Influence of soy fortification on microbial diversity during cassava fermentation and subsequent physicochemical characteristics of garri

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Abstract

This study investigated the influence of the addition of soy products on the microbiology, nutritional and physico-chemical characteristics of garri, a fermented cassava product. Malted soy flour (MSF) and soy protein (SP) were separately added (12% w/w) to cassava mash prior to controlled fermentation, while non-supplemented cassava mash served as a control. Identification of lactic acid bacteria (LAB) and aerobic mesophilic bacteria was accomplished by repetitive sequence based (rep)-PCR analysis and 16S rRNA gene sequencing. Physicochemical, nutritional and sensory characterisation of control and soy-fortified garri was performed using conventional methods. rep-PCR allowed differentiation of 142 isolates into 41 groups corresponding to 6 species of LAB and 25 species of aerobic mesophiles. LAB isolates belonged to the genera *Lactobacillus, Weissella*, *Leuconostoc and Lactococcus* with *Leuconostoc mesenteroides being* the dominant species in control and MSF-cassava while *Weissella cibaria* dominated SP-cassava fermentation. Aerobic mesophiles included Gram positive and negative bacteria such species of the genera *Bacillus*, *Clostridium*, *Staphylococcus*, *Serratia, Acinetobacter* and *Raoultella.* Diversity of aerobic mesophiles varied between control, MSF- and SP- cassava mash. Protein content of soy-fortified garri increased from 0.73% to 10.17% and 10.05% in MSF and SP garri respectively with a significant decrease in total cyanide from 26 to 11 ppm. Results from physicochemical and organoleptic evaluation indicate that supplementation of cassava with soy products prior to fermentation can produce acceptable garri. Soy products can be considered a viable option for protein fortification of garri, a low protein food with the aim of combating malnutrition.

Keywords: garri; cassava; soy products; fortification; lactic acid bacteria; aerobic mesophiles

1. Introduction

Cassava (*Manihot esculenta* Crantz*)* and associated fermented products provide a cheap source of calories and play an important role in combating hunger in many cassava-growing regions of the world. The use of cassava roots as food is limited as it is nutritionally deficient in terms of protein, vitamins and minerals (Ahaotu et al., 2011; Obatolu and Osho, 1992; Oboh and Akindahunsi, 2003). Another drawback is the presence of toxic cyanogenic glucosides in unprocessed cassava. If cassava tissue is damaged during harvest or storage, endogenous enzymes can hydrolyse these glucosides to hydrocyanic acid. Cassava processing, usually via fermentation, is thus vital in improving food security.

Garri is a gelatinized, granular, dry, coarse product obtained by roasting fermented, dewatered cassava mash. It is by far the most popular form in which cassava is consumed and sold in many African countries, Nigeria in particular (Ernesto et al.*,*2000; Oluwole et al., 2008). It is usually consumed as a stiff paste, eba, after mixing with boiling water and eaten with stews as a main meal, or mixed with cold water as a snack between meals. Garri is a good source of energy and fibre, with other nutrients of marginal nutritional significance (Ikegwuet al., 2009). However, continuous consumption of garri without supplementation with meat, fish and/or other protein-rich sources may result in protein deficiency (Agbon et al., 2010; Dakwa et al., 2005). West African diets are largely based on starchy staples such as cassava, maize, rice, and sorghum, as access to high quality animal proteins can be limited due to expense and lack of availability. Supplementation of cassava with good quality protein foods may aid in combating problems of protein malnutrition associated with high carbohydrate diets.

Soybean is a highly nutritious food material with a high percentage of amino acids and fatty acids. It is an important source of protein for many groups of people around the world. Soy protein is made from dehulled, defatted, soybean meal which can be processed into three kinds of high protein commercial products: soy flour, concentrates and isolates (Igoe and Hui, 2001). The addition of soy products such as soy protein (SP: 80-90% protein) or malted soy flour (MSF; 55-65% protein) to cassava mash prior to fermentation may improve the protein content of the final fermented product, garri. Improving the protein content of cassava based products has been the focus of previous scientific investigations (Agbon et al., 2010; Ahaotu et al., 2011; Arisa et al., 2011; Eke et al., 2008). However, there is limited information regarding the use of soy products as a source of high quality protein for garri production with respect to both the microbiology of the fermentation process and nutritional properties of fortified garri. The purpose of this study was two-fold. First, to evaluate the influence of two soy products, malted soy flour (MSF) and soy protein (SP) on the microbial population involved in cassava mash fermentation, using molecular typing techniques to identify the microorganisms involved. Secondly, to investigate the effect of soy fortification on nutritional and sensory characteristics of garri.

