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# 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 including 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.


## 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

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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 (Ikegwu et 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 L 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 55–60 °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.

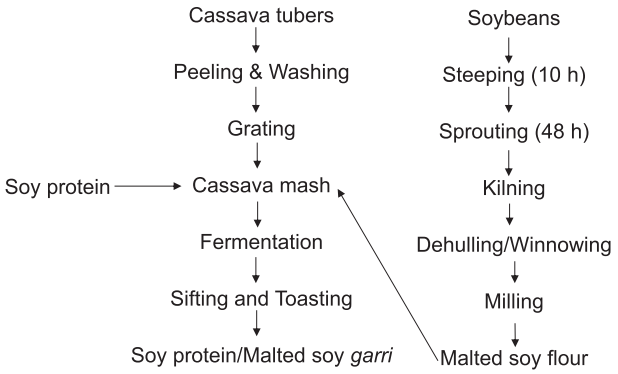


**Fig. 1.** Flow chart of the preparation of soy protein and malted soy flour fortified *garri*.

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™ 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-