.0	G	G	G	G	G	V	V	V	V	V	V	V	V
5.0	G	G	G	G	G	G	G	G	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}	3.0^{a}	3.0^{a}	3.0^{a}	$4.0^{a,b}$	$5.0^{b,c}$	$5.0^{b,c}$	$5.0^{b,c}$	$5.0^{b,c}$	$5.0^{b,c}$	$6.0^{c,d}$	$6.0^{c,d}$	$6.0^{c,d}$

Note: F*: Unfermented sample, NF: Spontaneously fermented, MF: Fermented with LAB consortium from maize, SF: Fermented with LAB consortium from sorghum, V: Viscous, G: Gel, LGC: Least gelation concentration. Values with the same superscript are not significantly different (P >0.05)

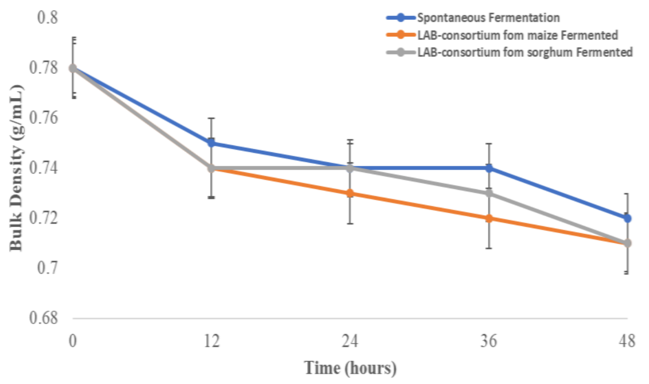

Figure 1. The bulk density (g/mL) of bambara flours fermented with lactic acid bacteria-consortium. The error bars represent standard error; LAB: Lactic Acid Bacteria

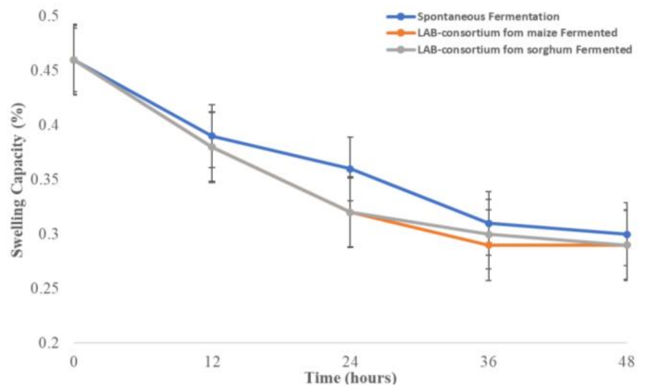

Figure 2. The swelling capacity (%) of bambara flours fermented with lactic acid bacteria-consortium. The error bars represent standard error; LAB: Lactic Acid Bacteria

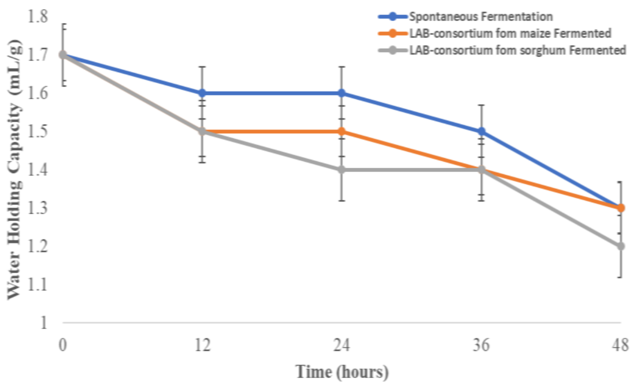

Figure 3. The water holding capacity (mL/g) of bambara flours fermented with lactic acid bacteria-consortium. The error bars represent standard error; LAB: Lactic Acid Bacteria

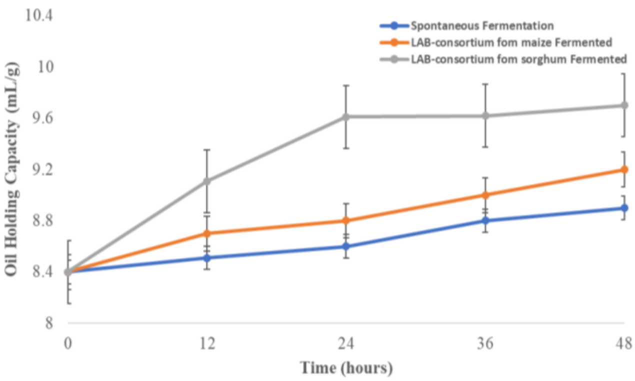

Figure 4. The oil holding capacity (mL/g) of bambara flours fermented with lactic acid bacteria-consortium. The error bars represent standard error; LAB: Lactic Acid Bacteria