2. Materials and Methods

2.1. Preparation of soy products

Soy protein (SP) was obtained from the National Soybean Research Laboratory (NSRL) Illinois, United States. To prepare malted soy flour, soybeans were purchased from Ekeonunwa market in Imo state, Nigeria. Malted soyflour (MSF) was produced by steeping 2 kg of clean soybeans in 3 litres of water at ambient temperature (*ca* 28°C) for

10 h. Water was drained and soybeans spread on a moistened, sterile jute bag, covered, and allowed to germinate for 48 h. The sprouts were sprinkled with water at appropriate intervals during the germination period. Germinated soybeans were dried in an air oven at 55 to 60˚C for 24 h after which they were dehulled prior to milling into flour (Fig. 1).

2.2 Production and sampling of soy fortified garri

Cassava tubers were obtained from a farm in Obinze, Imo state, Nigeria and washed, peeled and rewashed three times with water to remove sand particles prior to grating (Kenwood Food Processor, FP 110). Cassava mash (1300 g) was combined with 180 g of either MSF or SP. Cassava mash (1480 g) without soy supplementation served as control. Control, MSF and SP cassava mash were transferred into separate polyurethane bags and fermented at 30˚C for 72 h. During fermentation, 250 g of samples of the fermenting mash were collected aseptically at 0, 24, 48 and 72 h for microbiological analysis and garification. The garification procedure was conducted as described by Akingbala et al., (2005) with slight modifications. Cassava mash (200 g) was dewatered using a hydraulic press. The dewatered cake was manually crushed on a stainless-steel sifter, before roasting the filtrate on a hot pan over a low fire. The garified cassava granules were spread out in a thin layer and left to cool at ambient temperature in a sterile environment before being packaged in zip lock airtight packs and stored at - 2˚C for further analysis. Three independent fermentation trials were conducted.

2.3 Microbiological analysis

2.3.1 Enumeration and isolation of bacteria from fermenting cassava mash. For all samples, 10 g of fermenting cassava mash were aseptically transferred into stomacher bags and homogenised in 90 ml sterile Maximum Recovery Diluent (MRD, Oxoid CM0733, Oxoid, Basingstoke, UK) for 2 min using a paddle-type blender (Colworth 400, AJ Seward, London, UK). From appropriate ten-fold dilutions, lactic acid bacteria (LAB) were enumerated and isolated on deMan, Rogosa and Sharpe agar (MRS; Oxoid CM0361) incubated anaerobically at 35°C for 72 h. Aerobic mesophiles were enumerated and isolated on Nutrient agar (NA; Oxoid CM0003) incubated at 37°C for 48 h. Morphological characteristics of colonies recovered from MRS agar and NA were examined and representative colonies were selected from appropriate dilutions. Bacteria were separately isolated on NA or MRS agar and purified by streaking several times on the same media as appropriate.

2.3.2 Phenotypic characterisation

Purified isolates were initially examined by colony and cell morphology as well as Gram, catalase and oxidase reactions. Cell morphology was determined by light microscopy (Nikon Model Eclipse, E400, Japan) and isolates were examined for Gram reaction using the KOH method (Gregersen, 1978).

2.3.3 Differentiation of isolates at species and subspecies levels using rep-PCR

DNA extraction was carried out using InstaGene^{TM} matrix (Bio-Rad, 732-6030, Hemel Hempstead, UK) following the manufacturer's instructions. Isolates were grouped at species and subspecies levels using repetitive sequenced based PCR (rep-PCR) and primer GTG5 (5'-GTG GTG GTGGTG GTG-3'; 5 pmol ml^{-1}) under the following conditions: initial denaturation at 94°C for 4 min followed by 30 cycles of denaturation at

94˚C for 30 s, annealing at 45˚C for 1 min, elongation at 65°C for 8 min and final extension at 65°C for 16min (Ouoba et al., 2008). Amplified PCR products were separated by agarose gel electrophoresis. Gels were documented using the Gel Doc It Imaging System (M-26X, UVP, Cambridge UK). Profiles were analysed using the Bio-numerics system (Bio-Numerics 2.50, UPGMA Pearson Correlation, Applied Maths, Sint-Martens-Latem, Belgium).

