USE OF *LACTOBACILLUS PLANTARUM A6,* AN AMYLOLYTIC LACTIC ACID BACTERIUM, FOR PARTIAL STARCH HYDROLYSIS IN A PEARL MILLET-GROUNDNUT SLURRY

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INTRODUCTION

Bensaalga is a traditional fermented gruel prepared from pearl millet (*Pennisetum glaucum*) and regularly consumed for breakfast in Burkina Faso. It has been shown that it is consumed as complementary food to breast feeding by 75% of children less than 5 years old (Guyot et al., 2003). This gruel is produced by women at household or in small production units according to five main steps: soaking, milling, sieving, settling and cooking. Like other fermented traditional cereal gruels in developing countries, *bensaalga* is of poor nutritional value. Due to its low dry matter content, and therefore a low starch content, it has a very low energy density of about 30 Kcal/100 g of gruel (Tou et al, 2006), lower than 84 Kcal/100 g of gruel, the minimum value required for complementary food (Dewey and Brown, 2003). The use of different methods such as co-fermentation of cereals and grain legumes, extrusion cooking, enzymatic starch hydrolysis after gelatinisation by adding α-amylase source (e.g. malt, commercial enzymes) are ways for improving the nutritional quality of traditional cereal-based gruels. In the case of *bensaalga*, a process combination which favours partial starch hydrolysis has been investigated to improve the energy density of this gruel. It includes a pre-cooking step before the fermentation step, the addition of malt flour and back slopping inoculation (Tou et al, 2007). However, the nutritional and enzymatic characteristics of malted flour show variability mainly due to the nature and origin of the raw seeds or to technological variants used during their preparation. Indeed the industrial malt is expensive and the problems of microbial quality of traditional malt can lead to increased risk of infection. To investigate an alternative to the use of industrial or traditional malt, this work aims to explore the starch hydrolysis capacity of *Lactobacillus plantarum* A6, a selected amylolytic lactic acid bacterium, on a pre-gelatinized slurry using a millet-groundnut blend as the raw material in order to produce a fermented gruel with a higher dry matter content than the traditional gruel.

MATERIALS AND METHODS

Raw material

A local variety of pearl millet (*Pennisetum glaucum*) called Gampela was purchased from a farmer. Groundnuts (*Arachis hypogea*) were purchased at local market in Ouagadougou, Burkina Faso. The raw materials were cleaned to eliminate the various impurities before being used.

Preparation of cultures starters

The amylolytic strain *L. plantarum* A6 (LMG 18053, BCCM, Gent, Belgium), isolated from retted cassava in Congo was used in this study. The culture is stored at -80°C in sterile cryo-tubes containing MRS broth with 40% (v/v) glycerol. A liquid culture of the strain was

streaked on MRS agar plate and incubated at 30°C for 48 h. A colony was picked from the culture plate and grown in MRS broth to obtain 16 h pre-cultures and then 19 to 20 h cultures. These cultures were centrifuged at 8000×g for 10 min at 4°C, afterward the cells were washed with sterile distilled water and suspended in sterile distilled water. This procedure allowed the preparation of a culture containing 10^9colony-forming units (cfu)/ml, checked as viable on MRS agar.

Preparation of modified *ben-saalga*

A modified *ben-saalga* composed of pearl millet-groundnut blend was prepared according to the method described by Tou and al (2007). Pearl millet and groundnut are blended in the proportions of 76 and 24 % (DM) in order to obtain 250g (dry weight -d.w.-). The blend was first soaked in water (1:1.5 w/w) for 16 h, washed with water (1:2 w/w), and drained for 30 min. It is then milled for 3 min using a laboratory blender (Warring) sieved with water (1:3.5 w/w) through a 0.710mm sieve and boiled for 10 min, then cooled around 35°C before inoculation with a starter culture of lactic acid bacteria (1-2%, v/w) or by back slopping (3%, w/w), a small amount of fermented paste from a previous successful fermentation used as inoculum. A negative control was made by natural fermentation without precooking like in the traditional process. After 24 h fermentation, the porridge was prepared by diluting fermented paste in water and boiling for 5 min. The experiments were done in triplicate for each condition of fermentation.

pH

Changes in pH in the paste during fermentation were recorded on-line using a pH-meter register (WTW 340 i) Fisher Bioblock Scientific, Illkirch, France). Recorded data were thereafter transferred into an Excel file.

