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Effect of fermentation by amylolytic lactic acid bacteria, in process combinations, on characteristics of rice/soybean slurries: A new method for preparing high energy density complementary foods for young children

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Abstract

The use of amylolytic lactic acid bacteria (ALAB) in new methods to prepare high energy density complementary foods for young children was investigated. Using gelatinised slurry, composed of a mixture of rice with soybean, fermentation kinetics by the ALAB *Lactobacillus plantarum* A6, and chemical and rheological changes were studied at different dry matter (DM) contents. At high DM content (23%), it was possible to obtain a semi-liquid fermented gruel presenting the necessary characteristics to fulfil the energy requirement of young children. Furthermore, combination with spray-drying, as a post-treatment after fermentation by strain A6, had the additional advantage of enabling the use of spray-dried fermented flour to prepare gruels with higher DM content (30–33%) corresponding to 128–140 kcal/100 g of gruel.

Keywords: Gruel; Rice; Soybean; Energy density; Amylolytic lactic acid bacteria

1. Introduction

In many developing countries, cereal-based gruels are used as complementary foods for young children. To obtain gruels with a semi-liquid consistency after cooking, suitable for feeding young children, the starchy flour is traditionally diluted with a large quantity of water. At a low flour content (5–10 g dry matter/100 ml), the gruel has a free-flowing consistency and is easy to swallow but its energy density (20–40 kcal/100 g) is lower than the minimum value of 84 kcal/100 g of gruel recommended for children aged 9–11 months fed at a rate of 2 meals/day in addition to average breast milk intake (Dewey & Brown, 2003). At higher dry matter content, the gruel is stiff, due to starch gelatinisation and is thus unsuitable for feeding young children.

Several methods based on partial starch hydrolysis are used to prepare high energy density (HED) gruels with suitable consistency at high dry matter content. They generally include hydro-thermic treatments, such as drum-drying or extrusion cooking (Mouquet, Salvignol, Van Hoan, Monvois, & Trèche, 2003), or enzymatic starch hydrolysis which can be performed after gelatinisation by adding either germinated cereal flour (power flour), combined or not, with lactic acid fermentation (de Benoist, 1999; Lorri & Svanberg, 1993) or industrial amylases (Trèche, 1995). A limited

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decrease in the viscosity of cereal gruels has also been observed after natural lactic acid fermentation of amylaceous raw material but additional treatments are needed to prepare HED gruels (Lorri & Svanberg, 1993). Amylolytic lactic acid bacteria (ALAB) have repeatedly been isolated from traditional cereal- or cassava-based fermented foods (Johansson, Sanni, Lonner, & Molin, 1995; Morlon-Guyot, Guyot, Pot, Jacobe de Haut, & Raimbault, 1998; Nwankwo, Anadu, & Usoro, 1989; Olympia, Fukuda, Ono, Kaneko, & Takano, 1995; Sanni, Morlon-Guyot, & Guyot, 2001). Due to the ability of their α-amylases to partially hydrolyse raw starch (Rodriguez-Sanoja et al., 2000), ALAB can ferment different types of amylaceous raw material, such as corn (Nakamura, 1981), potato (Chatterjee, Chakrabarty, Chattopadhyay, & Mandal, 1997), or cassava (Giraud, Champailler, & Raimbault, 1994). However, under natural fermentation conditions, with limited hydrolysis of raw starch granules, ALAB are not efficient enough to enable the preparation of HED gruels. Nevertheless, the use of selected ALAB as starter cultures could facilitate the development of new biotechnological methods for the preparation of HED-fermented gruels, provided the starchy fraction of the raw material receives appropriate pre-treatment. In addition to starch hydrolysis, an additional benefit of lactic acid fermentation would be improving the nutritional and sanitary quality of the food (Holzapfel, 2002; Motarjemi, 2002), in contexts where the nutritional and health status of the consumers (e.g., young children) and local production conditions may be unsatisfactory, which is often the case in many developing countries.

In Asia, given the availability of rice and soybean, as well as the traditional consumption of fermented foods, it would be of interest to investigate the ability of ALAB to ferment rice–soybean mixtures prepared at high dry matter content to obtain new functional foods, such as HED-fermented complementary foods. For this reason, the present work investigates the effect of combining gelatinisation and lactic acid fermentation by ALAB or non-amylolytic LAB on the characteristics of rice/soybean slurry as a basis for developing a new (bio-) technological process to produce HED-fermented gruels. *Lactobacillus plantarum* A6, a strain isolated from cassava retting in Congo (Giraud, Brauman, Keleke, Lelong, & Raimbault, 1991), was selected because of its ability to efficiently hydrolyse starch and also because it is a well-documented strain for which molecular and physiological studies are available (Florencio et al., 2000; Giraud et al., 1994; Giraud & Cuny, 1997). For the sake of comparison, a non-amylolytic strain of *Lb. plantarum* was used as negative control.

2. Materials and methods

2.1. Preparation of raw material

Rice (*Oryza sativa* L.) and soybean (*Glycine max* (L.) Merill) were purchased at a market in Hanoi, Vietnam.

Rice was dehulled and milled in a hammer mill with a 150 μm pore mesh.

Soybeans were processed into fullfat soy flour. The beans were first soaked for 15 min and then sterilised at 121 °C for 15 min after the water had been drained from the beans (Kratzer, Bersch, Vohra, & Ernst, 1990). They were dried in a hot-air oven at 65 °C to a final moisture content of about 8%. The dried beans were dehulled and then milled with the same hammer mill.

The flour was stored at 4 °C.

A blend of 80% rice flour and 20% fullfat soy flour was used throughout this study. This formulation, which corresponds to the energy and macronutrient requirements of young children, was developed using the Alicom non-commercial software created by IRD.

