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INFLUENCE OF STARTER CULTURE LACTIC ACID BACTERIA ON THE PHYTIC ACID CONTENT OF SORGHUM-*OGI* (AN INDIGENOUS CEREAL GRUEL)

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Abstract
Sorghum-ogi is a traditional infant weaning food and as breakfast meal for adults. Sorghum in the production of ogi has the presence of anti-nutritional factors such as phytic acid. The influence of phytase-producing lactic acid bacteria (LAB) on phytic acid content of sorghum-ogi was studied. A total of sixty-three lactic acid bacteria were isolated from fermenting sorghum-ogi. Twenty-three of the isolates exhibited extracellular phytase production. The isolates were identified based on conventional lactic acid bacteria procedures as Lactobacillus plantarum, Streptococcus cremoris and S. mutans. The assay of phytase produced by isolates showed that L. plantarum W723 and S. cremoris W722 had the highest activity of 86U/ml and 77U/ml respectively. Sorghum-ogi fermented with S. cremoris W723 had the least production of lactic acid 8.7g/l at 0h while the consortium of S. cremoris W723 and L. plantarum W722 had the highest production of 26.8g/l at 72h. Fermentation with L. plantarum W723 had the highest reduction of 56.8% in phytic acid content after 72h. Proximate analysis of products from controlled fermentation of sorghum-ogi showed that there was increase in the content of ash and protein as compared with that observed for the raw sorghum. This suggests the reduction in phytic acid content of products and release of some minerals and proteins bound by phytic acid. It can be concluded that fermentation with the starter cultures singly and in combination had a great potential for reduction of phytic acid in cereal-based food thereby leading to bioavailability of minerals and proteins.

Keywords: Sorghum-ogi, Lactic acid bacteria, phytic acid, starter culture, fermentation

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1.INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is an important food crop in the world, following wheat, rice, maize and barley (FAO, 1997). Grain sorghum provides the staple food for a large population of Africa, India and the semi-arid parts of the tropics. Sorghum acts as a principal source of energy, protein, vitamins and minerals for millions of the people living in these regions. Besides being a staple food, it is used as feed for animals; its stalk provides fodder, fuel, shelter and syrup (Klopfenstein and Hoseney, 1995).

Unfortunately, sorghum has the presence of anti-nutritional factors which make complexes with food ingredients. In addition, *in vivo* and *in vitro* studies indicate that the proteins of wet cooked sorghum are significantly less digestible than the proteins of other similar cooked cereals such as wheat and maize (Doudou *et al*., 2003). The anti-nutritional effect of tannin and phytate in sorghum has been demonstrated by many researchers (Butler *et al*., 1984; Ryden and Selvendran, 1993; Agarwal and Chitnis, 1995).

Salts of phytic acid, designated as phytates, are regarded as the primary storage form of both phosphate and inositol in plant seeds and grains. Phytate is formed during maturation of the plant seed and in dormant seeds it represents 60–90 % of the total phosphate (Loewus, 2002). Phytate is therefore a common constituent of plant-derived foods (Konietzny and Greiner, 2003; Kvist *et. al.*, 2005).

The major concern about the presence of phytate in the human diet is its negative effect on mineral uptake. Minerals of concern in this regard include zinc, iron, calcium, magnesium, manganese and copper (Lopez *et. al.*, 2002; Konietzny and Greiner, 2003). The formation of insoluble mineral-phytate complexes at physiological pH values is regarded as the major reason for the poor mineral

bioavailability, because these complexes are essentially non-absorbable from the human gastrointestinal tract. Furthermore, the human small intestine has only a very limited capability to hydrolyze phytate (Iqbal *et al.*, 1994) due to the lack of endogenous phytate-degrading enzymes and the limited microbial population in the upper part of the digestive tract.

Lactic acid fermentation of cereals is a long established processing method, being used in Asia and Africa for the production of foods like beverages, gruels, and porridge (Charalampopoulos *et al.*, 2002). Some mineral deficiencies are common in developing countries, but marginal mineral deficiencies also occur in developed countries, particularly in vulnerable human, minerals can be improved by reducing of the phytate content in foods and feeds (Türk *et al*., 2000). Due to these nutritional consequences, the degradation of phytate during food processing is desirable. Fermentation is widely used to improve the nutritional and functional qualities of food and enzymatic processing of food and ingredients to achieve products (Fredrikson *et al.*, 2002; Bergqvist *et al.*, 2005). Food fermentation covers a wide range of microbial desirable characteristics such as prolonged shelf life, improved safety, attractive flavour, nutritional enrichment, elimination of anti-nutrients and promotion of health (Bergqvist *et al.*, 2005). Microorganisms used for food fermentation may be part of the natural micro flora found in the raw material that is fermented or specially cultivated cultures designed to bring about specific changes in the material that is being fermented. Today, defined starter cultures and controlled conditions are generally used in food fermentation. The type of microorganism, the fermentation conditions used, and the starting amount of phytate present in the raw material significantly affect the extent of phytate removal during the fermentation process (Raghavendra and Halami, 2009). In particular, the capability of lactic acid bacteria to produce a phytate-degrading enzyme is still in some dispute. Some studies seem to establish the capability of lactic acid bacteria to hydrolyze phytate (Lopez *et al.*, 2000; De Angelis *et al.*, 2003), whereas others failed to identify a phytate-degrading enzyme (Greiner *et al.*, 2003).