Determination of dry matter content

Dry matter content of samples was determined by standard AOAC methods (AOAC, 1990).

Fermentable sugars and fermentation products

Malto-dextrins were analyzed by high-performance ion chromatography (HPIC) using an anion exchange chromatograph (Dionex S.A., Voisins-Le-Bretonneux, France) with a carbopac PA1 column. The following conditions were used: mobile phase, H2O MQ, NaOH (150 mM), sodium acetate gradient of 0 to 300 mM; flow rate, 0.1 $ml.min^{-1;}$ and temperature, 34°C. For the analyses, samples (2 g) of paste collected at 0, 2, 4, 6, 8, 22, 24 h were homogenized with 8 g of H_2O. 0.2 ml of 2N H_2SO_4 was added to each 1.3 ml sub-sample in a microtube, the microtubes were centrifuged for 10 min at 8000g, and the supernatants were frozen at – 20°C until they were analyzed as described below. Before analyses, supernatants were filtered through 0.20 µm pore size filters, diluted and 25 µl were then injected onto the HPIC system.

Rheological measurements

A *Bostwick* consistometer and Haake VT 500 model viscometer were used for the characterization of gruel consistency at a constant temperature of 45°C over a period of 30 s and 10 min respectively.

RESULTS AND DISCUSION

During the fermentation step, kinetics of pH and starch hydrolysis products was determined. For the three different conditions of fermentation studied (i.e. natural fermentation, back-slopping inoculation, inoculation with strain A6), pH values varied from 6.52 ± 0.05 to 6.64 ±

0.11 at the beginning of the fermentation with an average of 6.58. During the fermentation process, pH decrease to reach a final value below 4.5 between 4.19 ± 0.24 and 3.94 ± 0.07, at which the inhibition of food borne pathogens would be expected. The similar values were observed in traditional processing of *bensaalga* (Tou et al, 2006). This decrease in pH is due to organics acids production such as lactic, acetic acids which are the result of metabolic activities. The maximum acidification rate of 0.42 U/pH was reached after 7 h for the control. In the case of inoculation of the precooked slurry by back-slopping or with *L. plantarum A6,* the maximum acidification rates of 0.45 and 0.46 U/pH were reached quickly after 3 h and 4 h fermentation, respectively. These results show the ability of *L. plantarum* A6 to reduce rapidly the pH as fast as back-slopping, a mixture of many microorganisms.

The major products analysed which result from starch hydrolysis were maltodextrins (maltotriose to maltoheptaose). As shown in figure 1, malto-dextrines concentrations remained quite constant or increase according to the fermentation condition used. With the control, no changes in the concentration in maltodextrins during all fermentation were observed. That could be explained by the fact that amylase activity of amylolytic lactic acid microflora was very weak or had no effect on the native starch of the uncooked flour. However, in the modified process including a pre-cooking step and inoculation, there was an accumulation of maltodextrins during the fermentation. These results indicate starch hydrolysis and show that the amylolysis of the gelatinized starch was much more efficient than that of the native starch. The gelatinisation is a common pre-treatment which could be implemented to enhance the action of amylases (Nguyen et al, 2006). The concentration of maltodextrins was very different in the end of the fermentation for the two type inoculums used. The highest maltodextrins concentration was observed with *L. plantarum* A6 strain. The maltotriose was the main product of starch hydrolysis with a final content of 2 mmol/kg of slurry at the end of fermentation. However, when the back-slopping method was used, maltoheptaose was the main product of starch hydrolysis with a final content of 0.53 mmol/kg of slurry.

