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Microbiological and physico-chemical analysis of fermented protein-fortified cassava (*Manihot esculenta* Crantz) flour

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ABSTRACT

Lactobacillus plantarum strain 6710 was used as the starter for wet fufu using protein (zeolin, sporazein, sporazein plus pro-vitamin A) and pro-vitamin A-fortified and wild-type cassava flours that were fermented with or without the addition of starter culture. Lactic acid bacteria count of the non-inoculated and inoculated wild type flours increased 4 and 1 log units in 24 h, respectively. The added strain rapidly increased titratable acidity when using wild-type cassava from 0.24 to 0.96% lactic acid at 24 h and 1.18% at 96 h. Titratable acidity also increased with sporazein, sporazein plus pro-vitamin A, and pro-vitamin A samples. A similar aroma profile was found in all samples suggesting that protein fortification does not have a detrimental effect on the aroma of fufu. This study showed that use of *L. plantarum* is feasible for production of wet fufu from protein-fortified cassava making the increased consumption of a nutritious product possible.

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a major source of calories in under-developed countries such as Ghana and Nigeria (Nweke, 2004) and ranks sixth in overall global crop production. However, a cassava-based diet does not provide complete nutrition. Cassava is cultivated mainly for its edible storage root, which contains 85% starch and only 1–2% protein (Han et al., 2001; Sheffield, Taylor, Fauquet, & Chen, 2006).

Another limitation of cassava is the potential toxicity of cassava roots due to the accumulation of the cyanogenic glycosides linamarin and lotaustralin. In Africa, improperly processed cassava is a major problem associated with cyanide-related health disorders (Maziya-Dixon, Dixon, & Adebowale, 2007). Numerous cassava processing methods have been devised, including fermentation followed by drying or roasting, to reduce toxicity while converting the highly perishable cassava into stable products (Vasconcelos, Twiddy, Westby, & Reilly, 1990).

Fufu is a fermented wet paste product, usually consumed in many parts of West Africa. For the production of fufu, cassava roots are peeled, washed, cut into pieces and submerged in water at room temperature for 5 days, but it has been found that local processors ferment cassava for different lengths of time (Oyewole & Ogundele, 2001; Ray & Sivakumar, 2009). Cassava roots release hydrogen cyanide into the soak water, reducing the pH and imparting the characteristic flavor of the retted cassava meal. Fufu is sold to consumers in wet or cooked form (Ray & Sivakumar, 2009).

Species of lactic acid bacteria (LAB) belonging to the genera *Lactobacillus, Leuconostoc* and *Streptococcus* are the predominant microorganisms in fufu (Ray & Sivakumar, 2009). In Africa, most food fermentations are done at the household level, and are conducted as spontaneous processes taking a relatively long time that can result in spoilage and survival of pathogens. Thus, the use of starter cultures is recommended, as rapid acidification of the product can inhibit growth of undesirable bacteria.

Lactobacillus plantarum has been found to have the highest acid producing ability in cassava tubers (Kostinek et al., 2007), therefore, a strain of this species was used in an attempt to reduce fermentation time that may cause the production of unacceptable aroma compounds. While traditional fufu processing involves the

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utilization of cassava roots, this study proposes the use of cassava flour as starting material.

Specifically, this study was undertaken to evaluate the microbiological and physico-chemical characteristics of *L. plantarum* 6710 on the course of fermentation of protein – and pro-vitamin A-fortified cassava flours in comparison to wild type flour, and their suitability to produce wet fufu.

2. Materials and methods

2.1. Cassava flours

Protein-fortified cassava flours (zeolin (Z), sporazein (S), sporazein plus pro-vitamin A (SPRO)), pro-vitamin A-fortified (PRO) and wild-type cassava (WT) flours were provided by the International Laboratory for Tropical Agricultural Biotechnology (ILTAB) (St. Louis, MO).

2.2. Microbiological assessment

2.2.1. Microorganisms and growth conditions

L. plantarum BFE 6710 provided by the Max Rubner-Institut (Germany) was grown from a stab culture in Lactobacillus de Man, Rogosa and Sharpe (MRS) broth at 32 °C for 24 h under aerobic conditions. Cultures were placed in cryogenic vials with 50% glycerol (1.5:1 ratio) and stored at −72 °C. Working cultures were obtained by streaking stock culture on MRS agar, incubated at 32 °C for 48 h and a single colony transferred into MRS broth and incubated under the same conditions. The broth culture was centrifuged at 8000× *g* at 4 °C for 10 min. The pellet was washed twice with sterile buffered peptone water (BPW), centrifuged as noted above and resuspended in 9 mL of BPW resulting in 7×10^{10} CFU/mL.