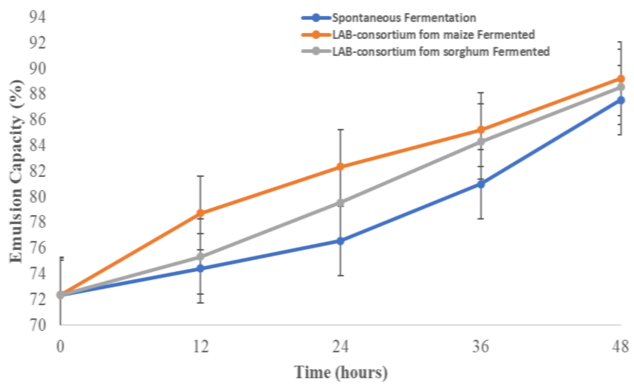

Figure 5. The emulsion capacity (%) of bambara flours fermented with lactic acid bacteria-consortium. The error bars represent standard error; LAB: Lactic Acid Bacteria

Discussion

In the present study, bambara groundnut's Bulk Density (BD) gradually decreased with increasing fermentation time. It decreased from 0.78±0.02 g/mL to 0.72±0.02 g/mL (spontaneous fermentation), 0.78±0.02 g/mL to 0.71±0.03 g/mL (LAB-consortium from maize fermented), and from 0.78±0.02 g/mL to 0.71±0.03 g/mL in LAB-consortium from sorghum fermented samples. The samples' bulk density variations were not differ significantly (P >0.05). Eltayeb et al. (2011) observed higher values of bulk density of bambara groundnut flour and protein. Adebowale and Maliki (2011) reported a gradual decrease in BD in the range of 0.80 to 0.63 g/mL with an increasing fermentation period of pigeon pea flours, comparable to the values obtained in the present investigation. Decreases in bulk density have been reported in products such as maize (Ogodo et al. 2016), sorghum, millet (Singh et al. 2012; Ocheme et al. 2015; Ogodo et al. 2017) and soybean (Ogodo et al. 2018c). The bulk density of the flour represents the load it can carry when allowed to be directly on top of one another (Singh et al. 2012). Moreover, Singh et al. (2012) reported that low-density foods obtained through fermentation are good for preparing weaning foods and infant food formulations (Singh et al. 2012; Ogodo et al. 2017). This indicates that the product of the present study can be used in infant food formulations.

Results of present study showed decrease in bambara groundnut flour's Swelling Capacity (SC) as fermentation progressed. The decreased ranged from 0.46±0.01%-0.30±0.02%, 0.46±0.01%-0.29±0.03%, and 0.46±0.01%-0.29±0.03% for spontaneous, LAB-consortium from maize and LAB-consortium from sorghum fermented samples, respectively. The variations showed significant differences (p <0.05) when the unfermented flour was compared to the fermented flour. The present observation is consistent with that of Singh et al. (2012), who reported a decrease in SC of pigeon peas with increasing fermentation time. Ogodo et al. (2018c) have also reported a decrease in the swelling capacity of fermented soybean flour. Similarly, the swelling capacity of fermented bambara groundnut-ogi formulation decreased with increasing percentage concentration of bambara groundnut flour in a study by Chude et al. (2018). However, the decrease in swelling capacity did not affect the organoleptic properties of the formulated food.