Therefore, the aim of this study was to investigate the influence of phytase-producing lactic acid bacteria (LAB) on phytic acid content of sorghum-*ogi*.

2.MATERIALS AND METHODS

Sample Collection and Preparation

Sorghum grains were obtained from a retail market in Ibadan, South west Nigeria. The grains were carefully sorted to free them from foreign materials as well as broken and shrunken seeds. The grains were divided into parts and kept in air-tight containers for processing.

Spontaneous Fermentation

Two hundred grams of sorghum was soaked with tap water in clean plastic containers and steeped for 48 hr. After 48 hr the water was drained and the grains wet-milled. The milled slurry was sieved through a fine mesh sieve into clean containers to remove the over tails which was discarded. The slurry obtained was allowed to stand and ferment for 72 hours at room temperature after which water was decanted leaving behind the semi-wet sorghum-*ogi* (Adelekan and Oyewole, 2010).

pH Determination

Samples were aseptically drawn every 12 hr for pH determination during the fermentation using a pH meter (Humboldt H4382 model).

Determination of Total Titratable Acidity

The production of lactic acid was determined by titrating 25ml of samples (drawn every 12 hours from the fermenting *ogi*) with 0.1M NaOH using 3 drops of phenolphthalein as indicator. The titratable acidity represents the amount of lactic acid produced and was calculated as 1ml of 0.1M NaOH equivalent to 0.9008g (w/v) Lactic acid (AOAC, 1990).

Microbiological Analyses of Samples
Isolation of Microorganisms
At the start of fermentation, 1ml of sample was aseptically transferred into 9ml of sterile distilled water and ten-fold serial dilution was carried out. 1ml each of samples from 10^6, 10^7 and 10^8 dilutions was aseptically transferred into sterile Petri dishes. About 10 – 15 ml of sterilized MRS agar was poured into each of the plates. The plates were allowed to set and incubated at 37^0C for 24-48 hours under anaerobic conditions. This procedure was repeated at, 24, 48 and 72 hours of fermentation.

Purification and Preservation of Isolates: Distinct colonies of organisms were randomly picked from the mixed cultures obtained from the pour plates. These were streaked on dried MRS agar plates. Pure cultures of organisms were obtained through successive streaking of isolates on MRS agar. Pure cultures of isolates were kept in triplicates on MRS agar slants as working and stock cultures and stored at 4 ^{0}C.

Phenotypic characterization
Presumptive LAB strains isolated from the different samples were characterized and isolates were Gram-stained and catalase activity was determined. The strains were preliminarily identified based on the phenotypic properties such as the ammonia production from arginine, growth at 15 ^{o}C and 45 ^{o}C, ability to grow at 6.5% of sodium chloride(NaCl), hydrogen peroxide production, pH in MRS broth and gas (CO_2) production from glucose according to Dykes (1994). All the strains were tested for the sugar fermentation patterns. The conventional sugar fermentation was further complemented using API 50CH strips and API 50CHL medium according to manufacturer's instruction (API System, Bio-Merieux, France).

Screening and Assay of LAB Isolates for Extracellular Phytase Production
Screening: The isolates were screened for extracellular phytase production according to the method of Bae *et al.* (1999) using phytase screening agar (PSA) described by Taheri *et al.* (2009) with slight modification. The medium was sterilized at 121^0C for 15 minutes, poured in sterile plates and allowed to set. Streaks of isolates were made on the dried plates and the plates incubated at 37^0C for 24 hours. Colonies surrounded by clear zones on the PSA medium were selected and flooded with 2% (w/v) aqueous $CoCl_2$ solution and incubated at room temperature for 30 minutes. To eliminate false positive results cause by acid production, a freshly prepared mixture of 6.25% (w/v) Ammonium molybdate $(NH_4)_6Mo_7O_{24}$ and 0.42% (w/v) Ammonium heptavanadate (NH_4VO_7) solutions were added to the plates. The plates were incubated at room temperature for 5 minutes. The colonies that maintained the zone of hydrolysis were selected (Bae *et al.*, 1999).

Assay: The assay of phytase enzyme was done using ammonium-molybdate blue method as described by Nielsen *et al.* (2008). Cells of isolates were cultivated in sterile modified Phytase Screening Broth (PSB with pH adjusted to 7) in test tubes (Taheri *et al.*, 2009). Culture supernatant was collected by centrifuging at 8000 g for 10 minutes. A 0.2ml of the supernatant (sample) was transferred into a clean test tube. The test tube was allowed to stand in water bath at 37^0C for 5 minutes. 0.8ml of buffered sodium phytate (substrate) solution was added to the sample, mixed and incubated in the water bath at 37^0C for 30 minutes to allow for reaction. The reaction was stopped by adding 1ml of 5% Trichloroacetic acid (TCA). 1ml of freshly prepared colour reagent was added and the mixture shaken properly. The mixture was then centrifuged at 8000 g for 10 minutes. It was allowed to stand at room temperature for 10 minutes before the absorbance was read at 700nm using spectrophotometer (Jenway 6310 model). The sample blank was prepared as described above except that the stop solution was added before the substrate solution. To obtain the standard curve from which the amount of liberated inorganic phosphate was extrapolated, the above procedure was followed using potassium

di-hydrogen phosphate (KH_2PO_4) as the sample solution. Standard blank was also prepared as for the sample blank. Absorbance was read accordingly and a graph of absorbance against concentration was drawn. The slope of the curve obtained was used to calculate the activity of the crude enzyme in U/ml.

