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<http://dx.doi.org/10.1016/j.fm.2017.04.019>

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

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23 **Abstract**

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

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

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71 **1. Introduction**

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

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

94 Soybean is a highly nutritious food material with a high percentage of amino acids and
95 fatty acids. It is an important source of protein for many groups of people around the
96 world. Soy protein is made from dehulled, defatted, soybean meal which can be
97 processed into three kinds of high protein commercial products: soy flour, concentrates
98 and isolates (Igoe and Hui, 2001). The addition of soy products such as soy protein (SP:
99 80-90% protein) or malted soy flour (MSF; 55-65% protein) to cassava mash prior to
100 fermentation may improve the protein content of the final fermented product, garri.
101 Improving the protein content of cassava based products has been the focus of
102 previous scientific investigations (Agbon et al., 2010; Ahaotu et al., 2011; Arisa et al.,
103 2011; Eke et al., 2008). However, there is limited information regarding the use of soy
104 products as a source of high quality protein for garri production with respect to both the
105 microbiology of the fermentation process and nutritional properties of fortified garri. The
106 purpose of this study was two-fold. First, to evaluate the influence of two soy products,
107 malted soy flour (MSF) and soy protein (SP) on the microbial population involved in
108 cassava mash fermentation, using molecular typing techniques to identify the
109 microorganisms involved. Secondly, to investigate the effect of soy fortification on
110 nutritional and sensory characteristics of garri.

111 2. Materials and Methods

112 2.1. Preparation of soy products

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

117 10 h. Water was drained and soybeans spread on a moistened, sterile jute bag,
118 covered, and allowed to germinate for 48 h. The sprouts were sprinkled with water at
119 appropriate intervals during the germination period. Germinated soybeans were dried in
120 an air oven at 55 to 60°C for 24 h after which they were dehulled prior to milling into
121 flour (Fig. 1).

122 2.2 Production and sampling of soy fortified garri

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

138 2.3 Microbiological analysis

139 2.3.1 Enumeration and isolation of bacteria from fermenting cassava mash. For all
140 samples, 10 g of fermenting cassava mash were aseptically transferred into stomacher
141 bags and homogenised in 90 ml sterile Maximum Recovery Diluent (MRD, Oxoid
142 CM0733, Oxoid, Basingstoke, UK) for 2 min using a paddle-type blender (Colworth 400,
143 AJ Seward, London, UK). From appropriate ten-fold dilutions, lactic acid bacteria (LAB)
144 were enumerated and isolated on deMan, Rogosa and Sharpe agar (MRS; Oxoid
145 CM0361) incubated anaerobically at 35°C for 72 h. Aerobic mesophiles were
146 enumerated and isolated on Nutrient agar (NA; Oxoid CM0003) incubated at 37°C for
147 48 h. Morphological characteristics of colonies recovered from MRS agar and NA were
148 examined and representative colonies were selected from appropriate dilutions.

149 Bacteria were separately isolated on NA or MRS agar and purified by streaking several
150 times on the same media as appropriate.

151 2.3.2 Phenotypic characterisation

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

156 2.3.3 Differentiation of isolates at species and subspecies levels using rep-PCR

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

162 94°C for 30 s, annealing at 45°C for 1 min, elongation at 65°C for 8 min and final
163 extension at 65°C for 16min (Ouoba et al., 2008). Amplified PCR products were
164 separated by agarose gel electrophoresis. Gels were documented using the Gel Doc It
165 Imaging System (M-26X, UVP, Cambridge UK). Profiles were analysed using the Bio-
166 numerics system (Bio-Numerics 2.50, UPGMA Pearson Correlation, Applied Maths,
167 Sint-Martens-Latem, Belgium).