2.3.4 Identification of bacteria using 16S rRNA gene sequencing

Bacteria were tentatively identified by 16S rRNA gene sequencing. Amplification of the 16S rRNA gene was performed using forward and reverse primers; pA (5′-AGA-GTT-TGA-TCC-TGC-CTC-AG-3′; 100 pmol μl^{-1}) and pE (5′-CCG-TCA-ATT-CCT-TTG-AGT-TT-3′; 100 pmol μl^{-1}) based on conserved regions of the 16S rRNA gene as previously described (Ouoba et al., 2008). Reaction conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min followed by a final extension at 72 °C for 5 min. Purified PCR products were sequenced using the internal primer - pD (5′-GTA-TTA-CCG-CGG-CTG-CTG-3′; 3.2 pmol μl^{-1}). To determine the closest known relative species on the basis of 16S rRNA gene homology, sequences were analysed using the Basic Local Alignment Tool (BLAST) programme (National Centre for Biotechnology, MD, USA) against the GenBank/EMBL/DDBJ sequence database and the EzTaxon server (Kim et al., 2012). Sequences demonstrating the highest similarity in terms of closest relative species and 98.96 – 100.00 % homology were considered to belong to the same species.

2.4 Physicochemical analysis

184 At each time point, two samples were taken for analysis and each sample analysed in
185 duplicate.
186 2.4.1 Determination of pH and titratable acidity
187 At each sampling point, 10 g of either cassava mash or garri was homogenised in 90 ml
188 distilled water using a stomacher and pH measured using a calibrated pH meter (Hanna
189 Instruments, UK). To measure titratable acidity, 10 g of the sample was homogenised in
190 100 ml of distilled water and filtered (Whatman, UK). 10 ml of the filtrate was titrated
191 against 0.1M NaOH using 1% (v/v) phenolphthalein as indicator.
192 2.4.2 Proximate analysis
193 Moisture, ash, fat and protein content of garri was determined according to standard
194 analytical methods (AOAC, 2006).
195 2.4.3 Determination of total cyanide
196 Cyanide content of fortified and non-fortified garri was determined using the picrate
197 paper kit method (protocol B2) as described by Bradbury et al., (1999).
198 2.5 Sensory Analysis
199 Eba is a stiff porridge made from mixing garri with boiled water. Twenty semi-trained
200 panellists familiar with both garri and eba, were selected from the students and staff of
201 the Federal University of Technology, Owerri to determine the preference and
202 acceptability of the soy fortified garri samples when made into eba. The qualities
203 assessed were texture, aroma, bolus formation, colour and general acceptability. Each
204 attribute was scored using a nine-point hedonic scale scorecard with 1 representing
205 ‘extremely dislike’ and nine representing ‘extremely liked.’ (Weaver and Daniel, 2003).
206 2.6. Statistical analysis

Statistical differences between mean values were determined by analysis of variance (ANOVA) and Least Significance Difference using Statistical Package for the Social Sciences (SPSS version 10.0 SPSS Inc. Chicago, Illinois, USA).

3. Results

3.1 Microbiological analysis

During the control fermentation, there was an increase in the total aerobic count from 1.6 x 10^4 to 6.0 x 10^8 cfu/g and the LAB from 1.5 x 10^4 to 7.0 x 10^8 cfu/g during the 72 h fermentation period. A similar pattern was observed for LAB and aerobic mesophiles growth in cassava mash supplemented with soy products over the same fermentation period. In MSF- and SP- cassava mash, there was an increase in the total aerobic count from 3.24 x 10^5 to 1.51 x 10^8 and 3.0 x 10^5cfu/g to 2.29 x 10^9 cfu/g respectively. With respect to the presumptive LAB population, there was an increase from 1.1 x 10^4 to 2.2 x 10^8cfu/g in MSF-cassava and from 1.1. x 10^3 to 2.6 x 10^9cfu/g in SP-cassava.