2.2. Experimental design

Gelatinised/homogenised blends, prepared at 12%, 16%, 18%, 20% and 22% DM, were fermented (Fig. 1). Particular attention was paid to the fermented slurries obtained from blends with 12% DM (low energy density) and 22% DM (high energy density). The blend with 22% DM was used to prepare HED fermented gruels without any further treatment after fermentation (Fig. 1). The blend with 12% DM enabled investigation of an alternative pathway, consisting in preparing fermented flours from the fermented slurry, either by spray-drying or freeze-drying; these "pre-treated" flours were subsequently used to prepare HED gruels by cooking in water (Fig. 1). The characteristics of HED gruels prepared either directly or through the use of fermented flours were compared.

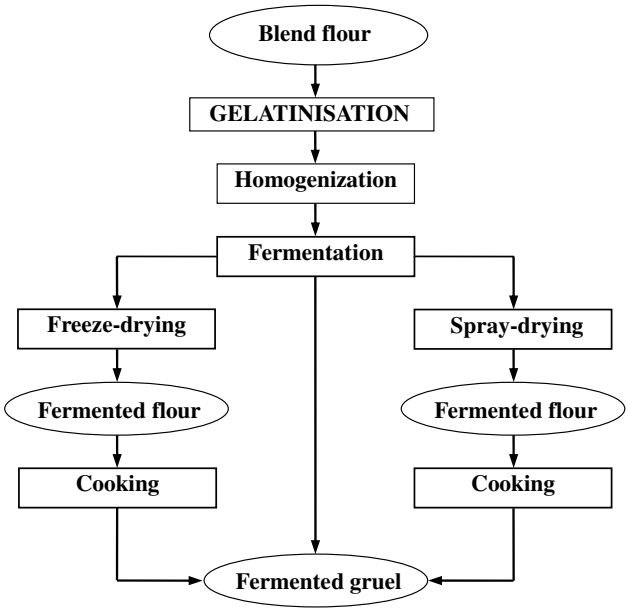

Fig. 1. Experimental design showing the different process combinations tested to produce fermented gruels or flour from a rice/soybean slurry.

2.3. Pre-treatment of the rice–soybean blend

2.3.1. Gelatinisation

450 ml of the slurry was heated until it started to bubble (~94 °C); gentle boiling was maintained for 15 min under stirring.

After gelatinisation, the gruel was cooled to 30 °C, bacterial cells (either *Lb. plantarum* A6 or *Lb. plantarum* Lacto Labo) were inoculated, then the preparation was homogenised with a Braun 600W mixer (MR 5550 CA) at maximum speed for 1 min.

2.4. Microorganisms and cultivation methods

The amylolytic strain, *Lb. plantarum* A6 (LMG 18053, BCCM, Gent, Belgium), was isolated during the process of cassava retting in Congo (Giraud et al., 1991). *Lb. plantarum* Lacto Labo (France) is a non-amylolytic strain used in a previous study (Giraud, Lelong, & Raimbault, 1991). Both strains are maintained in the stock culture collection of the laboratory and kept in 40% glycerol at −80 °C. The cells are routinely propagated by 10% (v/v) inoculation in sterile MRS broth (de Man, Rogosa, & Sharpe, 1960) and incubated at 30 °C overnight. For inoculation in the experiments made with the rice/soybean slurry, the absorbance at 600 nm wavelength (A_{600}) of the overnight culture was measured. Calibration curves (A_{600} vs. cfu/ml) enabled measurement of the cell concentration of the inoculum from the absorbance values; initial concentrations are presented in figures. After determining A_{600} of the inoculum, the cells were harvested by centrifugation at 14,000*g* for 10 min. Cell pellets were washed once in physiological sterile solution (0.9%, w/v saline) and then centrifuged once more before being suspended in the physiological sterile solution and used for inoculation.

A_{600} was measured with a Spectronic 401 spectrophotometer (Milton Roy, Paris, France). Before measurement, cell cultures were appropriately diluted in sterile medium to an A_{600} of less than 0.4 for appropriate readings in the linear range of the relationship (A_{600} vs. cell concentration).

Microbial growth during fermentation was measured by plate-count on MRS agar after serial decimal dilutions from an initial suspension containing 10 g of sample homogenised in 90 ml of physiological sterile solution. Incubation was at 30 °C for 24 h.

2.5. Fermentation conditions

Fermentation of the gelatinised slurries was performed in batches using 200 ml sterile fermentors filled to 170 ml. Temperature was maintained constant at 30 °C. pH was continuously recorded on-line, using steam sterilizable pH combination electrodes (Broadley James Corporation, California) connected to a TR20A pH transmitter (Demca, France). The rate of pH decrease was calculated as the first derivative of $pH = f(\text{time})$ allowing the maximum rate $(-\mathrm{dpH}/\mathrm{d}t)_{max}$ to be determined graphically.

Inoculation was performed with washed cells and the initial cell concentration was estimated as described above.

Samples were regularly taken from the fermentor for microbiological, chemical and physical analysis. All experiments were performed in triplicate.

2.6. Analytical methods

2.6.1. Proximate analysis of raw material and gruels

Crude protein content was determined according to the NF V03-050 (1975) standard method (nitrogen content determination by the Kjeldahl method) with a conversion factor of 6.25.

Crude lipid content was determined by HT6 Soxtec system (Tecator, Höganäs, Sweden), following the instructions in Tecator No. 3144.

Ash content was determined according to the NF 03-720 (1971) standard method.

Acid detergent fibre content (ADF), (cellulose and lignin), was determined according to the gravimetric method of Van Soest (1963) using a Dosi-fiber (Selecta, Barcelona, Spain).