Controlled Fermentation

L. plantarum W723 and *S. cremoris* W722 with the highest phytase activity of 86 and 77U/ml respectively were used singly and in combination as starters for the controlled fermentation of sorghum-*ogi* according to the method of Teniola and Odunfa (2001). The isolates were cultured on MRS agar slants for 24 hours at 37^0C. The cultures on agar slants were rinsed with 3ml sterile distilled water. The slants were gently shaken with the distilled water to dislodge the microbial cells. A 1ml of each cell suspension was used to inoculate 100 ml of sorghum flour–water mixture (1:2 g/ml) for the single starter while 0.5ml each of the culture was used for the combined starter. Fermentation was allowed to proceed for 72 hours. Uninoculated mixture served as the control. Prior to inoculation, the components of the mixture, including the dried flour, were sterilised at 121^0C for 20 min in an autoclave to prevent cooking. Samples were drawn at 12 – hour interval for pH and total titratable acidity determination. To determine the phytic acid content of the fermenting sorghum-*ogi*, samples were drawn at intervals of 24 hours for analysis.

Determination of Phytic Acid

Phytic acid content of the raw sorghum and sorghum-*ogi* from controlled fermentation were determined using the method of Wheeler and Ferrel (1971). Samples of the fermenting *ogi* were drawn every 24 hours, dried in Gallenkamp oven at 50^0C for 24 hours and then analyzed. 1g of a finely ground sample (40 mesh) was weighed into a 50ml centrifuge tube. Extraction was done with 25ml 3% Trichloroacetic acid (TCA) for 15 minutes with mechanical shaking. The suspension was centrifuged for 15 minutes at 3000rpm and 10ml aliquot of the supernatant transferred into another 50ml centrifuge tube. 4ml $FeCl_3$ solution (made to contain 2mg ferric iron per ml in 3% TCA) was added to the aliquot by blowing rapidly from the pipette. The tube and contents was heated in a boiling water bath for 45 minutes. The suspension was centrifuged at 2000rpm for 10 to 15 minutes and the clear supernatant carefully decanted. Precipitate was washed twice by dispersing well in 20 to 25ml of 3% TCA, heated in boiling water bath for 10 minutes and centrifuged for 10 min at 2000rpm. Washing was repeated once with distilled water. The precipitate was dispersed in about 5ml of distilled and 3ml of 1.5N NaOH was added with mixing. Volume was brought to approximately 30ml with water and heated in boiling water bath for 30 minutes. The hot mixture was filtered through Whatman No 2 filter paper into a conical flask. The precipitate was washed with 60-70ml of hot distilled water and the filtrate discarded. Precipitate was dissolved from the filter paper with 40ml of hot 2N HNO_3 into a 100ml volumetric flask. The filter paper was washed with several portions of distilled water and the washings collected in the same flask. The flask and content was cooled to room temperature and diluted to volume (100ml) with distilled water. 0.5ml of aliquot was transferred into a test tube and diluted with 6.5ml of distilled water. 2ml of 1.5M KSCN and 1ml of distilled water was added to make 10ml. The absorbance was read immediately within 1 minute at 480nm. A reagent blank was run with each set of sample. The iron content was calculated from a previously prepared standard curve. The phytate phosphorus was calculated from the iron results assuming a 4:6 iron: phosphorus molecular ratio (Wheeler and Ferrel, 1971).

Proximate Analysis of Products from Controlled Fermentation of Sorghum-*ogi*

Moisture content: The moisture content of products from controlled fermentation of sorghum-*ogi* was determined by weighing 5g of each sample into crucible (AOAC, 1990). This was dried to constant weight at 111^0C for

3 hours. The dried weight obtained was deducted from the initial 5g to give the amount of water present in the sample. The percentage moisture is given by:

$$\frac{\text{Weight of water}}{\text{Weight of sample}} \times 100\%$$

Fat: This was done using Rose-Gottlieb process (AOAC, 1990). 10g of the sample was weighed into a tube. 1ml of 0.88 ammonia solution was added and mixed. 10ml of 95% alcohol was then added and the mixture shaken very well. 25ml peroxide-free diethyl ether was added. The tube was covered with a stopper and shaken vigorously for 1 minute. 25ml of light petroleum (b.p. 40-60^{0}C) was added and the tube shaken vigorously. After the separation, the fat solution was transferred into a suitable flask (previously dried at 100^{0}C, cooled and weighed). To the tube, two successive lots of 5ml of mixed ethers were transferred (without shaking) to the flask. The extraction was repeated (with 15ml of ether and 15ml of light petroleum) and the subsequent operations repeated twice. The solvent from the flask was distilled of and the fat dried for 1 hour at 100^{0}C. It was then cooled and weighed.

