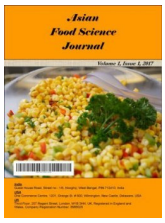

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Effect of *Lactobacillus* species on the Fermentation of Acha (*Digitaria exilis*) Grain

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Authors' contributions

This work was carried out in collaboration between all authors. Author OTI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AOO and OFO managed the analyses of the study. Author AOO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To study the effects of Acha fermented with *Lactobacillus* spp on the nutritional composition.
Study design: Acha was fermented in two forms (Local fermentation and controlled fermentation). Acha was weighed into a fermenting container of 100g and water of 1litre was added to submerge it for 72 hours in the ratio 1:3.
Place and Duration of Study: Sample: Department of Microbiology, Federal University of Technology Akure, Ondo State between January 2016 - October 2016.
Methodology: Microbial analysis was carried out using potato dextrose agar, nutrient agar and De man Rogosa agar. pH and total titratable acidity analysis were carried out. A proximate and mineral composition of the fermented acha was also carried out.
Results: A total number of thirteen (13) microorganisms were isolated from the locally fermented acha; these comprise of 8 bacteria (*Bacillus* spp*, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii, Staphylococcus aureus* and *Streptococcus* spp), two moulds and three yeasts (*Aspergillus niger, Aspergillus flavus, Mucor mucedo, Sacharromyces cerevisae* and

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Candida albicans). The pH values, reduce with increased days of fermentation. The titratable acidity in the fermentation of acha grains increased with hours of fermentation. Increase in protein, fibre content was evident in the controlled fermented Acha compared to the locally fermented Acha.

Keywords: Casei; acha; controlled fermentation.

1. INTRODUCTION

Cereals constitute the major Sources of energy, protein, vitamins, world population. The cereal grain, of economic importance, is the cool-season crops, mainly wheat, barley, oats, warm-season cereals like rice, maize, sorghum and millet [1]. Besides other African traditional cereals, acha (fonio) grains have played a central role in the emergence and development of traditional agriculture, nutrition and indigenous medicine in the West African savannah [2]. Traditionally, fonio is a useful diet for those suffering from diabetes or for women after delivery. Fonio is one of the most nutritious and best-tasting of African cereals [3].

Lactic Acid Bacteria (LAB) ferment glucose primarily to lactic acid, or to lactic acid, CO_2 and ethanol. Lactic acid bacteria are among the most important groups of microorganisms used in food fermentations. They contribute to the taste and texture of fermented products and inhibit food spoilage bacteria by producing growth-inhibiting substances and large amounts of lactic acid. As agents of fermentation LAB are involved in making yoghurt, cheese, cultured butter, sour cream, sausage, cucumber pickles, olives and sauerkraut, but some species may spoil beer, wine and processed meats [4].

Fermented foods are of great significance because they provide and preserve vast quantities of nutritious foods in a wide diversity of flavours, aromas and textures which enrich the human diet [5]. This process preserves the food and creates beneficial enzymes, B-vitamins, Omega-3 fatty acids, and various strains of probiotics. Natural fermentation of foods has also been shown to preserve nutrients in food and break the food down to a more digestible form [6].

2. MATERIALS AND METHODS

2.1 Source of Materials

Acha was bought from Sabongari market Kano, Kano State, Nigeria.

2.2 Preparation and Fermentation of Acha Floury

Acha sample was fermented in two different forms; the local fermentation and controlled fermentation. For the local fermentation, the acha sample was weighed into a fermenting container of 100 g and water of 1litre was added to submerge it for 72 hours in the ratio 1:3. The fermented sample was milled using a sterile milling machine and then lyophilsed. For the controlled fermentation, water was added to a weighed sample and allowed to submerge in ratio 1:6. The sample and water were sterilised at 121℃ for 15 minutes. It was allowed to cool and fermented with the 10^5 cfu/ml of the test isolates under a sterile condition by centrifugation. It was left to ferment for 72hours. The fermented sample was milled using a sterile milling machine and then lyophilsed.