Total starch content was estimated by measuring the glucose concentration using a colorimetric method (560 nm) after enzymatic degradation of starch with α-amylase (EC 3.2.1.1) (Termamyl 120L, Novozymes, Bagsvaerd, Denmark), followed by amyloglucosidase (EC 2328722) (Fluka 10115; Rankonkoma, NY, USA) according to Batey (1982) and Holmes et al. (1986) and using a conversion factor of 0.9. The results obtained also included mono- and disaccharides which were disregarded because they were only present in small quantities in raw cereal grains.

Damaged starch was determined by a method based on the evaluation of susceptibility to amyloglucosidase hydrolysis (Chiang & Johnson, 1977; Kainuma, Matsunaga, Itagawa, & Kobayashi, 1981). The damage rate is the ratio of the starch fraction susceptible to amyloglucosidase hydrolysis to total starch.

Analyses of crude protein, crude lipid and ADF fibres were made in triplicate. Analyses of total starch and damaged starch were made in duplicate.

The dry matter content of gruels was determined by oven-drying at 105 °C to constant weight.

Data were assessed by analysis of variance (ANOVA).

2.6.2. Chemical analysis

2 g of gruel sample was added to 8 ml of sterile water and homogenised with an Ultra Turrax-T8 (IKA, Staufen, Germany) at 10,000 tpm for 1 min. 1.3 ml of this suspension was mixed with either 0.2 ml of 2 N H_2SO_4 in microtubes (for lactic acid analysis) or 0.2 ml of 5 N NaOH (for sugar analysis) and centrifuged at 10,000*g* for 10 min. The supernatant was frozen at −20 °C until analysis.

Lactic and acetic acid concentrations were determined by HPLC using an Aminex HPX-87H column (Biorad, Yvry-sur-Seine, France) and sugars were analysed by

HPIC using a Dionex Carbopac PA1 column as previously described (Calderon, Loiseau, & Guyot, 2001).

2.6.3. Rheological properties

Apparent viscosity measurements were performed on gruels at 30 ± 0.5 °C with a Haake viscometer VT550 with SV-DIN coaxial cylinders driven by a PC computer with the Rheowin 2.67 software. The shear rate and the shear time were 83 s^{-1} and 10 min, respectively (Mouquet & Trèche, 2001).

The consistency of the gruels was assessed by measurement in a Bostwick consistometer (CSC Scientific Company Inc., Fairfax, Virginia, USA) (Bookwalter, Peplinski, & Pfeifer, 1968). Measurements were made at 30 °C and the Bostwick flow was expressed in mm/30 s.

2.6.4. Energy density

The energy values were calculated with 16.7 kJ/g (4 kcal/g) for protein, 37.4 kJ/g (9 kcal/g) for fat and 16.7 kJ/g (4 kcal/g) for carbohydrates (Atwater & Benedict, 1902).

3. Results

3.1. Fermentation kinetics of gelatinised rice/soybean slurries prepared with different dry matter contents

*3.1.1. Growth of the amylolytic and non-amylolytic **Lb. plantarum** strains in gelatinised rice/soybean slurries*

The ability of the amylolytic and non amylolytic *Lb. plantarum* strains A6 and Lacto Labo, respectively, to grow on gelatinised mixtures of rice and soybean flours was investigated by inoculating the slurries at low and high DM content (12.7% and 23.4% DM).

As shown in Fig. 2, the slurries were able to support a limited growth of the two strains which reached a maximum population concentration of 8.7–8.9 log cfu/g in less than 6 h. It was difficult to distinguish a clear single logarithmic phase during growth, and consequently the specific growth rate was not calculated.

3.1.2. Kinetics of pH decrease, organic acid formation and sugar changes during fermentation

pH decrease is an important parameter for assessing how fast the process will reach conditions (pH lower than 4.5) which can hinder or inhibit the growth of food-borne pathogens. pH was recorded during fermentation by the strain A6 of slurries prepared at DM contents ranging from 12.7% to 23.4%. For the strain Lacto Labo used as negative control, the pH decrease was only recorded during fermentation of the slurries prepared at 12.7% (low DM content) and 23.4% DM (high DM content). The kinetics of organic acid formation were investigated at low and high DM contents for both strains.

In all assays, pH decrease was observed without any lag from the beginning of fermentation. With the strain A6, pH dropped from an initial value of 6.40 to final values

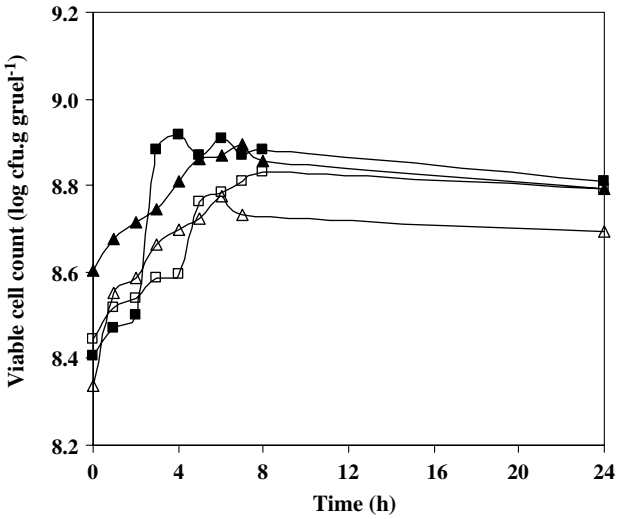

Fig. 2. Growth of strains *Lb. plantarum* A6 and Lacto Labo in gelatinised rice/soybean slurries. Symbols: (△) 12.7% (A6); (□) 12.7% (Lacto Labo); (▲) 23.4% (A6) and (■) 23.4% (Lacto Labo).

of 3.79 and 4.01 at 12.7% and 23.4% DM contents, respectively, whereas final pH was higher with the Lacto Labo strain (Fig. 3(a)). As for the maximum rate of pH decrease $(-\mathrm{dpH/d}t)_{max}$, whereas no major variations were observed at the different DM contents (Fig. 3(b)), the time to reach the maximum rate of pH decrease varied from 1 to 2.5 h (Fig. 3(c)). Shorter times were obtained at higher DM contents (between 18.9% and 23.4%) for both strains. The time required to reach a pH of 4.5, below which inhibition of food-borne pathogens could be expected, was 8–11 h for the A6 strain at different DM contents. For the Lacto Labo strain, a longer time (12 h) was observed at 23.4% DM content (Fig. 3(d)).

