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Screening of lactic acid bacteria potentially useful for sorghum fermentation

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ABSTRACT

A screening of commercial lactic acid bacteria potentially useful in the improvement of sorghum nutritional quality was done. The aim of this study was to test starter cultures to meet the prerequisites for the establishment of small-scale industrial production of sorghum fermented foods in Africa.

Sorghum was fermented with commercial strains of *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus paracasei*, *Lactobacillus fermentum*, *Pediococcus pentosaceus* and *Streptococcus thermophilus*.

As a result of sorghum prolamins hydrolysis, an increase in the *in vitro* protein digestibility (IVPD) was promoted. After pepsin digestion, changes occurred in the electrophoretic profile of prolamins, with a decrease of almost all fermented sample spots in comparison with the unfermented sample. Samples in which the decrease of 45 kDa and 66 kDa oligomers was more pronounced, presented higher IVPD.

Fourier Transform Infrared spectroscopy in tandem with multivariate analysis showed starch structural changes in samples fermented with *Lactobacillus brevis*, *Lactobacillus fermentum*, *Streptococcus thermophilus* and *Pediococcus pentosaceus.*

This work demonstrates that all tested bacteria promoted beneficial effects on sorghum nutritional quality and are suitable to be used as commercial starters for industrial applications. *Streptococcus thermophilus*, *Lactobacillus brevis* and *Lactobacillus fermentum* are the most promising starters as they lead to higher IVPD values (46.48, 39.19 and 36.73%, respectively).

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1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench], together with millet, is a major food for around 60 million people concentrated in the inland areas of tropical Africa, who consume them mostly in the form of fermented or unfermented preparations (FAO, 2004). Fermented porridges are prepared in many African countries for human consumption, and appear to be one of the most common types of food prepared from sorghum (Duodu et al., 2003). Heterolactic bacteria are the dominant microorganisms found in sorghum natural fermentation. A wide variety of lactic acid bacteria (LAB) are found in these sorghum products (Calderon et al., 2003).

Fermentation plays an important role in sorghum food preparation as it provides an improvement of sorghum nutritional quality. Lactic acid fermentation, using natural mixed cultures, has been shown to improve the digestibility of sorghum proteins (Belton and Taylor, 2004). This is of great importance as sorghum is known to become less digestible than other cereals after cooking (Axtell et al., 1981; Eggum et al., 1983; Hamaker et al., 1987; MacLean et al., 1981). Fermentation also leads to an increase of sorghum protein content (El Tinay et al., 1979), enhancement of carbohydrates accessibility (Elkhalifa et al., 2004, 2006), improvement of amino acid balance (Au and Fields, 1981; El Tinay et al., 1979), decrease of anti nutritional factors, like tannins and phytic acid (Osman, 2004), and increase of vitamin content (El Tinay et al., 1979; Kazanas and Fields, 1981).

Household fermentation technologies have been upgraded to an industrial scale in order to provide value added products that meet urban population demand for traditional products (Belton et al., 2004; Gadaga et al., 1999).

One of the prerequisites for the establishment of small-scale industrial production of fermented foods in Africa is the development of starter cultures (Sanni, 1993).

A screening of lactic acid bacteria potentially useful in the improvement of sorghum nutritional quality was done in this work. The aim of this study is to better understand the role played by these LAB's and to evaluate their application for achieving an industrial scale production of sorghum foods.

Abbreviations: ATR, Attenuated total reflection; CFU, Cell Forming Units; DNS, 3,5- Dinitrosalicylic Acid; FT-IR, Fourier Transform Infrared; HMW, High Molecular Weight; IVPD, *in vitro* Protein Digestibility; LAB, lactic acid bacteria; OD, Optical Density; PCA, Principal Components Analysis; SDS-PAGE, Sodium Dodecyl Sulfate Polycrylamide Gel Electrophoresis; SNV, Standard Normal Variate.

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2. Materials and methods

2.1. Sorghum flour

Sorghum grains, of the Australian variety Jumbo were purchased in retail trade and ground with a coffee mill to pass through a 3×10^{-4} m sieve. This sorghum cultivar has a soft endosperm (Lazaro and Favier, 2000) The characterization of the reddish brown grains showed that this sorghum is a non-tannin (Type I) variety (determined by the chlorox bleach test described by Waniska et al., 1992), has 9% of total protein (determined by the Kjeldahl method described by Nunes, 1998) and 0.32% of polyphenol content (determined by the method described by Price and Butler, 1977).

2.2. Bacterial strains and growth conditions

Lactobacillus plantarum subsp. *argentoratensis* (DSM 16365), *Lactobacillus brevis* (DSM 6235), *Lactobacillus paracasei* subsp. *paracasei* (DSM 20006), *Lactobacillus fermentum* (DSM 20052), *Pediococcus pentosaceus* (DSM 20283) and *Streptococcus thermophilus* (DSM 20617) were obtained from DMSZ (Braunschweig, Germany) in lyophilized form. All these species have been found in traditionally fermented sorghum products (Mugula et al., 2003b, c; Muyanja et al., 2003).