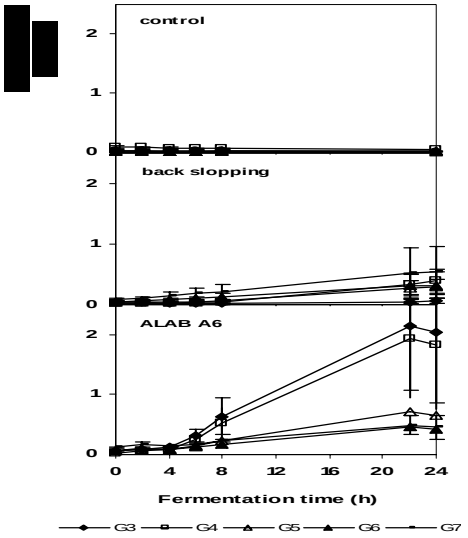

Figure 1. Evolution of the maltodextrins during the fermentation of the pearl-millet /groundnut slurry

The rheological properties of the gruels obtained from fermented slurries were assessed by the measurement of the apparent viscosity and the bostwick flow at different dry matter contents (Figure 2). At a Bostwick flow of 120 mm/30s corresponding to a consistency suitable for young children consumption, the corresponding dry matter content of the gruel from the

control experiment was 8 g DM/100 g, lower than the values obtained, 15 and 14 g DM/100g, for the gruels obtained by inoculation with *L. plantarum* A6 or by back-slopping, respectively. Similarly, compared to the control experiment, the viscosity of the gruels decreased at higher dry matter content when the slurry was inoculated with strain A6 or by back slopping, with the higher effect observed with strain A6.

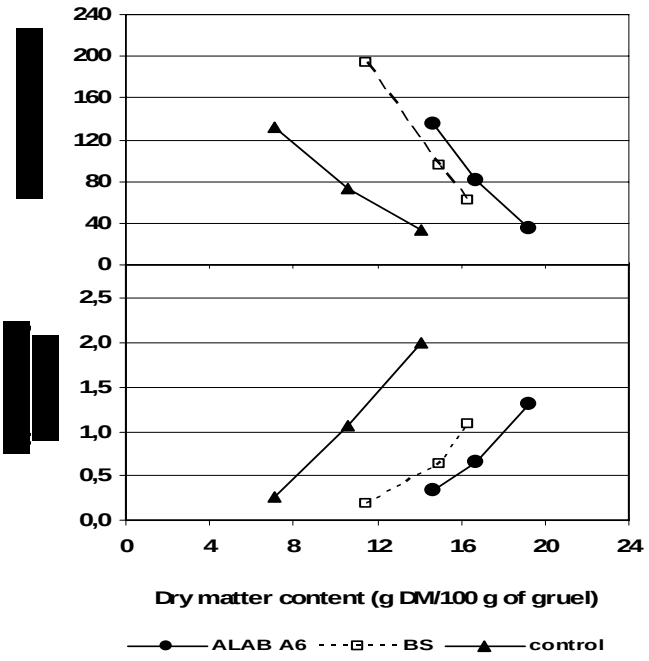

Figure 2. Evaluation of fermented gruel consistency

CONCLUSION

These works showed that the additional step of pre-cooking favoured the partial starch hydrolysis of the slurry by *L. plantarum* A6 or by the native microflora brought by back slopping. Starch hydrolysis enabled to increase the starch content up to 15 g DM/100g of gruel whereas the gruel remained at a suitable consistency for young children consumption. Hydrolysis conditions should be improved in order to be able to reach higher values of dry matter content (i.e; around 20g DM/100g of gruel). Notwithstanding, the amylolytic strain A6 showed a promising potential for being used as starter culture for such a purpose.

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