For both strains, lactic and acetic acids were the only end-products detected during fermentation (Fig. 4); acetic acid being produced in small amounts, as expected, from the metabolic characteristics of the *Lb. plantarum* strains, variations in pH were consequently mainly due to lactic acid, which is a stronger organic acid than is acetic acid. A higher rate of metabolite production was obtained at high DM contents than at low DM contents. At high DM contents, lactic acid was produced at higher concentrations with the amylolytic strain A6 than with the non-amylolytic strain Lacto Labo. At low DM content, lactic acid production started to level off early with the strain Lacto Labo whereas it continued to increase strongly with the strain A6 (Fig. 4), suggesting the involvement of an additional carbon source. Continued production of acetic acid, at an extremely low concentration by strain Lacto Labo at the same time as a progressive levelling off of lactic acid production, is consistent with the ability of *Lb. plantarum* species to convert some lactic acid into acetic acid (Pintado, Raimbault, & Guyot, 2005).

Concerning sugar changes during fermentation (Fig. 5) at low DM content, acid formation by the strain A6 cannot

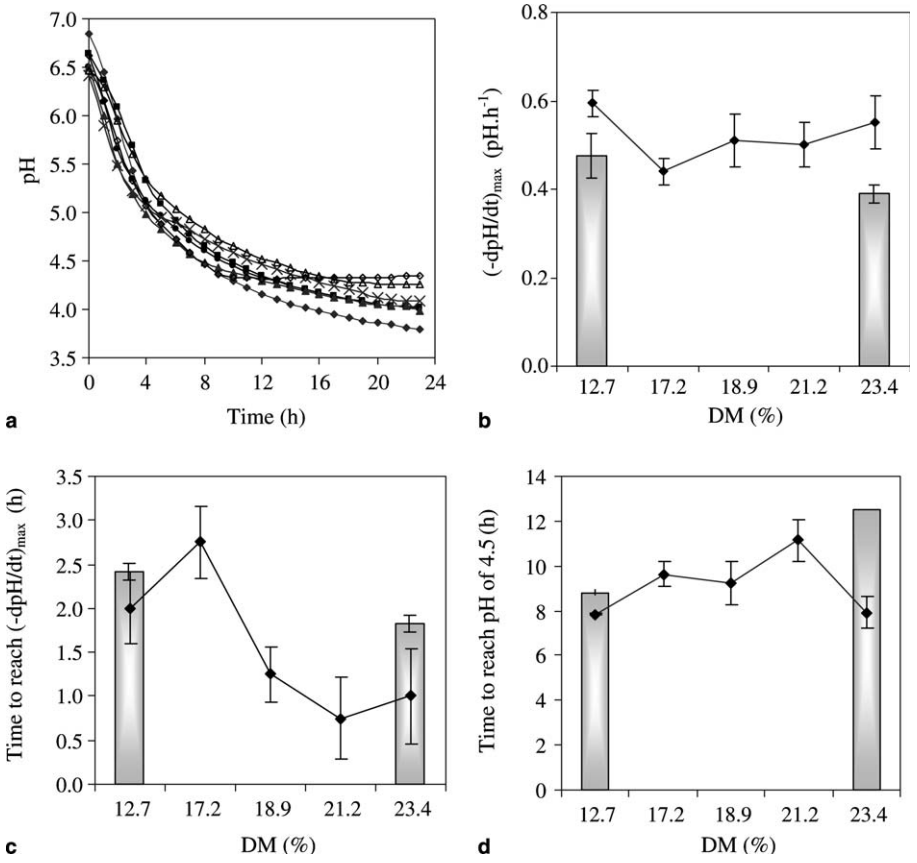

Fig. 3. Changes in pH during fermentation by strains *Lb. plantarum* A6 and Lacto Labo at different dry matter content. (a) Kinetics of pH decrease during fermentation of the slurries. Symbols: (◆) 12.7% (A6); (■) 17.2% (A6); (●) 18.9% (A6); (×) 21.2% (A6); (▲) 23.4% (A6); (◇) 12.7% (Lacto Labo) and (△) 23.4% (Lacto Labo). (b) Maximum rate of pH decrease. (c) Time to reach $(-\mathrm{dpH/d}t)_{max}$. (d) Time to reach pH 4.5. Symbols: (◆) A6; grey bars Lacto Labo.

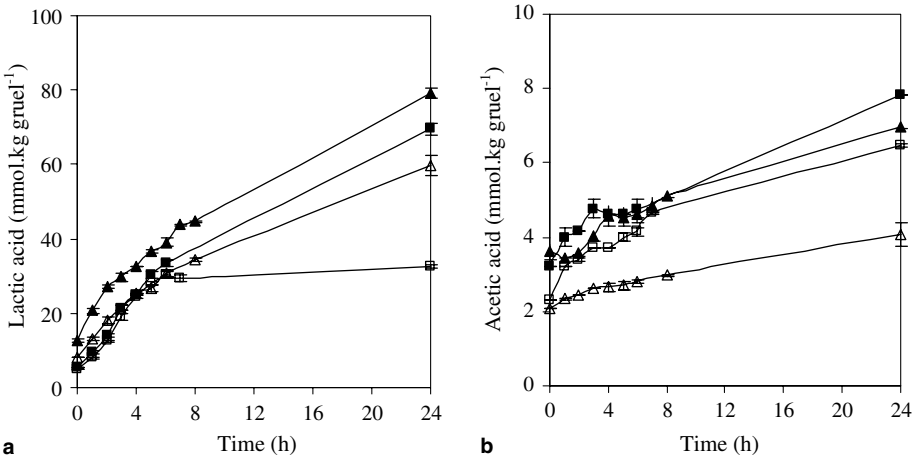

Fig. 4. Organic acid production during fermentation by strains *Lb. plantarum* A6 and Lacto Labo at low (12.7%) and high (23.4%) dry matter contents. (a) Lactic acid; (b) Acetic acid. Symbols: (△) 12.7% (A6); (□) 12.7% (Lacto Labo); (▲) 23.4% (A6); and (■) 23.4% (Lacto labo).