Ash: This was done by heating the dried residue of 5g sample of the *ogi* at a temperature of 500^{0}C in a muffle furnace for about 5 hours (AOAC, 1990). The ash content was obtained by deducting the weight obtained after the heating process from the dry weight. Percentage ash is given by:

$$\frac{\text{Ash}}{\text{Dry weight}} \times 100\%$$

Protein: This was done using Formol titration method (AOAC, 1990). To 10ml of each sample, 0.5ml of 0.5% phenolphthalein indicator and 0.4ml of neutral saturated potassium oxalate was added. The mixture was gently shaken and then allowed to stand for about 3minutes. The mixture was neutralized with 0.1M sodium hydroxide to a standard pink colour. 2ml of formalin was added to the solution and mixed. It was allowed to stand for a few minutes and the new acidity produced titrated with 0.1M sodium hydroxide to the same pink colour (titration a ml). Also, 2ml of the formalin plus 10ml of water was titrated separately with 0.1M sodium hydroxide (b ml) as blank. The protein content of the sample (equivalent to M x 6.38 from the Kjedahl method) is 1.7(a-b).

Carbohydrate (CHO): The amount of carbohydrate present in the samples was obtained by difference.

Statistical Analysis

Phytic acid determination and proximate analysis carried out on the samples were analyzed in duplicate and averaged. Duncan's multiple range test was used to separate means and significance was accepted At $P \leq 0.05$ (Eltayeb *et al*., 2007).

3.RESULTS AND DISCUSSION

Table 1 shows the phenotypic characteristics of the six groups. A total of sixty-three organisms suspected to be lactic acid bacteria were isolated from red and white sorghum-*ogi.* The isolates were gram positive and negative for catalase, motility, indole, starch hydrolysis, gelatin liquefaction, oxidase, citrate, and nitrate reduction. Six of the isolates were positive for arginine deamination while fifty-seven were negative. Eight of the isolates showed positive reaction for casein hydrolysis while fifty-five were negative. The isolates were grouped into six clusters as: single cocci, paired cocci, single and paired cocci, single and chained cocci, single rods and single and paired rods. The probable identity of the isolates with reference to Nair and Surendran (2004, 2005), indicated that there were fifteen *Lactobacillus* sp. and eight *Streptococcus* sp.

Table 2 represents the pattern of carbohydrate fermentation of the three groups of isolates using the Analytical Profile Index (API systems, Biomerieux Sa. France). *Lactobacillus plantarum, Streptococcus cremoris* and *S. mutans* were isolated from *sorghum-ogi.* The presence and effect of lactic acid bacteria cannot be ruled out since *ogi* fermentation is lactic acid fermentation.

Table 1: Phenotypic Characteristics of Isolates

	LAB Group					
	A	B	C	D	E	F
No of Strains	13	11	2	10	18	9
Percentage	20.6	17.4	3.2	15.9	28.6	14.3
Cell Morphology	Single cocci	Paired cocci	Single and paired cocci	Single and chained cocci	Single rods	Single and paired rods
Gram Reaction	13/0	11/0	2/0	10/0	18/0	9/0
Catalase	0/13	0/11	0/2	0/10	0/18	0/9
Motility	0/13	0/11	0/2	0/10	0/18	0/9
Indole	0/13	0/11	0/2	0/10	0/18	0/9
Starch Hydrolysis	0/13	0/11	0/2	0/10	0/18	0/9
Casein Hydrolysis	3/10	1/10	0/2	3/7	1/17	0/9
Gelatin Liquefaction	0/13	0/11	0/2	0/10	0/18	0/9
Oxidase	0/13	0/11	0/2	0/10	0/18	0/9
Citrate	0/13	0/11	0/2	0/10	0/18	0/9
Nitrate Reduction	0/13	0/11	0/2	0/10	0/18	0/9
Arginine Deamination	0/13	1/10	0/2	2/8	2/16	1/8

Key:
+/-: Positive/Negative
A: Single cocci
B: Paired cocci
C: Single and paired cocci
D: Single and chained cocci
E: Single rods
F: Single and paired rods