168 2.3.4 Identification of bacteria using 16S rRNA gene sequencing

169 Bacteria were tentatively identified by 16S rRNA gene sequencing. Amplification of the
170 16S rRNA gene was performed using forward and reverse primers; pA (5'-AGA-GTT-
171 TGA-TCC-TGC-CTC-AG-3'; 100 pmol μl^{-1}) and pE (5'-CCG-TCA-ATT-CCT-TTG-AGT-
172 TT-3'; 100 pmol μl^{-1}) based on conserved regions of the 16S rRNA gene as previously
173 described (Ouoba et al., 2008). Reaction conditions consisted of an initial denaturation
174 at 95 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C
175 for 1 min followed by a final extension at 72 °C for 5 min. Purified PCR products were
176 sequenced using the internal primer - pD (5'-GTA-TTA-CCG-CGG-CTG-CTG-3';
177 3.2 pmol μl^{-1}). To determine the closest known relative species on the basis of 16S
178 rRNA gene homology, sequences were analysed using the Basic Local Alignment Tool
179 (BLAST) programme (National Centre for Biotechnology, MD, USA) against the
180 GenBank/EMBL/DDBJ sequence database and the EzTaxon server (Kim et al., 2012).
181 Sequences demonstrating the highest similarity in terms of closest relative species and
182 98.96 – 100.00 % homology were considered to belong to the same species.

183 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

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

210 **3. Results**

211 **3.1 Microbiological analysis**

212 During the control fermentation, there was an increase in the total aerobic count from
213 1.6×10^4 to 6.0×10^8 cfu/g and the LAB from 1.5×10^4 to 7.0×10^8 cfu/g during the 72 h
214 fermentation period. A similar pattern was observed for LAB and aerobic mesophiles
215 growth in cassava mash supplemented with soy products over the same fermentation
216 period. In MSF- and SP- cassava mash, there was an increase in the total aerobic count
217 from 3.24×10^5 to 1.51×10^8 and 3.0×10^5 cfu/g to 2.29×10^9 cfu/g respectively. With
218 respect to the presumptive LAB population, there was an increase from 1.1×10^4 to 2.2
219 $\times 10^8$ cfu/g in MSF-cassava and from 1.1×10^3 to 2.6×10^9 cfu/g in SP-cassava.

220 A total of 142 bacterial isolates with variable macroscopic and microscopic
221 characteristics was obtained from the control and soy supplemented cassava mash.
222 Presumptive LAB isolates (88) were characterised as Gram positive, catalase and
223 oxidase negative, cocci, bacilli and coccobacilli. Cluster analysis of rep-PCR profiles of
224 these isolates allowed classification into six groups representing four genera and six
225 species (Fig. 2). Sequencing of the 16S rRNA gene of isolates within each group
226 allowed identification at genus and species level (Table 1). Overall, *Leuconostoc* was
227 the most dominant genus and encompassed the species *Leuconostoc mesenteroides*
228 (61.36%), *Leuconostoc lactis* (2.27%) and *Leuconostoc fallax* (2.27%). Other LAB

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

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

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

248 **3.3 Physicochemical characteristics of soy fortified garri**

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

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

264 **3.4 Sensory attributes of eba made from soy extract fortified *garri***

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

271 **4. DISCUSSION**

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

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

278 Supplementation of cassava mash with soy extracts did not have a marked effect on the
279 microbiology of cassava fermentation. The role of LAB during cassava fermentation is
280 well documented (Amoa-Awua et al., 1996; Kostinek et al., 2005; Oyewole and Odunfa,
281 1988). Lactic acid bacteria play an important role in acidification of the cassava,
282 contributing to desirable organoleptic characteristics of the final fermented product.