2.3 Microbiological Analysis

The microbial population of total viable bacteria, fungi and lactic acid were determined using standard plate count agar, Nutrient Agar (NA), Potato Dextrose Agar (PDA), and Man Rogosa Sharpe Agar (MRS) respectively. One gram of the acha was homogenised with 9 ml of sterile distilled water. One ml of the aliquot was transferred into a test tube already containing 9ml sterile distilled water. Serial dilution of this was carried out with up to six test-tubes. At suitable dilutions, 0.1 ml was transferred aseptically into a Petri dish. A sterile molten agar (cooled to between 45℃ to 50℃) was poured over the inoculation, and the mixture was swirled round to ensure even distribution of the aliquot. After solidifying, Nutrient agar plates were incubated at 37℃ for 24 hours, MRS plates were incubated at 45℃ for 48 hours, and PDA plates were incubated at 25℃ for 48 hours. After incubation, plates were observed for growth of discrete colonies. Isolates were then subcultured to obtain pure cultures which were labelled appropriately. Purification of the culture was confirmed by Gram staining. Biochemical tests were also performed for identification purposes according to the scheme of [7,8].

2.4 Determination of pH and Total Titratable Acidity (TTA)

Ten grams of the sample each was mixed with sterile distilled water 50 ml. The mixture was allowed to stand for 10 minutes, after which the supernatant was decanted and the pH measured using a Lah meter (Model PHM 92) which had been standardised using buffer solutions. Readings (in triplicates) were taken on the pH meter scale. This was done at 0, 24, 48 and 72 hours of fermentation. The TTA analysis was done using [9] method. A 10 ml of the sample was pipetted into a beaker and 3 drops of Phenolphthalein indicator was added. Titration was done using 0.1 M NaOH to a faint pink colour for at least one minute compared against a white background. Acidity was calculated as gram lactic acid/100 g sample.

2.5 Determination of Proximate Analysis

The proximate analysis of the sample was carried out using the standard procedure of [9].

2.6 Statistical Analysis

All results are means of three independent trials ± standard error. Data were subjected to 1-way Analysis of Variance (ANOVA) using SPSS version 16.0. Duncan's multiple range tests was used to separate means at the 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 Microorganisms Isolated from Acha Grains

Microorganisms isolated from locally fermented acha were bacteria and fungi. Eight bacteria were isolated from fermented acha grain. They were *Bacillus* spp*, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii, Staphylococcus aureus* and *Streptococcus* spp. This is shown in Table 1. Table 2 shows the *Lactobacillus* species that were isolated. The Majority of the lactic acid bacteria isolated from Acha is *Lactobacillus* spp. These organisms, increased early in the fermentation of Acha grain. The decrease in sugar concentration could be largely due to the activities of these organisms which metabolized and converted sugars into organic acids during acha fermentation [10]. While for the fungi, there were two moulds and three yeasts. They are *Aspergillus niger, Aspergillus flavus, Mucor mucedo, Sacharromyces cerevisae* and *Candida albicans* presented in Table 3. The fungi community showed a mix of moulds and yeasts. The presence of the yeasts further shows high sugar fermentative activities during the acha fermentation. The yeast, *Saccharomyces* proliferated rapidly during fermentation and then fell in counts disappearing towards the end of fermentation [11].

3.2 Changes in Microbial Population during Fermentation

The microbial population of all the *Lactobacillus* fermented samples as well as in the locally fermented Acha increased with the duration (0-72 hours) of fermentation except for *L. delbrueckii.* For the locally fermented sample, D increases from 6.40 $x10^{-6}$±0.30 to 8.00 x 10^{-6} ± 0.40, *L. casei* (A) increased from 4.73 x 10^{-6} ±0.31 to 8.43 x 10^{-6} ±0.50 and *L. acidophilus* (B) increased from 5.57 x 10^{-6} ±0.35 to 9.23 x 10^{-6} ±0.35 while *L. delbrueckii* (C) decreases from 3.33 $x10^{-6}$±0.31 to 2.53 x 10^{-6} ±0.31 during 0-72 hours fermentation. This is shown in Table 4.There were significant differences within the bacteria count of inoculated acha fermentation and between the un-inoculated. These significant differences in the bacteria counts could be due to differences in the types of inoculated bacteria and the indigenous bacteria involved in the fermentation process.The bacteria counts during Acha fermentation increased with hours of fermentation. This is probably because the fermentation conditions were conducive for their proliferation and more substrate for use by the indigenous, inoculated and colonizing bacteria released as the fermentation time increases. This increase in microbial growth is similar to the observation of Chang et al. [12] who reported that Lactobacillus increases during fermentation due to the acidity of the medium. The un-inoculated Acha fermentation (locally fermented Acha) had the highest bacteria counts throughout the period of fermentation. The non-inoculation of the medium might be responsible for the high bacteria counts, since the natural fermentative process was not interfered with by inoculation. Inoculation could lead to positive or negative relationship between the inoculated and the indigenous bacteria. Acha fermented with *L. acidophilus* had the highest bacteria counts among those Acha fermentation inoculated throughout the 72 hours period. It is possible *L. acidophilus* had a better positive interaction with the indigenous bacteria compared to the Acha fermentation inoculated by *L. casei and L. delbrueckii.*