A total of 142 bacterial isolates with variable macroscopic and microscopic characteristics was obtained from the control and soy supplemented cassava mash. Presumptive LAB isolates (88) were characterised as Gram positive, catalase and oxidase negative, cocci, bacilli and coccobacilli. Cluster analysis of rep-PCR profiles of these isolates allowed classification into six groups representing four genera and six species (Fig. 2). Sequencing of the 16S rRNA gene of isolates within each group allowed identification at genus and species level (Table 1). Overall, *Leuconostoc* was the most dominant genus and encompassed the species *Leuconostoc mesenteroides* (61.36%), *Leuconostoc lactis* (2.27%) and *Leuconostoc fallax* (2.27%). Other LAB

species identified were *Lactococcus lactis* (3.41%), *Weissella cibaria* (14.77%) with the sole lactobacilli species being *Lactobacillus plantarum (15.92%).*

The LAB profile for fermenting unfortified and MSF – cassava mash was similar. Both fermentations were dominated by *Leuconostoc mesenteroides* particularly during the first 48 h of fermentation, followed by *Lactobacillus plantarum*. In cassava fortified with SP, *Weissella cibaria* was the dominant LAB during the fermentation, followed by *Lactobacillus plantarum* (Table 1)

Fifty-four (54) aerobic mesophiles in total were recovered on NA from both control and fortified fermenting cassava mash and clustered based on 35 unique rep-PCR profiles corresponding to 15 genera and 26 species (Fig 2, Table 1). The dominant genus within this group was *Bacillus* (25.93%), isolated from all three cassava samples, while the dominant species was *Bacillus cereus sensu lato* (16.67%). Four species of *Staphylococcus* including *Staphylococcus gallinarium*, *Staphylococcus epidermidis*, *Staphylococcus warneri* and *Staphylococcus sciuri* made up 16.67% of total aerobic count. Gram negative bacteria isolated from control and soy-supplemented mash included *Raoultella planticola* (7.41%), *Serratia nematodiphila* (7.41%) *Pantoea dispersa* (1.85%)*, Pantoea vagans* (1.85%), *Pseudomonas hibiscicola* (1.85%) *and Klebsiella variicola* (1.85%). Apart from the common presence of *Bacillus,* diversity of aerobic mesophiles varied according to sample (Table 1).

3.3 Physicochemical characteristics of soy fortified garri

The effect of soy fortification on the pH, titratable acidity, total cyanide and proximate composition of control, MSF- and SP- garri was determined (Table 2). Comparisons were considered significant where $p < 0.05$.

The pH of both soy-fortified garri samples was significantly higher than that of the control sample with SP garri significantly higher at 5.16 than both MSF and control. No significant changes were observed in the titratable acidity of both unfortified and soy-fortified garri. Fortification significantly improved the protein content of garri. Compared to unfortified garri with an average protein content of 0.73%, the protein content in MSF- and SP-fortified garri increased to 10.17% and 10.05% protein respectively. Additionally, fortified garri had significantly lower cyanide concentrations. The cyanide content of MSF and SP garri was 11 mg kg^{-1} compared to 26 mg kg^{-1} in the control. MSF garri had significantly higher fat content of 4.13% compared to the other two samples although SP garri had an increased fat content than the control. Control and SP garri had a significantly higher moisture content compared to MSF. Fortification with SP significantly increased ash content of garri compared to MS fortification.

3.4 Sensory attributes of eba made from soy extract fortified *garri*

In eba produced from control and soy-fortified garri, features such as bolus formation, texture, colour, aroma and general acceptability was assessed (Table 3). The combined data of the sensory attributes of eba indicated no significant differences in the mean scores ($p<0.05$) for all samples and parameters studied. Soy fortified garri compared favourably with control in overall acceptability, however, the colour of MSF-fortified eba scored lower than both control and SP-fortified samples.

4. DISCUSSION

Cassava is an important food for millions of people who live in the tropics but its use as a staple is limited due to its low protein content and potential cyanide toxicity. In many Nigerian homes, cassava products such as garri are an essential part of the diet.

Strategies for fortifying local food to improve its nutritive quality without affecting safety and quality attributes is an important research focus as part of the effort to combat malnutrition and food insecurity (Oboh and Akindahunsi, 2003).