be explained by the consumption of mono- and disaccharides since sucrose was not consumed and glucose concentration increased, strongly suggesting that starch is the extra-carbon source responsible for lactic acid production. In the strain Lacto Labo, sucrose was the only sugar which decreased. In contrast, at high DM content, while the strain Lacto Labo still presented the same pattern of substrate consumption, sucrose was fermented by strain A6 and maltose and glucose were produced at low concentrations. As for the α-galactosides (raffinose, stachyose and verbascose), neither strain was able to ferment any of these antinutritional factors (Fig. 5).

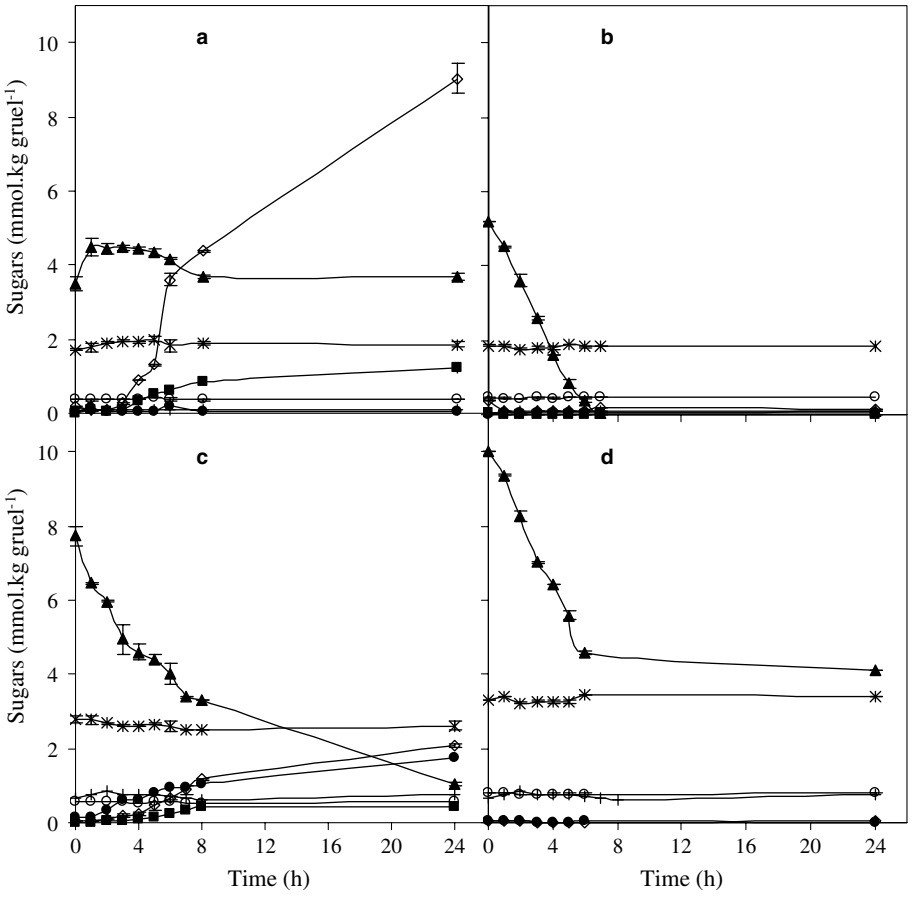

Fig. 5. Sugar changes during fermentation of slurries by strains *Lb. plantarum* A6 and Lacto Labo at low (12.7%) and high (23.4%) dry matter content (a) 12.7% (A6); (b) 12.7% (Lacto Labo); (c) 23.4% (A6); (d) 23.4% (Lacto Labo). Symbols: (◇) glucose; (■) fructose; (▲) sucrose; (●) maltose; (○) raffinose; (✳) stachyose and (+): verbascose.

In addition, as shown in Table 1, no major change in the global composition of the gruel was observed due to the fermentation, suggesting that at least the gross estimate of the nutritional value of gruels, based on the initial blend composition, is not affected by the fermentation process. The initial rate of damaged starch in the blend flour was 12%. These rates in gelatinised gruels before fermentation were 83 and 81% at 12.7 and 23.4% DM, respectively, and did not vary to a great extent in either strain after fermentation.

3.1.3. Rheological changes due to fermentation

Apparent viscosity and Bostwick flow were determined after gelatinisation of the slurry just before inoculation, and thereafter at time 0 just after the inoculation and at homogenisation steps and during fermentation.