Table 1. Morphology and biochemical characteristics of bacteria Isolates Isolated during fermentation of Acha grains

	Cultural characteristics			Microscopic characteristics				Biochemical characteristics										Probable identity
Isolates	**Colour**	**Shape**	**Elevation**	**Gram rxn**	**Cell shape**	**Spore**	**Motility**	**Catalase**	**Coagulase**	**Oxidase**	**Citrate**	**Maltose**	**Mannitol**	**Glucose**	**Galactose**	**Sucrose**	**Lactose**	
N1	Yellow	Round	Raised	+	Cocci	_	_	+	_	_	+	+	+	_	+	_	+	*Micrococcus luteus*
N4	Cream	Round	Raised	_	Rod	_	+	+	_	_	_	+	+	+	_	_	+	*E. coli*
N5	Cream	Irregular	Flat	+	Cocci	+	+	+	_	_	_	+	+	+	_	_	+	*Staphylococcus aureus*
N7	Cream	Irregular	Flat	+	Rod	+	+	+	_	+	+	+	+	+	_	+	_	*Bacillus substilis*
N9	White	Irregular	Flat	+	Rod	+	_	_	_	_	_	+	+	+	_	_	+	*Streptomyces spp.*

Legend: + = Positive, - = Negative

Table 2. Morphology and biochemical characteristics of *Lactobacillus* Species Isolated during fermentation of Acha Grains

	Cultural characteristics			Microscopic characteristics				Biochemical characteristics										Probable identity
Isolate	**Colour**	**Shape**	**Elevation**	**Gram Rxn**	**Cell shape**	**Spore**	**Motility**	**Catalase**	**Coagulase**	**Oxidase**	**Citrate**	**Maltose**	**Mannitol**	**Glucose**	**Galactose**	**Sucrose**	**Lactose**	
M1	Yellow	Round	Raised	+	Rod	_	_	+	_	_	+	+	+	_	+	_	+	*Lactobacillus casei*
M2	Cream	Irregular	Raised	+	Rod	+	+	+	_	_	+	+	+	+	+	+	+	*Lactobacillus acidophilus*
M3	Yellow	Round	Flat	+	Rod	_	+	+	_	_	_	_	_	+	_	+	_	*Lactobacillus delbrueckii*

**Values are means of triplicate determinations ± SD. Means in the same column with different superscripts are significantly different (P≤0.05)*
Legend: + = Positive, - = Negative

Table 3. Morphology and microscopic characteristics of fungal isolates during fermentation of Acha Grains

Isolate	Colour	Description of microscopic appearance	Probably fungus
1	White fluffy mycelia	White fluffy mycelia with Septate hyphae, no stolon, sporangiosphere on branched aerial mycelium.	*Mucor rmucedo*
2	White to cream	Blastoconidia (cell buds) are unicellular, globose, and ellipsoid to elongate in shape. Multilateral (multipolar) budding is typical. Pseudohyphae, if present, are rudimentary. Hyphae are absent.	*Sacharromyces cerevisae*
3	Black	Simple, upright conidiophores terminating in ovoid swelling. One-celled conidia, coloured in mass and dry.	*Aspergillus niger*
4	Green	Simple, upright conidiophores terminating in ovoid swelling.	*Aspergillus flavus*
5	Cream	Spherical to sub-spherical budding blastoconidia.	*Candida albicans*