Supplementation of cassava mash with soy extracts did not have a marked effect on the microbiology of cassava fermentation. The role of LAB during cassava fermentation is well documented (Amoa-Awua et al., 1996; Kostinek et al., 2005; Oyewole and Odunfa, 1988). Lactic acid bacteria play an important role in acidification of the cassava, contributing to desirable organoleptic characteristics of the final fermented product. Acidification and production of other antimicrobial compounds by fermenting LAB strains may prevent the growth and/orsurvival of foodborne pathogens, thereby improving food safety (Anyogu et al., 2014; Mante et al., 2003). The dominance of LAB strains during cassava fermentation was not affected by the addition of soy extracts to cassava mash prior to fermentation. Cassava supplemented with MSF had the same LAB species profile as the control, unfortified sample. Similar to reports by Coulin et al., (2006) and Tsav-Wua et al., (2004) the predominant LAB recovered in this study was *Leuconostoc mesenteroides*. However, this is not in agreement with other authors, who have reported *Lactobacillus plantarum* as the predominant LAB present during cassava fermentation (Kostinek et al. 2005; Obilie et al., 2004). In cassava supplemented with soy protein *Weissella* spp. was the dominant LAB present. Although infrequently associated with cassava fermentation, Anyogu et al. (2014) noted the presence of *Weissella* during submerged fermentation of cassava. This supports the view that diversity of LAB is influenced by geographical origin, as well as the nature of the

297 fermentation process and underscores the importance of investigating the influence of
298 fortification on the microbial fermenting population.
299 Aerobic bacteria, particularly *Bacillus* spp., form a significant proportion of the microbial
300 population of fermenting cassava, where they are responsible for textural modification of
301 cassava tissue (Amoa- Awua and Jakobsen, 1995). The presence of soy products in
302 fermenting cassava mash appeared to have a more noticeable effect on the diversity of
303 the aerobic population than on LAB. The addition of MSF in particular led to the
304 dominance of *Bacillus* spp., including *B. cereus sensu lato* compared to the control
305 fermentation. This may be due to the increased protein content available during
306 fermentation as various species of *Bacillus* have repeatedly been associated with the
307 fermentation of protein rich soyfoods such as *iru* (Adewunmi et al., 2013), *afiyo*
308 (Ogunshe et al., 2007) and *soy dawadawa* (Dakwa et al., 2005; Omafvube, et al. 2000).
309 In addition, the pH of soy fortified *garri* was significantly higher than control. At pH
310 values below 4.2, as has been reported for garri (Achinewu et al., 2008; Tawo et al.,
311 2009), *B. cereus* will generally exist as spores but at higher pH values, there may be an
312 increased likelihood of spore germination, outgrowth and multiplication of vegetative
313 cells. Some studies aimed at evaluating the microbiological quality of fermented
314 cassava products have reported the presence of potentially pathogenic bacteria,
315 including *Bacillus* spp. and Enterobactericeae (Adebayo-Oyetoro et al., 2013;
316 Omafuvbe et al., 2007; Tsav-Wua et al., 2004). Consequently, our observation of *B.*
317 *cereus* and Gram negative bacteria such as *Serratia nematodiphila*, *Pantoea dispersa*,
318 *Raoultella planticola* is cause for concern and warrants further investigation.
319 Observations by Udoro et al., (2014) suggest that lengthening the cassava fermentation

period could lead to lower pH values of garri. However, it is not uncommon for processors to utilise shorter fermentation periods of 24 or 48 h, particularly when demand for garri is high.

Previous studies aimed at improving the protein content of garri have focused on inoculating starter cultures (Ahaotu et al., 2011; Akindahunsi et al., 1999; Oboh and Akindahunsi, 2003), protein rich biomass obtained from palm wine (Ogbo et al., 2009) and groundnut flour (Arisa et al., 2011). The inclusion of high protein soy products in fermenting cassava markedly improves the protein content of the final product garri and can aid in combating malnutrition associated with predominantly carbohydrate diets. The protein content of fortified garri (11%) was a considerable improvement on the unfortified garri (0.73%). Results further indicate that processing of cassava mash during garri production does not lead to significant loss of protein content, confirming the results of Eke et al., (2008), although other authors have noted that the pressing, sieving and frying of cassava mash for garri production can lead to a marked reduction in protein content (Oboh and Akindahunsi, 2003). Of particular interest was the significant reduction in cyanogenic glucosides of fortified garri. Fortification either improved or at least did not negatively impact the proximate composition of garri. Supplementation of cassava mash with MSF and SP prior to fermentation did not affect the general acceptability of garri, although slight modifications to the concentration of MSF can be made to improve the colour of the final product to make it more desirable to consumers.

Malted soy flour and soy protein may be considered viable options for protein fortification of garri. Addition of soy products does not affect the LAB fermenting

population and can significantly improve the protein content of a high carbohydrate meal. These advantages must be balanced against a potential increase in *Bacillus* population. Further research will focus on investigating the influence of soy fortification on microbial diversity during storage of garri.