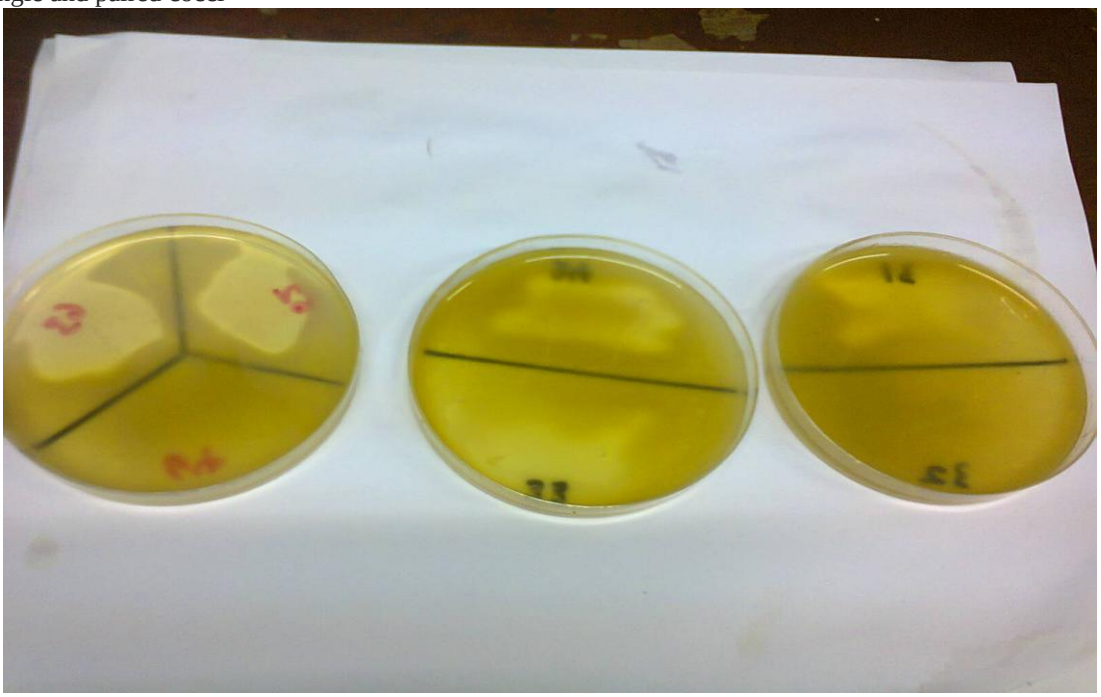

Figure 1: Zone of phytate hydrolysis after counterstaining

A : Very clear zone of phytate hydrolysis shown by *S. cremoris* W725 and *L. plantarum* W726
B : Clear zone of phytate hydrolysis shown by *L. plantarum* W245 and *L. plantarum* W246
C : No zone of phytate hydrolysis shown by *L. plantarum* W244

The presence of *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Streptococcus* has been reported in *ogi* and many cereal- based fermentations (Mbugua, 1985; Odunfa and Adeyele, 1985).

The presence of *L. plantarum* and *L. lactis* spp *cremoris* in *brown-ogi* has also been reported by Adebayo-Tayo and Onilude (2008). They opined that LAB were present in fermenting foods, because of their ability to produce high levels of lactic acid as well as being able to survive under high acidic conditions. Teniola *et al.* (2005) also reported that *Lactobacillus* species are the predominant micro-organisms in fermentation of *ogi-baba* and that there is some therapeutic value associated with *ogi-baba*. The most predominant LAB species isolated in this present study was *Lactobacillus plantarum*. This could be due to the fact that the substrate used in the preparation of the fermented food is of plant origin. Earlier reports by Olukoya *et al.* (1993) on the occurrence of lactic acid bacteria spectrum indicated that *L. plantarum* constituted the highest number of LAB isolated from fermented plant materials.

When the isolates were screened for extracellular phytase production on solid medium, sixty-one of them showed zone of hydrolysis before counterstaining. After counterstaining, only twenty-three retained their zone of hydrolysis (not shown). Figure 1 shows the zones of hydrolysis of phytate after counterstaining ranging from very clear zone to no zone of hydrolysis. The isolates that retained their zones of hydrolysis after counterstaining indicated extracellular phytase production.

Table 2: Analytical Profile Index (API) Sugar Fermentation Pattern of Isolates

	Strain		
Sugars	***Lactobacillus plantarum***	*Streptococcus cremoris*	*Streptococcus mutans*
Glycerol	-	-	-
Erythritol	-	-	-
D-arabinose	-	-	-
L-arabinose	+	-	-
Ribose	+	+	+
D-Xylose	-	-	-
L-Xylose	-	-	-
Adonitol	-	-	-
β-Metyl-Xyloside	-	-	-
Galactose	+	+	-
D-Glucose	+	+	+
D-Fructose	+	+	+
D-Mannose	+	+	+
L-Sorbose	-	-	-
Rhamnose	-	-	-
Dulcitol	-	-	-
Inositol	-	-	-
Mannitol	+	-	+
Sorbitol	-	-	+
α-Methyl-Mannoside	+	-	-
α-Meth-D-Glucoside	+	-	-
N Acetylgluco-samine	+	+	+
Amygdaline	+	-	+
Arbutin	+	-	+
Esculin	+	+	+
Salicin	+	+	+
Cellobiose	+	+	+
Maltose	+	+	+
Lactose	+	-	+
Saccharose	+	-	+
Trehalose	+	+	+
Inulin	-	-	-
Melezitose	+	-	-
D-Raffinose	+	-	-
Amidon	-	-	-
Glycogen	-	-	-
Xylitol	-	-	-
β-Gentiobiose	+	+	+
D-Turanose	-	-	-
D-Lyxose	-	-	-
D-Tagatose	-	+	-
D-Fucose	-	-	-
L-Fucose	-	-	-
D-Arabitol	+	-	-
D-Arabitol	-	-	-
Gluconate	+	-	-
2 Keto-gluconate	-	-	-
2 Keto-gluconate	+	-	-

Table 3: Activity of phytase enzyme produced by isolates

	Screening		
Isolate	Before counterstaining	After counterstaining	Activity (U/ml)
S. mutans R728	+++	++	25
S. cremoris R7211	++	++	55
S. cremoris W722	++	++	**77**
L. plantarum W723	++	++	**86**
L. plantarum W724	++	++	20
S. mutans W725	+++	++	18
L. plantarum W726	+++	++	36