2.2.2. Growth of starter culture in cassava flour

All wild-type and fortified cassava flours were stored in sealed plastic containers at 4 °C. Ninety g of cassava flour samples were transferred to sterilized plastic containers. Initial inoculation by *L. plantarum* was done by transferring a cell pellet suspension of 7×10^{10} CFU/mL into cassava flour. Moisture content of cassava flours was adjusted to 68% with sterile water. A second set of control (non-inoculated) flours was also prepared. Non-inoculated (NF) and inoculated (LF) cassava flour samples were covered and incubated at 32 °C for 96 h.

2.2.3. Monitoring lactic acid bacteria (LAB) growth

Cassava samples were removed at 0, 24, 48, 72 and 96 h of incubation and assessed as follows: 5 g sample was placed in 80-mL sterile stomacher bag and homogenized for 2 min with 45 mL of sterile BPW. One hundred μL of appropriate ten-fold serial dilutions of samples prepared in 0.2% peptone water were duplicate spread-plated on MRS agar for enumeration of LAB after incubation at 32 °C for 48 h.

2.3. Physico-chemical analyses

Fermented cassava flours (wet fufu) were removed at predetermined incubation times and kept at −70 °C for 7 days until evaluation.

2.3.1. Chemical composition

Moisture and ash of cassava flours was determined by the air oven method 925.10 and the direct method 923.03, respectively (AOAC, 2000). Soluble protein was determined in wet fufu. Samples were extracted with 100 mM Tris–HCl, pH 7.8, 200 mM NaCl, 1 mM EDTA, 0.2% Triton X-100 with 4% 2-mercaptoethanol in a ratio of 0.1:1 (wet fufu:solvent). The homogenate was vortexed for 5 min at 2500 rpm and centrifuged at 1500× *g* for 10 min at 4 °C. The supernatant was analyzed by the Bradford method (CBX kit) (G-Biosciences, St. Louis, MO) according to the manufacturer's protocol. A calibration curve was constructed with bovine serum albumin. Total nitrogen content of cassava flours was determined by Approved Method 46-30.01 (AACC, 2010) with a LECO Instrument FP-528 nitrogen analyzer. Total starch of cassava flours was determined by the total starch assay procedure (amyloglucosidase/α-amylase) K-TSTA (Megazyme International Ltd., Wicklow, Ireland).

2.3.2. pH and titratable acidity (TA)

Modified methods 943.02, sec. 32.1.20 and 942.15, sec. 37.1.37B were used for determination of pH and titratable acidity, respectively (AOAC, 2000).

2.3.3. Pasting properties

Cassava flour starch pasting properties were measured with the Newport Scientific Pty Rapid Visco Analyzer (RVA) interfaced with a personal computer equipped with Thermocline software for Windows 2.1 according to the AACC Approved Method 76-21.01 (AACC, 2000). The samples were analyzed by transferring 3.5 g (14% moisture basis) flour with 25 mL of water into an RVA canister, held at 50 °C for 1 min, heated from 50 to 95 °C in 4.42 min, held at 95 °C for 2.7 min, cooled to 50 °C in 3.82 min, and held at 50 °C for 1.06 min.

2.3.4. Color evaluation

A Minolta colorimeter CM-2002 was calibrated using its white standard calibration cover. The cassava flour was placed in a plastic petri dish, slightly shaken to form a layer of 5 mm thickness, covered with the Petri dish lid and the color read on the meter. The L, a* and b* values were recorded and averages computed from two randomly selected points.

2.3.5. Volatile analysis

An optimized method of Iyer, Mattinson, and Fellman (2010) was used. A slurry sample of wet fufu at all fermentation times (0.5 g), sodium chloride (0.98 g) and distilled water (3.0 mL) was prepared in a 10 mL headspace amber vial. Automation of the procedure was achieved using a CTC CombiPal autosampler and programmed using CycleComposer software (A.01.04). A SPME stableflex fiber coated with 65 μm polydimethylsiloxane/divinylbenzene was conditioned for 1 h at 270 °C. The sample was stirred at 250 rpm, the fiber was exposed to the headspace of the slurry and volatile compounds were collected for 60 min.

The volatiles were thermally desorbed into the injection port of a gas chromatograph equipped with a 6890N GC split/splitless injector and a HP-5MS column (30 m × 0.248 mm × 0.25 μm film thickness). Data were collected using Chemstation software (E.02.00.493). Helium was the carrier gas. The injector and detector temperatures were 200 °C and 250 °C, respectively. The column temperature was initially at 33 °C for 5 min before increasing to 50 °C at a rate of 2 °C/min, then to 225 °C at a rate of 5 °C/min. The sample was desorbed for 5 min in the splitless mode. Wet fufu volatile compounds were identified using a mass spectrometer MS 5975C and the MS spectra were compared against a NIST library. The quantitative data were determined by running standards and developing response factors based on water matrices. The data were reported as μg/mL.

2.4. Data analysis

Cassava flour physico-chemical data were analyzed for