Table 4. Bacterial count (cfu/ml) during fermentation

Sample	0 HRS	24 HRS	48 HRS	72 HRS
A	4.73±0.31^{b}	5.27±0.06^{a}	6.47±0.25^{a}	8.43±0.50^{a}
B	5.57±0.35^{c}	6.30±0.36^{b}	7.37±0.25^{b}	9.23±0.35^{a}
C	3.33±0.31^{a}	5.47±0.32^{a}	4.47±0.38^{a}	2.53±0.31^{a}
D	6.40±0.30^{d}	7.60±0.30^{c}	7.80±0.36^{c}	8.00±0.40^{b}

**Values are means of triplicate determinations ± SD. Means in the same column with different superscripts are significantly different (P≤0.05)*

Values are mean of three replicates.

Legend: Acha fermented with L. casei, Acha fermented with L. acidophilus, Acha fermented with L. delbrueckii, Acha fermented locally

3.3 Changes in pH and Titratable Acidity

Changes in pH and total titratable Acidity are shown in Figs. 1 and 2. The pH of the fermented samples at the onset of the fermentation (0hrs) were 5.47±0.15**,** 6.03±0.15, 5.90±0.10, 6.03±0.15 and decreased to 4.13±0.15, 3.90±0.10, 4.00±0.10, 4.27±0.15 at the end of fermentation for A, B, C, and D respectively. For the total titratable acidity, there was increase from 0.18±0.01, 0.11±0.01, 0.14±0.01, 0.14±0.03 at 0 hours to 1.43±0.02, 1.60±0.01, 1.10±0.01, 0.94±0.01 for A, B, C, and D respectively at 72 hours. The pH values during the fermentation of Acha were below the neutral pH value of 7.0. The fermentation could be said to be basic lactose fermentation. The trend of the pH values showed it reduces with increased days of fermentation. The fermentation could have gradually become acidic due to the presence of lactic acid [13]. The titratable acidity in the fermentation of Acha grains increased with hours of fermentation. The amount of titratable acidity increases with corresponding decrease in the pH values. This agreed with the report of [14] that variation in titratable acidity and pH is a function of the fermentative activities of microorganisms, especially lactic acid bacteria present in a product and the length of fermentation.

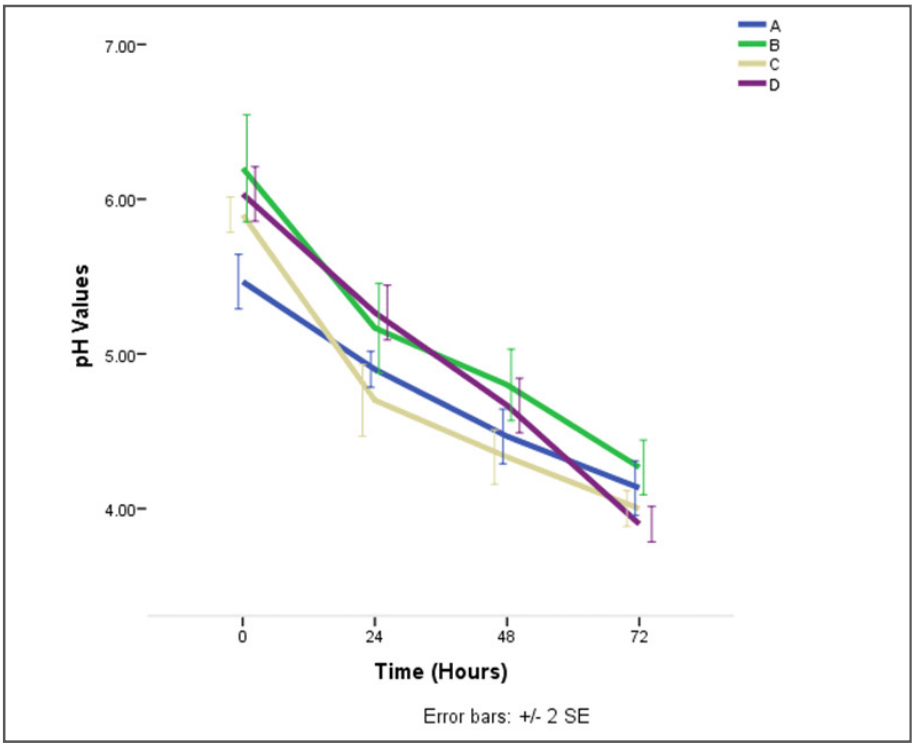

Fig. 1. pH Values during Acha Fermentation

Legend:A- Acha fermented with L. casei, B- Acha fermented with L. acidophilus C- Acha fermented L. delbrueckii, D- Acha fermented locally