+++: very clear zone, ++: clear zone, +: slight zone

Table 3 shows the activity (U/ml) of the enzyme produced by the isolates. The two isolates *L. plantarum* W723 and *S. cremoris* W722 had the highest activities of 86U/ml and 77U/ml respectively. These were selected for the controlled fermentation of sorghum-*ogi*. There is a pressing need for food grade LAB to be utilized in fermented food processes in order to promote functional foods or nutraceutical supplements (Famularo *et al.*, 2005). Studies with experimental animals as well as clinical studies have elucidated that the phytate content of certain foods such as whole wheat products, wheat bran and soy products is a foremost determinant negatively governing the nutritional balance of trace minerals and proteins in subjects on a regular vegetarian diet (Raboy, 2003). There are few studies dealing with the role of LAB in degrading phytic acid (De Angelis *et al*., 2003). In this study, lactic acid bacteria (LAB) isolated from spontaneous fermentation of sorghum-*ogi* was screened for extracellular phytase production. Production of extracellular phytase by the LAB isolates is in agreement with the report of De Angelis *et al.* (2003) and Raghavendra and Halami, (2009) but contrary to the report by Greiner *et al.* (2003) who were unable to identify phytase-degrading enzyme in LAB. The phytate degrading ability of the isolates might be due to the presence of phytase enzyme and this was confirmed when 23 of the isolates degraded sodium phytate in the presence of calcium chloride. Calcium may not be involved in the reaction but it is needed for enzyme activity (De Angelis *et al.*, 2003).

The pH of sorghum-*ogi* fermented with *Lactobacillus plantarum* W723 was 5.62 at the start of fermentation. The pH dropped to 4.32 at the end of fermentation. Sorghum-*ogi* fermented with *Streptococcus cremoris* W722 had a pH value of 5.61 at the start of fermentation and 4.16 at the end of 72 hours. Sorghum-*ogi* fermented with a consortium of *L. plantarum* W723 and *S. cremoris* W722 had a pH of 5.30 at the start of fermentation and 4.21 at the end of fermentation. The uninoculated control had a pH of 5.31 and 4.34 at the start and end of fermentation respectively (Figure 2). The fermentation of sorghum-*ogi* exhibited a general decrease in pH and an increase in lactic acid production up to 48 hours before there was slight decline. This observation agrees with the reports of Teniola and Odunfa (2001) and Ogunbanwo and Okanlawon (2009).

Figure 3 shows the changes in lactic acid production during controlled fermentation of sorghum-*ogi*. The amount of lactic acid produced in g/L during fermentation with *L. plantarum* W723 was 9.4 and 22.2 at the start and end of the fermentation respectively. Lactic acid production for sorghum-*ogi* produced with *S. cremoris* W722 in g/L at 0 hour was 8.7 and 23.5 at 72 hours of fermentation. Sorghum-*ogi* fermented with a consortium of *L. plantarum* W723 and *S. cremoris* W722 showed a production of 9.5g/L of lactic acid at the start of fermentation and 26.8g/L at the end.

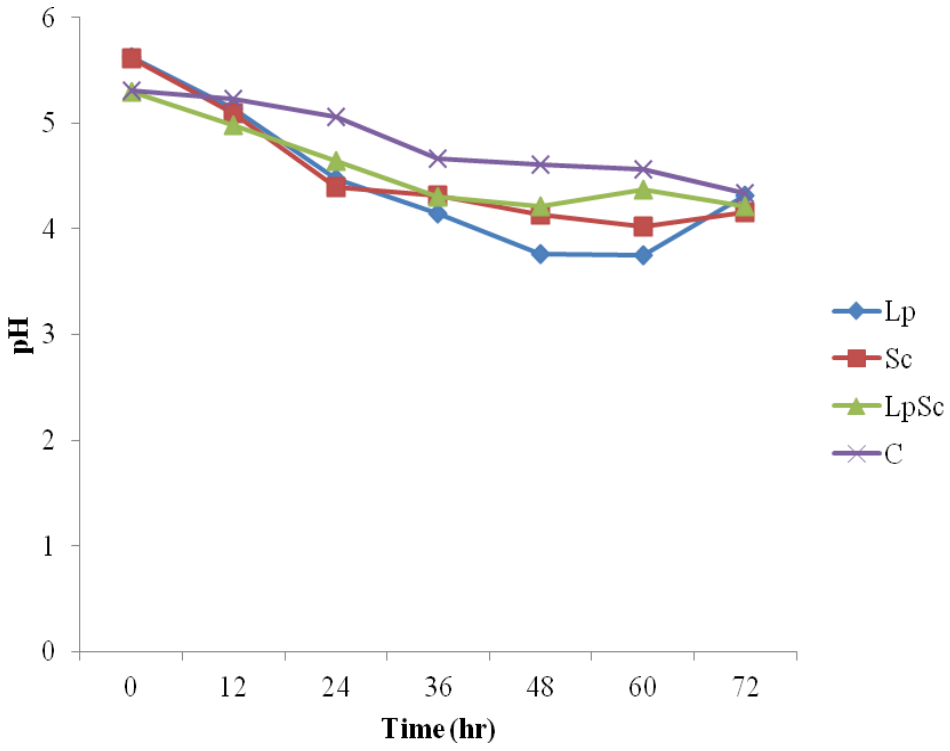

Figure 2: pH Changes during Controlled Fermentation of Sorghum-*ogi*
Lp – *Lactobacillus plantarum* W723; Sc – *Streptococcus cremoris* W722
LpSc – *L. plantarum* W723+ *S. cremoris* W722; C – Control