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469 Table 1: Identification of microorganisms from control and soy supplemented fermenting cassava mash

Time of fermentation/Origin[a]	Control			MSF -fortified			SP-fortified		
	Bacteria	Rep-PCR pattern[b]	Identification[c]	Bacteria	Rep-PCR pattern	Identification	Bacteria	Rep-PCR pattern	Identification
0 h	A1	1	*Leuconostoc mesenteroides*	A42	1	*Leuconostoc mesenteroides*	A64	5	*Weissella cibaria*
	A2	1	*Leuconostoc mesenteroides*	A36	1	*Leuconostoc mesenteroides*	NL40	29	*Staphylococcus gallinarium*
	A3	1	*Leuconostoc mesenteroides*	A37	1	*Leuconostoc mesenteroides*	NL43	30	*Staphylococcus gallinarium*
	A4	1	*Leuconostoc mesenteroides*	A38	1	*Leuconostoc mesenteroides*	NL42	33	*Staphylococcus sciuri*
	A5	1	*Leuconostoc mesenteroides*	A39	1	*Leuconostoc mesenteroides*	NL44	33	*Staphylococcus sciuri*
	A6	1	*Leuconostoc mesenteroides*	A40	1	*Leuconostoc mesenteroides*	NL41	32	*Staphylococcus epidermidis*
	A7	1	*Leuconostoc mesenteroides*	A41	1	*Leuconostoc mesenteroides*			
	NL51	7	*Pantoea dispersa*	NL56	15	*Clostridium beijerinckii*			
	NL1	9	*Microbacterium paraoxydans*	NL58	15	*Clostridium beijerinckii*			
	NL52	10	*Microbacterium azadirachtae*	NL53	16	*Clostridium beijerinckii*			
	NL53	11	*Microbacterium azadirachtae*	NL19	17	*Bacillus cereus sensu lato*			
	NL2	12	*Exiguobacterium indicium*	NL26	17	*Bacillus cereus sensu lato*			
	NL3	12	*Exiguobacterium indicum*	NL22	22	*Bacillus mojavensis*			
	NL4	13	*Pseudomonas hibiscicola*	NL29	26	*Bacillus pumilus*			
	NL5	14	*Acinetobacter oleivorans*	NL24	24	*Bacillus aerophilus*			
	NL7	14	*Acinetobacter oleivorans*	NL25	35	*Paenibacillus pabuli*			
	NL6	34	*Staphylococcus warneri*	NL55	35	*Paenibacillus pabuli*			
	NL8	37	*Brachybacterium rhamnosus*	NL31	35	*Paenibacillus pabuli*			
				NL21	28	*Chryseobacterium bernadetii*			
				NL23	12	*Exiguobacterium indicum*			
24 h	A14	1	*Leuconostoc mesenteroides*	A43	1	*Leuconostoc mesenteroides*	A65	5	*Weissella cibaria*
	A15	1	*Leuconostoc mesenteroides*	A44	1	*Leuconostoc mesenteroides*	A66	5	*Weissella cibaria*
	A8	1	*Leuconostoc mesenteroides*	A45	1	*Leuconostoc mesenteroides*	A67	5	*Weissella cibaria*
	A9	1	*Leuconostoc mesenteroides*	A46	1	*Leuconostoc mesenteroides*	A68	5	*Weissella cibaria*
	A10	1	*Leuconostoc mesenteroides*	A47	1	*Leuconostoc mesenteroides*	A69	5	*Weissella cibaria*
	A11	1	*Leuconostoc mesenteroides*	NL32	18	*Bacillus cereus* sensu lato	A70	5	*Weissella cibaria*
	A12	1	*Leuconostoc mesenteroides*	NL33	12	*Exiguobacterium indicum*	A71	4	*Lactococcus lactis*
	A13	1	*Leuconostoc mesenteroides*	NL34	36	*Serratia nematodiphila*	NL45	31	*Staphylococcus gallinarum*
	NL9	38	*Klebsiella variicola*				NL46	39	*Raoultella planticola*

470 [a]Origin – Non-supplemented (Control), MSF (Malted soy flour), SP (soy protein) [b]Rep-PCR, Repetitive sequence based
471 PCR [c]Identification based on 16S rRNA gene sequences
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Table 1(contd.): Identification of microorganisms from control and soy supplemented fermenting cassava mash