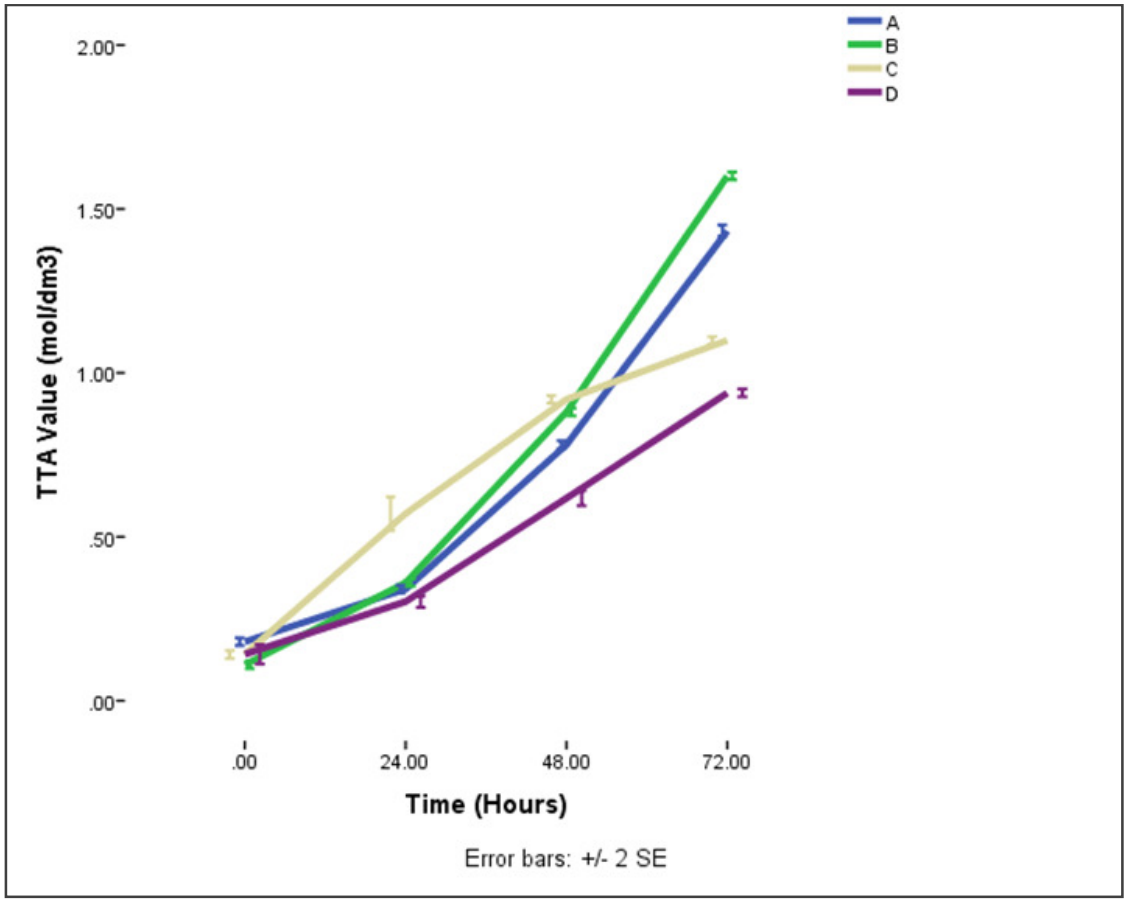

Fig. 2. TTA Values during Acha fermentation

Legend: *A- Acha fermented with L. casei, B- Acha fermented with L. acidophilus, C- Acha fermented L. delbrueckii, D- Acha fermented locally*

Table 5. Proximate composition of acha sample fermented with *Lactobacillus* species and locally