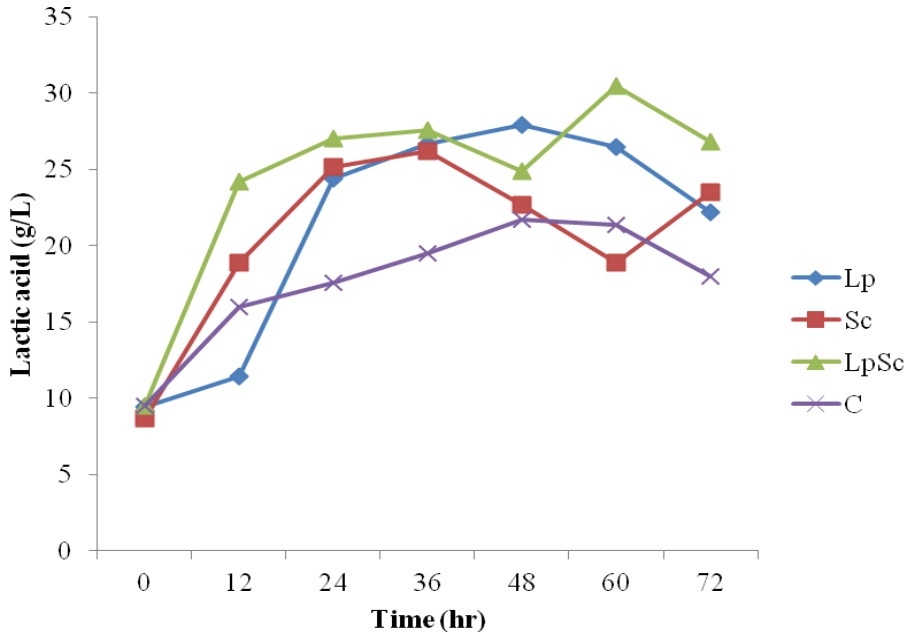

Figure 3: Changes in lactic acid production during controlled fermentation of sorghum-*ogi*
Lp – *Lactobacillus plantarum* W723; Sc – *Streptococcus cremoris* W722
LpSc – *L. plantarum* W723+ *S. cremoris* W722; C –Control

Production of lactic acid in the uninoculated control increased from 9.5g/L at the start to 18.0g/L at the end of fermentation.

The decrease in pH and increase in lactic acid production followed the same trend as reported for other traditional fermented foods (Mohammed *et al.*, 1991; Choi *et al.*, 1994; Dziedzoaze *et al.*, 1996). The general reduction in pH during fermentation is indicative of acid production by microorganisms present during the fermentation. The cause of the increase in acidity and consequent drop in pH during fermentation of cereal was likely due to utilization of free sugars by yeasts and LAB (Efiuvwevwere and Akona, 1995; Zvauya *et al*., 1997). Teniola and Odunfa (2001) opined that the increase in pH and decrease in total acidity after various periods of fermentation may be caused by the removal of volatile acids by evaporation and the reduced acidity of the microorganisms during fermentation.

The phytic acid content of the sorghum used for the fermentation was observed to be 4.10%. Fermentation of the sorghum into *ogi* using *L. plantarum* W723 as starter decreased this value to 3.03% at the start of fermentation representing a reduction of 26.1%. The value reduced to 1.77% at 72 hours of fermentation, indicating 56.8% reduction in the phytic acid content of the sorghum. *S. cremoris* W722 had a value of 3.83% of phytic acid with a reduction of 6.6% at the start of fermentation. Value of 2.07% was observed at 72 hours of fermentation with a corresponding percentage reduction of 49.5%. *L. plantarum* W723 and *S. cremoris* W722 had the value of 2.61% phytic acid content and 36.3% reduction at the start of fermentation. At 72 hours, the content of phytic acid reduced to 2.38% with corresponding percentage reduction of 41.9% respectively. The uninoculated control at the start of fermentation had phytic acid content of 4.06% and 0.9% reduction. The phytic acid content reduced to 2.07% at 72 hours of fermentation with corresponding percentage reduction of 49.5% respectively (Table 4). Screening of the isolates using the method of Bae *et al.* (1999) in which staining and counterstaining was employed, it was observed that the phytate degrading ability of the isolates was due to phytase, but not to acid hydrolysis. Sreeramulu *et al.* (1996) observed that decrease in phytate levels was due to the production of extracellular phytase by *Lactobacillus* and *Streptococcus.* They found production of extracellular phytase by *L. amylovorus* B4552. On the other hand, Zamudio *et al.* (2001) observed that *L. plantarum* produced non-specific acid phosphatase and it showed much less specificity towards sodium phytate. When the amount of phytase enzyme produced by the isolates which passed the screening test was quantified, *L. plantarum* W723 and *S. cremoris* W722 had the highest activities of 86U/ml and 77U/ml respectively. Zamudio *et al.* (2001) tested six lactic acid bacteria (*Pediococcus pentosaceus, Leuconostoc mesenteroides, Lactobacillus casei, L. fermentum, L. delbrueckii* and *L. plantarum*) for phytate degradation and for intracellular and extracellular phytase activity in culture medium.