Time of fermentation/Origin[a]	Control (Unfortified)			MSF-fortified			SP - fortified		
	Bacteria	Rep-PCR pattern[b]	Identification[c]	Bacteria	Rep-PCR pattern	Identification	Bacteria	Rep-PCR pattern	Identification
24 h	NL10	8	*Pantoea eucalypti*				NL47	19	*Bacillus cereus sensu lato*
	NL11	36	*Serratia nematodiphila*						
	NL12	36	*Serratia nematodiphila*						
	NL13	36	*Serratia nematodiphila*						
	NL14	41	*Staphylococcus saprophyticus*						
48 h	A16	1	*Leuconostoc mesenteroides*	A48	1	*Leuconostoc mesenteroides*	A72	2	*Leuconostoc lactis*
	A17	1	*Leuconostoc mesenteroides*	A49	1	*Leuconostoc mesenteroides*	A73	2	*Leuconostoc lactis*
	A18	1	*Leuconostoc mesenteroides*	A50	1	*Leuconostoc mesenteroides*	A74	4	*Lactococcus lactis*
	A19	1	*Leuconostoc mesenteroides*	A51	1	*Leuconostoc mesenteroides*	A75	5	*Weissella cibaria*
	A20	1	*Leuconostoc mesenteroides*	A52	1	*Leuconostoc mesenteroides*	A76	5	*Weissella cibaria*
	A21	1	*Leuconostoc mesenteroides*	A53	1	*Leuconostoc mesenteroides*	A77	5	*Weissella cibaria*
	A22	1	*Leuconostoc mesenteroides*	A54	1	*Leuconostoc mesenteroides*	A78	5	*Weissella cibaria*
	A23	1	*Leuconostoc mesenteroides*	A55	1	*Leuconostoc mesenteroides*	A79	5	*Weissella cibaria*
	A26	1	*Leuconostoc mesenteroides*				A80	6	*Lactobacillus plantarum*
	A24	6	*Lactobacillus plantarum*				A81	6	*Lactobacillus plantarum*
	A25	6	*Lactobacillus plantarum*				A82	6	*Lactobacillus plantarum*
	NL15	34	*Staphylococcus warneri*				NL48	39	*Raoultella planticola*
	NL16	21	*Bacillus cereus* sensu lato				NL49	39	*Raoultella planticola*
	NL17	21	*Bacillus cereus* sensu lato				NL50	39	*Raoultella planticola*
72 h	A27	1	*Leuconostoc mesenteroides*	A60	1	*Leuconostoc mesenteroides*	A83	4	*Lactococcus lactis*
	A28	1	*Leuconostoc mesenteroides*	A61	1	*Leuconostoc mesenteroides*	A84	5	*Weissella cibaria*
	A29	1	*Leuconostoc mesenteroides*	A62	1	*Leuconostoc mesenteroides*	A85	6	*Lactobacillus plantarum*
	A30	1	*Leuconostoc mesenteroides*	A63	1	*Leuconostoc mesenteroides*	A86	6	*Lactobacillus plantarum*
	A31	1	*Leuconostoc mesenteroides*	A57	1	*Lactobacillus plantarum*	A87	6	*Lactobacillus plantarum*
	A35	1	*Leuconostoc mesenteroides*	A58	1	*Lactobacillus plantarum*	A88	3	*Leuconostoc fallax*
	A32	6	*Lactobacillus plantarum*	A59	1	*Lactobacillus plantarum*	A89	3	*Leuconostoc fallax*
	A33	6	*Lactobacillus plantarum*	NL35	20	*Bacillus cereus sensu lato*			
	A34	6	*Lactobacillus plantarum*	NL36	23	*Bacillus cereus sensu lato*			
	NL54	27	*Bacillus aryabhattai*	NL37	25	*Bacillus aerophilus*			
	NL18	20	*Bacillus cereus sensu lato*	NL38	25	*Bacillus aerophilus*			
				NL39	40	*Lysinibacillus macroides*			

[a]Origin – Unfortified cassava (Control), MSF (Malted soy flour), SP (soy protein) [b]Rep-PCR, Repetitive sequence based PCR [c]Identification based on 16S rRNA gene sequences