After gelatinisation, when homogenisation was not performed, viscosity could not be measured in the mixtures prepared at different DM contents because of their solid consistency. Measurements were made possible once the gelatinised mixtures were homogenised after inoculation and viscosities at this stage were between 2 and 5 Pa s (Fig. 6) depending on the %DM of the blend. During fermentation by strain A6 of the gruels with different DM contents, viscosity decreased gradually and after 24 h of fermentation, values of 0.05 and 0.5–0.6 Pa s were obtained for the gruels prepared with 12.7% and 17.2–23.4% DM, respectively. At the end of fermentation, the Bostwick flow

Table 1
Chemical analysis of the rice–soybean flour and fermented gruels (% DM ± SD[a])

	Crude proteins (%)	Crude lipids (%)	Ash (%)	Fibre (%)	Damaged starch rate (%)
Rice/soybean flour	15.7 ± 0.45	5.54 ± 0.08	1.29 ± 0.03	1.87 ± 0.03	25.0
Fermented gruel at 12.7% DM-by A6	14.6 ± 0.07	5.05 ± 0.03	1.54 ± 0.02	1.47 ± 0.00	85.5
Fermented gruel at 12.7% DM-by Lacto Labo	14.5 ± 0.09	4.63 ± 0.05	1.48 ± 0.01	1.73 ± 0.07	84
Fermented gruel at 23.4% DM by A6	13.1 ± 0.26	4.78 ± 0.16	1.47 ± 0.03	1.91 ± 0.11	82
Fermented gruel at 23.4% DM by Lacto Labo	16.2 ± 0.14	5.01 ± 0.03	1.42 ± 0.00	2.20 ± 0.14	81.5

[a] SD: standard deviation.

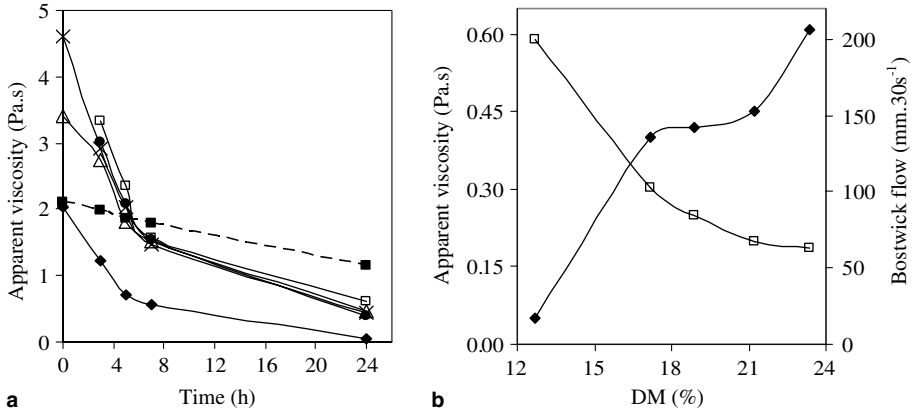

Fig. 6. Effect of fermentation by strains *Lb. plantarum* A6 and Lacto Labo on the viscosity and Bostwick flow of the rice/soybean slurry. (a) Kinetics of viscosity changes during fermentation of the gruel. Symbols: (◇) 12.7% (A6); (△) 17.2% (A6); (×) 18.9 % (A6); (●) 21.2 (A6); (□) 23.4% (A6) and (■) 12.7% (Lacto Labo). (b) Final viscosity and Bostwick flow of fermented gruels at different dry matter contents. Symbols: (◇) apparent viscosity and (□) Bostwick flow.

was above 200 mm for a few seconds in the gruel with 12.7% DM and 68 mm/30 s in the gruel with 23.4% DM (Fig. 6).

In the gelatinised blend prepared with 12.7% DM, which was fermented by the strain Lacto Labo, a lower decrease in viscosity was observed, with a final value at 24 h of 1.15 Pa s and a Bostwick flow of 26 mm/30 s. Furthermore, in contrast to the experiments performed with strain A6, it was impossible to measure the gruel with 23.4% DM fermented by strain Lacto Labo because it remained very thick.

3.2. Rheological comparison of gruels prepared from sour flours obtained by either freeze-drying or spray-drying of fermented gelatinised blends

As an alternative to direct processing of the blends into a fermented gruel with high DM content, investigations were undertaken to see if HED fermented gruels could also be prepared from flours obtained from dehydrated fermented slurries with low DM contents. Flours were prepared by either freeze-drying or spray-drying of gelatinised blends with low DM contents fermented by strain A6. Subsequently, rheological measurements were made on gruels prepared with flours with different DM contents.

Rheological characteristics of gruel prepared at different DM contents from freeze-dried flours were similar to those obtained with the gelatinised suspensions fermented by strain A6. In particular, at 12.7% DM and 23.4% DM, the viscosities of the gruels were 0.05 and 0.5 Pa s, and the Bostwick flows were more than 200 mm/30 s for a few seconds and 80 mm/30 s, respectively. After freeze-drying, the number of viable cells of strain A6 in the sour flours was 3.2×10^4 cfu/g DM.

The gruels prepared at different DM contents from fermented flours obtained by spray-drying gave very interesting results: at 32% DM, the viscosity of gruel prepared with the atomised flour was 1.02 Pa s and the Bostwick flow was 65 mm/30 s. From 12% to 24% DM, the viscosity of the resulting gruels was 0.01–0.1 Pa s, and for a few seconds, the Bostwick flow was above 200 mm. In addition to these results, it is interesting to note that spray-drying did not drastically affect the viability of strain A6, since high counts (2×10^6 cfu/g DM of sour flour) were obtained after the treatment.

4. Discussion

Poor growth of both strains on the gelatinised mixture suggests limitations in some nutrients or growth factors for LAB. It is also possible that heat-sensitive growth factors were destroyed during cooking. Furthermore, the fact that it was not possible to clearly identify a single logarithmic growth phase could be a consequence of the complex mixture of nutrients and growth factors which comprised the blend. Consequently, in such conditions, such a growth pattern is more realistic than a classical smooth "S-shape" logarithmic growth curve, since simultaneous or successive depletion of nutrients could have been responsible for the non-homogeneous growth behaviour during the fermentation process.