Sample	% MC	% ASH	% CP	% FAT	% FIBRE	% CHO
A	12.31±0.00^{d}	0.09±0.00^{b}	14.07±0.04^{d}	4.32±0.03^{b}	0.99±0.01^{d}	68.22±0.01^{a}
B	11.34±0.03^{c}	0.07±0.01^{a}	15.85±0.01^{e}	3.70±0.27^{a}	1.00±0.00^{d}	68.23±0.51^{a}
C	12.97±0.02^{e}	0.07±0.01^{a}	12.76±0.02^{b}	4.58±0.06^{c}	0.83±0.02^{c}	68.80±0.01^{b}
D	11.16±0.02^{b}	0.07±0.01^{a}	11.79±0.08^{a}	4.90±0.01^{d}	0.79±0.02^{b}	72.27±0.07^{d}

**Values are means of triplicate determinations ± SD. Means in the same column with different superscripts are significantly different (P≤0.05)*

3.4 Changes in Proximate Composition of the Fermented Acha Samples

Table 5 show the changes in Proximate Composition of the Fermented Acha Samples

3.4.1 The crude protein content of the fermented samples

A significant ($p<0.05$) increase of crude protein was observed in Acha sample fermented with *L. acidophilus* (15.85±0.01) compared to locally fermented which was 11.79±0.08. The percentage of protein and fibre of the inoculated fermentation showed improvement over the un-inoculated sample. A significant ($p<0.05$) increase of crude protein was observed in Acha samples fermented with *L. acidophilus* (15.85±0.01), *L. casei* (14.07±0.04), and *L. Delbrueckii* (12.76±0.02) compared to locally fermented, which was (11.79±0.08). According to [15], the changes could be attributed to increase

in microbial mass during fermentation causing extensive hydrolysis of the protein molecules to amino acids and other simple or lower molecular weight peptides. It may also be due to the structural proteins that are an integral part of the microbial cells. The increase in crude protein in the inoculated fermented Acha sample is an indication that they will, therefore, be good sources of high-quality plant proteins for animal and man that cannot afford animal protein in their diets.

3.4.2 The Ash content of the fermented samples

The ash content of Acha sample fermented with *L. delbrueckii, L. acidophilus* and the samples fermented locally are same (0.07±0.01) while it increases in the samples fermented with *L. Casei* to 0.09±0.01.

3.4.3 The carbohydrate content of the fermented samples

There was a significant ($p<0.05$) decrease in Carbohydrate content of the sample fermented with *L. casei* (68.22±0.01) compared with the samples fermented locally (72.27±0.07).

3.4.4 The fat content of the fermented samples

The fat content of acha fermented locally showed a significant ($p\leq0.05$) increase compared to acha fermented with *L. acidophilus* (from 4.90±0.01 to 3.70±0.27). The fat content (4.90±0.01) of Acha fermented locally showed a significant ($p\leq0.05$) increase compared to acha fermented with *L. acidophilus, L. casei*, and *L. delbrueckii* (3.70±0.27, 4.32±0.03 and 4.58±0.06 respectively). The increase observed may be attributed to poor or in extensive breakdown of large molecules of fat of the samples into simple fatty acids during the fermentation process. This observation could also be due to poor utilization of oxidized lipids to generate energy for growth and cellular activities by microorganisms [16].

3.4.5 The crude fibre content of the samples

The crude fibre content obtained for acha fermented locally (0.79±0.02) was significantly ($p\leq0.05$) lower than the sample fermented with *L. acidophilus* (1.00±0.00). Crude fibre content obtained for Acha fermented locally (0.79±0.02) was significantly ($p\leq0.05$) lower than the sample fermented with the microorganism. This reduction in crude fibre may be due to the enzymatic breakdown of the fibre during fermentation by lactic acid bacteria which utilized them as carbon source and converted to microbial biomass, thereby reducing the fibre content of such food [17].

3.4.6 The moisture content of the samples a

The moisture content increased significantly ($p\leq0.05$) in acha sample fermented with *L. delbrueckii* (12.97±0.02) and decreased to 11.16±0.02 in acha fermented locally.

4. CONCLUSION

The acha from inoculated fermentation will therefore be good protein and fibre source. The protein will help in body repair and building, while the fibre will improve the digestive process in the gastrointestinal tract. The health benefits of whole grain cereal products are now widely recognized and considered to result from the presence of a range of nutritional components, including dietary fibre and protein. Hence, Acha can help millions in sub-Sahara Africa especially in weaning.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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