A MRS broth (Merck-Darmstadt, Germany) was used in their rehydration. After re-hydration, the bacteria were disseminated by streaking on MRS agar (Merck - Darmstadt, Germany) and incubated for 24 h at their optimal growth temperature (37 °C for *L. fermentum* and *S. thermophilus* and 30 °C for the other ones).

2.3. Preparation of starter cultures

Aqueous suspensions of starter cultures were prepared from 24 h cultures of each one of the LAB species on agar plates. With sterile loops, LAB cultures were transferred to physiological serum (NaCl 0.9% (w/v)) and stirred in a vortex. Dilutions were made in order to obtain inoculums containing about 10^7 CFU/mL, determined by optical densities. Optical densities at 600 nm (OD_{600}) were measured using a Shimadzu UV-160A spectrophotometer (Tokyo, Japan). Cellular concentrations of each inoculum were obtained from calibration curves between OD_{600} and the number of colonies/mL determined by standard plate count.

2.4. Flour fermentation

For lactic fermentation, 6 samples of sorghum flour (15 g each) were mixed with sterilized water (1:10 w/v), in sealed E-flasks. These mixtures were boiled, for starch gelatinization, during 1 minute under vigorous stirring prior to autoclaving at 121 °C for 15 min. After cooling, at room temperature (*ca.* 25 °C), each one of these samples was inoculated, respectively, with 5×10^{-3} L of *L. plantarum* subsp. *argentoratensis, L. brevis, L. paracasei* subsp. *paracasei*, *L. fermentum*, *P. pentosaceus* and *S. thermophilus* suspensions. All the samples were incubated for five days at the optimal growth temperature of the respective inoculum. Fermentation time was established according to previous work on sorghum fermentation (Correia, 2004)

An unfermented control sample was prepared as described before, omitting the inoculation step. To keep the same conditions of fermented samples, 5×10^{-3} L of physiological serum (NaCl 0.9% w/v) was added.

2.5. Preparation of samples

After fermentations were completed, pH was measured and each of the samples was divided in two portions. The first one was freeze dried and ground again. This portion was kept to determine total protein, soluble proteins, reducing sugars, total sugars and free amino acids. Total protein was determined directly on the freeze dried powder. To analyse each of following soluble components; soluble proteins, reducing sugars, total sugars and free amino acids, 1 g of the freeze dried samples was mixed with distilled water in a proportion of 1:20 w/v, with magnetical stirring for 1 h. All samples were centrifuged ($2500 \times g$ at room temperature) for 3 min and the analytes determined in the supernatants. In the case of soluble protein, pH was adjusted to 2 (with HCl) after water addition.

The second portion of each sample was centrifuged at $24,000 \times g$ for 20 min (Sigma 3K30 centrifuge – Osterode am Harz, Germany) and the residues were freeze dried and ground again. These residues were used for the determination of total starch, FT-IR analysis and *in vitro* protein digestibility (IVPD) assay.

2.6. pH measurement

The pH of the samples was measured with a glass electrode.

2.7. Reducing sugar determination

Reducing sugars were determined by the 3,5-dinitrosalicylic acid (DNS) colorimetric method, with glucose as the standard (Miller, 1959). 1×10^{-3} L of DNS reagent was added to 1×10^{-3} L of sample supernatant. The mix was kept in a boiling water bath for 5 min. After cooling to room temperature (*ca.* 25 °C) in a cold water bath, 1×10^{-2} L of distilled water was added. The absorbance at 540 nm was measured (Shimadzu UV-160A spectrophotometer), interpolating the value obtained with calculated values for glucose solutions of known concentration. The blanks were prepared by substituting sample solution for distilled water.

2.8. Total sugars determination

A modified phenol-sulfuric acid method was used to determine total sugars present in the samples (Dubois et al., 1956). 1×10^{-3} L of 5% phenol was added to 1×10^{-4} L of sample. Then, 1×10^{-3} L of concentrated sulfuric acid was added and the mixture was kept in a boiling water bath for 10 min. After cooling to room temperature (*ca.* 25 °C) in a cold water bath, the absorbance at 490 nm was measured (Shimadzu UV-160A spectrophotometer – Tokyo, Japan). The amount of sugars was then determined by reference to a standard curve prepared with glucose. The blanks were prepared by substituting sample solution for distilled water.

2.9. Soluble protein determination

Soluble proteins were determined with a TCA concentration – BCA assay protocol kit for protein determination (Sigma – Missouri, USA).

2.10. Free amino acids determination

The quantitative measurement of free amino acids was made using the ninhydrin reaction (Plummer, 1978). 2×10^{-3} L of buffered ninhydrin reagent (0.8 g of ninhydrin and 0.12 g of hydrindantin dissolved in 3×10^{-2} L of 2-methoxyethanol plus 1×10^{-2} L of acetate buffer 4 M, pH 5.5) were added to 2×10^{-3} L of sample and heated in a boiling water bath for 15 min. The mixture was cooled to room temperature (*ca.* 25 °C), 3×10^{-3} L of 50% ethanol was added and the absorbance was read at 570 nm after 10 minutes (Shimadzu UV-160A spectrophotometer). The amount of amino acids was determined by reference to

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