Preliminary experiments showed that the amylolytic strain A6 was incapable of efficient starch fermentation in the raw rice/soybean mixture (data not shown), making it necessary to gelatinise it. After gelatinisation, results at low DM contents strongly suggest that the formation of end-products by strain A6, as opposed to strain Lacto Labo used as negative control, was due to the use of starch. It has previously been reported that sucrose at a high concentration can negatively affect amylase production and consequently starch fermentation (Giraud et al., 1991). The tuning of starch fermentation by sucrose concentration is clearly illustrated here. At a low DM content, corresponding to a low sucrose concentration, starch fermentation was not affected, as indicated by glucose and maltose

production right from the onset of the fermentation, and competition between substrates facilitated the use of products of starch hydrolysis. However, at a high DM content, due to the higher availability of sucrose, in agreement with a previous report by Giraud et al. (1991), starch fermentation by strain A6 was hindered until sucrose decreased to a concentration which allowed starch fermentation, indicated by late maltose and glucose production.

Sucrose is a very common natural sugar in plants, and, in addition, other sugars, such as α-galactosides present in soybean, are antinutritional factors (ANF). Fermentation kinetics unfortunately showed that strain A6 was not able to ferment raffinose, stachyose and verbascose. Recently, LeBlanc et al. (2004) showed that complete removal of raffinose and stachyose in a commercial soymilk could be achieved by using a single culture of *Lactobacillus fermentum* strain CRL 722. Such a result, together with the results presented here, should facilitate the development of a mixed culture starter to address nutritional problems linked both to low energy density and to the α-galactoside content of the rice/soybean gruel.

In searching for ways to increase energy density by increasing DM content, direct evidence of starch hydrolysis by strain A6 was provided by changes in viscosity and in Bostwick flow, since fermentation by this strain induced a much greater decrease in viscosity than that observed when strain Lacto Labo was used. The limited decrease in viscosity during fermentation of the slurry by the non-amylolytic strain Lacto Labo is not surprising since it has been reported that a pH below 4.0 (Svanberg, 1995) decreased the viscosity of the paste in cereal starch gruels. Wanink, Van Viet, and Nout (1994), with maize–sorghum–soy porridges, showed that viscosity decreased when the pH was 5.0–5.5 but, at a lower pH, the viscosity of the porridge increased. These apparently contradictory results could be due to the composition of the different mixtures used; that is, mixing soybean with cereals might induce different rheological behaviour from that observed in fermented gruels prepared from a single cereal. Nevertheless, whatever the effect of pH on viscosity, previous results (Svanberg, 1995; Wanink et al., 1994) showed that fermentation with non-amylolytic LAB will not make it possible to prepare HED gruel with a high DM content, unless germinated cereals (power flours) are used as a source of amylase. In contrast, changes due to fermentation by the ALAB strain allowed preparation of fermented gruels with high DM contents without power flour or malt, with a calculated energy density of 98 kcal/100 g of gruel at 23% DM, which is higher than the recommended value of 84 kcal/100 g of gruel for children of 9–11 months of age at a rate of 2 meals/day, in addition to average breast milk intake (Dewey & Brown, 2003). Furthermore, the fact that flours can be prepared from the fermented gruels, could increase the number of possible ways to prepare such a functional food. In this respect, spray-drying as a post-treatment, combined with fermentation by ALAB, seems more promising than freeze-drying, since spray-drying has the additional advantage of allowing use of the resulting flour to prepare gruels with 30–33% DM content, corresponding to 128–140 kcal/100 g of gruel.

The fact that amylase and lactic acid production can be combined in a single fermentation step would not only provide a way to increase the energy density of gruels but also to improve its safety, given that lactic acid fermentation is an efficient way to inhibit food-borne pathogens. However, depending on the context, it might be preferable to prepare flours instead of ready-to use gruels, e.g. for handling, packaging and storage. Drying the fermented slurry by spray-drying would have the advantage of providing additional protection against spoilage and pathogenic microflora. Lardinois et al. (2003) successfully applied spray-drying in an African context (Senegal) to a millet-based fermented slurry to produce naturally fermented flours, thereby opening the way for the use of this technology in developing countries. Survival of LAB after this treatment would represent an additional advantage, providing the product with some resistance against microbial contamination and avenue for the development of a probiotic product in a dry form.

5. Conclusion

To our knowledge, this is the first time that ALAB have been used in a simple process combination which consists in gelatinisation, homogenisation and fermentation, making it possible to prepare HED fermented gruels. This represents a new alternative to existing biological methods, such as the addition of power flour, malt or commercial α-amylases (Mouquet et al., 2003; Onyango, Henle, Hofmann, & Bley, 2004; Trèche, 1999). This project focussed mainly on the increase in energy density of a cereal-soybean gruel; it intentionally did not tackle other nutritional problems which can be addressed by existing methods (e.g. fortification with micronutrients, or a removal of some antinutritional factors by roasting of soybean). However, experiments are now underway to investigate the biological removal of α-galactosides of soybean by means of co-cultures of ALAB with other LAB which ferment these compounds.

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References

NF 03-720. (1971). Céréales et produits de mouture, détermination des cendres. Association française de normalisation.

NF V03-050. (1975). Agricultural Food Products: General Directions for the Determination of Nitrogen by the Kjeldahl method. Association française de normalisation.

Atwater, W. O., & Benedict, F. G. (1902). Experiments on the metabolism of matter and energy in the human body, 1898–1900. United States.

Office of experiment stations. Bulletin No. 109. Government Printing Office, Washington, DC.

Batey, I. L. (1982). Starch analysis using thermostable α-amylase. *Starch, 4*, 125–128.

Bookwalter, G. N., Peplinski, A. J., & Pfeifer, V. F. (1968). Using a Bostwick consistometer to measure consistencies of processed corn meals and their CSM blends. *Cereal Science Today, 13*(11), 407–410.