283 Acidification and production of other antimicrobial compounds by fermenting LAB strains
284 may prevent the growth and/or survival of foodborne pathogens, thereby improving food
285 safety (Anyogu et al., 2014; Mante et al., 2003). The dominance of LAB strains during
286 cassava fermentation was not affected by the addition of soy extracts to cassava mash
287 prior to fermentation. Cassava supplemented with MSF had the same LAB species
288 profile as the control, unfortified sample. Similar to reports by Coulin et al., (2006) and
289 Tsav-Wua et al., (2004) the predominant LAB recovered in this study was *Leuconostoc*
290 *mesenteroides*. However, this is not in agreement with other authors, who have
291 reported *Lactobacillus plantarum* as the predominant LAB present during cassava
292 fermentation (Kostinek et al. 2005; Obilie et al., 2004). In cassava supplemented with
293 soy protein *Weissella* spp. was the dominant LAB present. Although infrequently
294 associated with cassava fermentation, Anyogu et al. (2014) noted the presence of
295 *Weissella* during submerged fermentation of cassava. This supports the view that
296 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 Enterobacteriaceae (Adebayo-Oyetero 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

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

323 Previous studies aimed at improving the protein content of garri have focused on
324 inoculating starter cultures (Ahaotu et al., 2011; Akindahunsi et al., 1999; Oboh and
325 Akindahunsi, 2003), protein rich biomass obtained from palm wine (Ogbo et al., 2009)
326 and groundnut flour (Arisa et al., 2011). The inclusion of high protein soy products in
327 fermenting cassava markedly improves the protein content of the final product garri and
328 can aid in combating malnutrition associated with predominantly carbohydrate diets.

329 The protein content of fortified garri (11%) was a considerable improvement on the
330 unfortified garri (0.73%). Results further indicate that processing of cassava mash
331 during garri production does not lead to significant loss of protein content, confirming
332 the results of Eke et al., (2008), although other authors have noted that the pressing,
333 sieving and frying of cassava mash for garri production can lead to a marked reduction
334 in protein content (Oboh and Akindahunsi, 2003). Of particular interest was the
335 significant reduction in cyanogenic glucosides of fortified garri. Fortification either
336 improved or at least did not negatively impact the proximate composition of garri.

337 Supplementation of cassava mash with MSF and SP prior to fermentation did not affect
338 the general acceptability of garri, although slight modifications to the concentration of
339 MSF can be made to improve the colour of the final product to make it more desirable to
340 consumers.

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

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

347 **Acknowledgments**

348 N. N. Ahaotu is grateful to the Federal University of Technology, Owerri for funding this
349 research through the Tertiary Education Trust Fund (TETFund) intervention programme.

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

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

470 ^aOrigin – Non-supplemented (Control), MSF (Malted soy flour), SP (soy protein) ^bRep-PCR, Repetitive sequence based
 471 PCR ^cIdentification based on 16S rRNA gene sequences

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474 Table 1(contd.): Identification of microorganisms from control and soy supplemented fermenting cassava mash