The water holding capacity in bambara groundnut flour decreased significantly (p <0.05) when compared between unfermented and fermented samples. The decreased value ranged from 1.7±0.02 mL/g-1.3±0.03 mL/g (spontaneous fermentation), 1.7±0.02 mL/g-1.3±0.03 mL/g (LAB-consortium from maize fermentation) and 1.7±0.02 mL/g-1.2±0.01 mL/g (LAB-consortium from sorghum fermentation). The result of this study is comparable to the work of Adebowale and Maliki (2011), who reported a decrease in WHC of pigeon peas at the end of a 5-day fermentation period. Similarly, decreases in water holding capacity after fermentation have been reported in products, such as maize (Ogodo et al. 2016), sorghum, millet (Singh et al. 2012), and soybean (Ogodo et al. 2018c). The report of Chude et al. (2018) showed a decrease in the water absorption capacity of bambara groundnut-ogi food formulation following an increase in the percentage of bambara groundnut flour. Similarly, in a study by Elkhalifa et al. (2005), there was a decrease in WHC after 8-24 h fermentation of sorghum. However, the report of an increase in the water absorption capacity of maize from 1.2-1.8 ml/g by Beugre et al. (2014) did not correspond to the present study. Water binding capacity is useful when flour is added to food formulation, especially when doughs are involved. It is a useful indicator of the quantity of water available for gelatinization. Low water absorption holding capacity is desirable in making thinner gruels (Singh et al. 2012), which agrees with the current findings. This indicates that the fermented flours in this study can be applied in the preparation and formulation of infant weaning foods and the production of product,s such as biscuits, snacks, and other baked foods when blended with other unfermented flours (Singh et al. 2012; Ogodo et al. 2016).

There is a significant increase ($p < 0.05$) in the Oil Holding Capacity (OHC) of the bambara groundnut flour in the present study as fermentation time increased. The OHC increased from 8.60±0.01 mL/g to 9.78±0.02 mL/g (spontaneous fermentation), from 8.92±0.02 mL/g to 9.69±0.03 mL/g (LAB-consortium from maize fermentation) and from 8.40±0.00 mL/g to 9.70±0.03 (LAB consortium from sorghum fermentation). The increase values observed in the present study is higher than the 1.43 ml/g reported by Acuña et al. (2012) for soybeans. This implies that these fermented flours could be used to formulate and fortify food, especially where the limiting factor is OHC (Singh et al. 2012). Moreover, the water and oil holding capacity depends on the food's inherent factors, such as surface hydrophobicity and polarity, protein conformation, and amino acid contents (Suresh and Samsher 2013; Ogodo et al. 2016); also, where oil absorption needs to be optimized in a food system, the binding of the flour proteins with oil makes it useful. This property could make the flour useful if food production includes sausages (Suresh and Samsher 2013).

Gelation power is an index of the gelling tendency of samples, and it is an important factor in food preparations (Adebowale and Maliki 2011). In the present study, the Least Gelation Concentration (LGC) decreased as the fermentation time increased, ranging from 3.0% (unfermented sample) to 6.0% in 48 h spontaneous and LAB-consortia fermentations. This observation is in line with Adebowale and Maliki (2011) and Ogodo et al. (2018c) who observed that fermentation decreased the gelation power of pigeon peas and soybeans, respectively. Gelation power may be affected by the proportion of carbohydrates, lipids, and proteins in the flour, which shows that the way these macromolecules interact within a product influences the behavior of the functional properties of the product greatly (Adebowale and Maliki 2011; Ogodo et al. 2017).

The result of the Emulsion Capacity (EC) of the bambara groundnut flour increased with increasing fermentation period ranging from 72.36±2.01% to 87.54±0.36% (spontaneous fermentation) from 72.36±2.01% to 89.22±1.44% and 72.36±2.01%-88.56±0.14% for LAB-consortium from maize and LAB-consortium from sorghum fermentation, respectively. The values recorded for the fermented products significantly ($p < 0.05$) differ from those from the unfermented samples. However, the values recorded for spontaneous fermentation, LAB-consortium from maize, and LAB-consortium from sorghum fermented products showed no significant difference ($p > 0.05$). The increase in the emulsion capacity of soybeans has been reported in a previous study (Ogodo et al. 2018c). The observation of EC has a relationship with the protein solubility pattern, as Suresh and Samsher (2013) asserted. Similarly, Kaushal et al. (2012) have reported that protein hydrophobicity in a particular product is a contributing factor to the emulsifying properties of the product.

In conclusion, the present study proved that spontaneous fermentation with lactic acid bacteria consortium isolated from maize, and fermentation with lactic acid bacteria consortium isolated from sorghum could improve the parameters of functionalities of bambara groundnut flour. The results of the present study revealed that fermentation with lactic acid bacteria consortium enhanced the functional properties of bambara groundnut flour more than spontaneous fermentation. Hence, this indicates the potential of lactic acid bacteria consortium from cereals in improving bambara groundnut flour's functional and nutritional qualities. This can be applied in natural food fortification by food industries.

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