480 Table 2 Effect of fortification with soy products on the chemical composition of garri

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Samples	Parameters						
	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Total cyanide (mg kg^{-1})	pH	Titratable acidity (%)
Control	0.73 ± 0.12^{b}	0.39 ± 0.02^{b}	1.06 ± 0.80^{b}	6.30 ± 0.55^{a}	26.41 + 9.80^{a}	4.79 + 1.14^{c}	0.54 + 0.003^{a}
MSF	10.17 ± 0.44^{a}	4.13 ± 0.09^{a}	1.90 ± 0.42^{ab}	5.56 ± 0.61^{b}	11.08 + 3.91^{b}	4.96 + 0.90^{b}	0.63 + 0.003^{a}
SP	10.05 ± 2.02^{a}	1.17 ± 2.91^{b}	2.09 ± 0.04^{a}	6.38 ± 0.69^{a}	11.02 + 2.53^{b}	5.16 + 0.86^{a}	0.81 + 0.004^{a}

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483 Values represent means of duplicate experiments ± standard deviation. Values with the same superscript in a column are

484 not significantly different ($p < 0.05$).

485 Keys: Control = Unfortified MSF = Malted soy flour SP = Soy protein.

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Table 3: Sensory attributes of eba produced from soy-fortified *garri*

Sample/Time of fermentation	Texture	Colour	Aroma	Bolus formation	General acceptability
Control/0 h	6.70 ± 1.66[a]	6.00 ± 2.00[b]	7.95 ± 1.05[a]	7.30 ± 2.00[a]	6.85 ± 1.76[a]
Control/24 h	6.55 ± 1.88[a]	6.45 ± 1.36[a]	5.30 ± 1.95[a]	5.40 ± 1.79[a]	5.85 ±1.60[a]
Control/48 h	7.25 ±1.62[a]	6.80 ± 1.96[a]	7.30 ± 1.38[a]	7.30 ± 1.26[a]	7.40 ± 1.60[a]
Control/72 h	7.70 ± 1.38[a]	7.20 ± 1.94[a]	7.50 ±1.15[a]	7.25 ± 1.59[a]	7.50 ± 1.47[a]
MSF/0 h	6.05 ± 2.31[b]	5.55 ± 1.93[b]	5.20 ± 1.99[a]	6.35 ± 1.95[a]	6.10 ± 1.92[a]
MSF/24 h	6.00 ± 2.00[b]	5.80 ± 2.09[b]	4.55 ± 2.33[a]	6.00 ± 1.86[a]	5.70 ± 1.87[a]
MSF/48 h	7.55 ± 1.51[a]	6.95 ± 1.64[a]	5.35 ± 2.03[a]	6.35 ± 2.06[a]	6.55 ±1.73[a]
MSF/72 h	7.45 ± 1.61[a]	6.75 ± 1.62[a]	5.20 ± 2.07[a]	6.05 ± 1.93[a]	6.40 ± 1.54[a]
SP/0 h	7.20 ± 1.67[a]	6.75 ± 1.59[a]	6.40 ± 1.96[a]	6.35 ± 1.84[a]	6.45 ± 1.57[a]
SP/24 h	6.80 ± 2.09[a]	7.05 ± 1.39[a]	6.95 ± 1.43[a]	6.50 ± 1.88[a]	6.65 ± 1.42[a]
SP/48 h	7.25 ± 1.65[a]	7.45 ± 1.36[a]	6.95 ± 1.23[a]	7.00 ± 1.59[a]	7.15 ± 1.69[a]
SP/72 h	7.65 ± 1.27[a]	7.00 ±1.49[a]	6.35 ± 1.76[a]	6.40 ± 1.76[a]	6.75 ± 1.77[a]

Values are means $\pm$ standard deviation of twenty panellists. Values with the same superscript in a column are not significantly different ($p \leq 0.05$).

Keys:

Control= Garri made from unfortified cassava mash

MSF = Malted soy flour

SP = Soy protein

0 h, 24 h, 48 h, 72 h = Time of cassava fermentation before garification

Figure Caption

Fig 1: Flow chart of the preparation of soy protein and malted soy flour fortified *garri*

Fig 2: Dendrogram of cluster analysis of rep-PCR fingerprints of lactic acid bacteria and aerobic mesophiles isolated from control and soy-fortified cassava mash. The dendrogram is based on Dice's coefficient of similarity with the unweighted pair method with arithmetic averages clustering algorithm (UPGMA). Numbers in brackets represent the rep group number.