Time of fermentation/Ori gin ^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	<i>Pantoea eucalypti</i>				NL47	19	<i>Bacillus cereus sensu lato</i>
	NL11	36	<i>Serratia nematodiphila</i>						
	NL12	36	<i>Serratia nematodiphila</i>						
	NL13	36	<i>Serratia nematodiphila</i>						
	NL14	41	<i>Staphylococcus saprophyticus</i>						
48 h	A16	1	<i>Leuconostoc mesenteroides</i>	A48	1	<i>Leuconostoc mesenteroides</i>	A72	2	<i>Leuconostoc lactis</i>
	A17	1	<i>Leuconostoc mesenteroides</i>	A49	1	<i>Leuconostoc mesenteroides</i>	A73	2	<i>Leuconostoc lactis</i>
	A18	1	<i>Leuconostoc mesenteroides</i>	A50	1	<i>Leuconostoc mesenteroides</i>	A74	4	<i>Lactococcus lactis</i>
	A19	1	<i>Leuconostoc mesenteroides</i>	A51	1	<i>Leuconostoc mesenteroides</i>	A75	5	<i>Weissella cibaria</i>
	A20	1	<i>Leuconostoc mesenteroides</i>	A52	1	<i>Leuconostoc mesenteroides</i>	A76	5	<i>Weissella cibaria</i>
	A21	1	<i>Leuconostoc mesenteroides</i>	A53	1	<i>Leuconostoc mesenteroides</i>	A77	5	<i>Weissella cibaria</i>
	A22	1	<i>Leuconostoc mesenteroides</i>	A54	1	<i>Leuconostoc mesenteroides</i>	A78	5	<i>Weissella cibaria</i>
	A23	1	<i>Leuconostoc mesenteroides</i>	A55	1	<i>Leuconostoc mesenteroides</i>	A79	5	<i>Weissella cibaria</i>
	A26	1	<i>Leuconostoc mesenteroides</i>				A80	6	<i>Lactobacillus plantarum</i>
	A24	6	<i>Lactobacillus plantarum</i>				A81	6	<i>Lactobacillus plantarum</i>
	A25	6	<i>Lactobacillus plantarum</i>				A82	6	<i>Lactobacillus plantarum</i>
	NL15	34	<i>Staphylococcus warneri</i>				NL48	39	<i>Raoultella planticola</i>
	NL16	21	<i>Bacillus cereus sensu lato</i>				NL49	39	<i>Raoultella planticola</i>
	NL17	21	<i>Bacillus cereus sensu lato</i>				NL50	39	<i>Raoultella planticola</i>
	72 h	A27	1	<i>Leuconostoc mesenteroides</i>	A60	1	<i>Leuconostoc mesenteroides</i>	A83	4
A28		1	<i>Leuconostoc mesenteroides</i>	A61	1	<i>Leuconostoc mesenteroides</i>	A84	5	<i>Weissella cibaria</i>
A29		1	<i>Leuconostoc mesenteroides</i>	A62	1	<i>Leuconostoc mesenteroides</i>	A85	6	<i>Lactobacillus plantarum</i>
A30		1	<i>Leuconostoc mesenteroides</i>	A63	1	<i>Leuconostoc mesenteroides</i>	A86	6	<i>Lactobacillus plantarum</i>
A31		1	<i>Leuconostoc mesenteroides</i>	A57	1	<i>Lactobacillus plantarum</i>	A87	6	<i>Lactobacillus plantarum</i>
A35		1	<i>Leuconostoc mesenteroides</i>	A58	1	<i>Lactobacillus plantarum</i>	A88	3	<i>Leuconostoc fallax</i>
A32		6	<i>Lactobacillus plantarum</i>	A59	1	<i>Lactobacillus plantarum</i>	A89	3	<i>Leuconostoc fallax</i>
A33		6	<i>Lactobacillus plantarum</i>	NL35	20	<i>Bacillus cereus sensu lato</i>			
A34		6	<i>Lactobacillus plantarum</i>	NL36	23	<i>Bacillus cereus sensu lato</i>			
NL54		27	<i>Bacillus aryabhatai</i>	NL37	25	<i>Bacillus aerophilus</i>			
NL18		20	<i>Bacillus cereus sensu lato</i>	NL38	25	<i>Bacillus aerophilus</i>			
				NL39	40	<i>Lysinibacillus macroides</i>			

475 ^aOrigin – Unfortified cassava (Control), MSF (Malted soy flour), SP (soy protein) ^bRep-PCR, Repetitive sequence based476 PCR ^cIdentification based on 16S rRNA gene sequences

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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	Titrateable 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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496 Table 3: Sensory attributes of eba produced from soy-fortified *garri*

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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

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

500 Keys:

501 Control= Garri made from unfortified cassava mash

502 MSF = Malted soy flour

503 SP = Soy protein

504 0 h, 24 h, 48 h, 72 h = Time of cassava fermentation before garification

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511 Figure Caption

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

513 Fig 2: Dendrogram of cluster analysis of rep-PCR fingerprints of lactic acid bacteria and

514 aerobic mesophiles isolated from control and soy-fortified cassava mash. The

515 dendrogram is based on Dice's coefficient of similarity with the unweighted pair method

516 with arithmetic averages clustering algorithm (UPGMA). Numbers in brackets represent

517 the rep group number.

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