Among the six lactic acid bacteria tested, *L. plantarum* exhibited the highest extracellular phytase activity. Phytase activity was detected extracellularly in the culture broth supernatant fluid at the stationary phase. These researchers found that substituting inorganic phosphate for sodium phytate in the culture medium clearly stimulated phytase activity (80U/ml). Sreeramulu *et al.* (1996) had also described *L. plantarum* as the next best producer to *L. amylovorus*. *L. plantarum* W723 produced the highest reduction in phytic acid content of the *ogi* produced (56.8%) while the consortium of *L. plantarum* W723 and *S. cremoris* W722 gave a non-synergistic effect (41.9%) as the reduction in phytic acid of their product was lower than that of single isolate fermentation (56.8% and 49.5% respectively for PLp and PSc). These results complement the report of De Angelis *et al.* (2003). They reported that the activity of *L. sanfranciscensis* led to a 64-74% decrease of phytate in sourdough after 8 hours of fermentation at 37°C. Lopez *et al.* (2000) reported that all the tested strains of lactic acid bacteria isolated from sourdough expressed phytase activity and were able to degrade

Table 4: Changes and Percentage Reduction in Phytic Acid Content of Product of Controlled Fermentation of Sorghum-*ogi*

	0hr		24hr		48hr		72hr	
Product	Phytic Acid Content (%)	% Reduction	Phytic Acid Content (%)	% Reduction	Phytic Acid Content (%)	% Reduction	Phytic Acid Content (%)	% Reducti
PLp	3.03	26.1	2.37	42.2	2.24	45.4	1.77	56.8
PSc	3.83	6.6	2.80	31.7	2.77	32.4	2.07	49.5
PLpSc	2.61	36.3	2.57	37.3	2.50	39.0	2.38	41.9
PC	4.06	0.9	2.53	38.3	2.47	39.8	2.07	49.5

Each value is expressed as the mean of three independent experiments.
PLp – *ogi* from *L. plantarum* W723
PSc – *ogi* from *S. cremoris* W722
PLpSc – *ogi* from *L. plantarum* W723 and *S. cremoris* W722
PC – Control

Table 5: Proximate Analysis (%) of Products from Controlled Fermentation of Sorghum-*ogi*

Product	Moisture content	Dry Matter	Fat	Ash	Protein	CHO
RSrg	7.52	92.48	1.99	1.01	10.79	78.69
PLp	9.34^{a}	90.66^{d}	4.57^{c}	1.03^{a}	11.65^{b}	73.42^{d}
PSc	12.54^{c}	87.47^{b}	4.95^{d}	1.11^{ab}	11.22^{ab}	70.20^{b}
PLpSc	14.06^{d}	85.95^{a}	4.03^{a}	1.06^{ab}	12.21^{c}	68.65^{a}

Means in each column with different superscripts represent significant difference (P≤0.05)
RSrg – Composition of raw sorghum (Control)
PLp – *ogi* from *L. plantarum* W723
PSc – *ogi* from *S. cremoris* W722
PLpSc – *ogi* from *L. plantarum* W723 and *S. cremoris* W722

approximately 30% of phytate in only 2 hours. Furthermore, Haros *et al.* (2008) tested the ability of the different strains of LAB to hydrolyse InsP6 and generate *myo*-inositols with lower numbers of phosphate groups (InsP3-InsP5) during growth using HPLC. The *myo*-inositol contents were determined in the culture supernatants of every strain grown until the stationary phase. The relative InsP6 hydrolysis was in the range from 0.0 to 8.83%. These researchers found that the highest degradation of InsP6 was carried out by *L. plantarum* W42 (8.53%) and *L. plantarum* 110 (8.83%), followed by *L. casei* 40W (6.56%).

Table 5 shows the result of proximate analysis (%) of the products obtained from the controlled fermentation of sorghum-*ogi*. The highest moisture content was observed for product from fermentation with the combination of *L. plantarum* and *S. cremoris* (PLpSc) which had a value of 14.06 while product from the fermentation with *L. plantarum* (PLp) had the lowest moisture content of 9.3. The amount of dry matter ranged from 85.95 to 90.66 for PLpSc and PLp respectively. The ash content of the products which is indicative of the amount of minerals contained in the product was observed to range from 1.03 to 1.11. Protein content of the controlled fermentation sorghum-ogi was slightly higher than observed in the raw sorghum composition. The carbohydrate present in controlled fermented products was observed to be lower than that in the raw sorghum. The moisture content of all the products from controlled fermentation was higher in values than in the raw sorghum. There was marked increase in the fat, ash and protein content of the products obtained from the controlled fermentation leading to a decrease in the carbohydrate content. The highest protein content was observed in the product from consortium of *L. plantarum* and *S. cremoris* (PLpSc). The ash content of the products also increased slightly for PLp and PLpSc while the increase was a little higher for PSc. The increase observed in the amount of protein and ash content is indicative that the reduction in phytic acid content of the products had led to the release of some of the minerals and proteins in the raw sorghum that the phytic acid formed complexes with.

CONCLUSIONS

The controlled fermented products had maximum levels of phytase activity and phytic acid degrading ability. These observations indicate that *L. plantarum* W723 and *Streptococcus cremoris* W722 had the potential to be used as starter cultures for developing several fermented cereal foods, thus decreasing phytate levels and facilitating the bioavailability of minerals.

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