Calderon, M., Loiseau, G., & Guyot, J. P. (2001). Nutritional requirements and simplified cultivation medium to study growth and energetics of a sourdough lactic acid bacterium *Lactobacillus fermentum* Ogi E1 during heterolactic fermentation of starch. *Journal of Applied Microbiology, 90*, 508–516.

Chatterjee, M., Chakrabarty, S. L., Chattopadhyay, B. D., & Mandal, R. K. (1997). Production of lactic acid by direct fermentation of starchy wastes by an amylase-producing *Lactobacillus*. *Biotechnology Letters, 19*, 873–874.

Chiang, C. J., & Johnson, J. A. (1977). Measurement of total and gelatinized starch by glucoamylase and o-toluidine reagent. *Cereal Chemistry, 54*(3), 429–435.

de Benoist, B. (1999). Complementary feeding: a challenge to both children and mother. In M. C. Dop, D. Benbouzid, S. Trèche, B. de Benoist, A. Verster, & F. Delpeuch (Eds.), *Complementary feeding of young children in Africa and the Middle East*. Geneva: World Health Organisation.

de Man, J. C., Rogosa, M., & Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology, 23*, 130–135.

Dewey, K. G., & Brown, K. H. (2003). Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *Food and Nutrition Bulletin, 24*(1), 5–28.

Florencio, J. A., Raimbault, M., Guyot, J. P., Eiras-Stofella, D. R., Soccol, C. R., & Fontana, D. (2000). *Lactobacillus plantarum* amylase acting on crude starch granules: native isoforms and activity changes after limited proteolysis. *Applied Biochemistry and Biotechnology, 84-86*, 721–730.

Giraud, E., Brauman, A., Keleke, S., Lelong, B., & Raimbault, M. (1991). Isolation and physiological study of an amylolytic strain of *Lactobacillus plantarum*. *Applied Microbiology and Biotechnology, 36*, 379–383.

Giraud, E, Lelong, B., & Raimbault, M. (1991). Influence of pH and initial lactate concentration on the growth of *Lactobacillus plantarum*. *Applied Microbiology and Biotechnology, 36*, 96–99.

Giraud, E., Champailler, A., & Raimbault, M. (1994). Degradation of raw starch by a wild amylolytic strain of *Lactobacillus plantarum*. *Applied and Environmental Microbiology, 60*, 4319–4323.

Giraud, E., & Cuny, G. (1997). Molecular characterization of the α-amylase genes of *Lactobacillus plantarum* A6 and *Lactobacillus amylovorus* reveals an unusual 3′end structure with direct tandem repeats and suggest a common evolutionary origin. *Gene, 198*, 149–158.

Holmes, J., Björck, I., Drews, A., & Asp, N. (1986). A rapid method for the analysis of starch. *Starch, 38*, 224–226.

Holzapfel, W. H. (2002). Appropriate starter culture technologies for small scale fermentation in developing countries. *International Journal of Food Microbiology, 75*, 197–212.

Johansson, M. L., Sanni, A., Lonner, C., & Molin, G. (1995). Phenotypically-based taxonomy using API 50 CH of lactobacilli from Nigerian Ogi, and the occurrence of starch fermenting strains. *International Journal of Food Microbiology, 25*, 159–168.

Kainuma, K., Matsunaga, F., Itagawa, M., & Kobayashi, S. (1981). New enzyme system β-amylase – pullulanase to determine the degree of gelatinization and retrogradation of starch or starch products. *Journal of Japanese Society on Starch Science, 28*(4), 235–240.

Kratzer, F. M., Bersch, S., Vohra, P., & Ernst, R. A. (1990). Chemical and biological evaluation of soyabean flakes autoclaved for different durations. *Animal Feed Science and Technology, 31*, 247–259.

Lardinois, M., Totté, A., Tounkara, L., Mbaye, C. T., Beye, C., Thonard, P., et al. (2003). Conservation of local products by mean of transfer of a drying technology: example of atomization in Senegal. In I. D. Brouwer, A. S. Traoré, & S. Trèche (Eds.), *Proceedings of the 2nd international workshop "food based approaches for a healthy nutrition in west africa* (pp. 615–621). Burkina Faso: University of Ouagadougou Press.

LeBlanc, J. G., Garro, M. S., Silvestroni, A., Connes, C., Piard, J. C., Sesma, F., et al. (2004). Reduction of α-galactooligosaccharides in soyamilk by *Lactobacillus fermentum* CRL 722: in vitro and in vivo evaluation of fermented soyamilk. *Journal of Applied Microbiology, 97*, 876–881.

Lorri, W., & Svanberg, U. (1993). Lactic acid-fermented cereal gruels: viscosity and flour concentration. *International Journal of Food Sciences and Nutrition, 44*, 207–213.

Morlon-Guyot, J., Guyot, J. P., Pot, B., Jacobe de Haut, I., & Raimbault, M. (1998). A new starch-hydrolyzing lactic acid bacterium isolated from cassava sour starch fermentation. *International Journal of Systematic Bacteriology, 48*, 1101–1109.

Motarjemi, Y. (2002). Impact of small scale fermentation technology on food safety in developing countries. *International Journal of Food Microbiology, 75*, 213–229.

Mouquet, C., & Trèche, S. (2001). Viscosity of gruels for infants: a comparison of measurement procedures. *International Journal of Food Sciences and Nutrition, 52*, 389–400.

Mouquet, C., Salvignol, B, Van Hoan, N., Monvois, J., & Trèche, S. (2003). Ability of a "very low-cost extruder" to produce instant infant flours at a small scale in Vietnam. *Food Chemistry, 82*, 249–255.

Nakamura, L. K. (1981). *Lactobacillus amylovorus* a new starch-hydrolyzing species from cattle waste-corn fermentations. *International Journal of Systematic Bacteriology, 31*, 56–63.

Nwankwo, D., Anadu, E., & Usoro, R. (1989). Cassava-fermenting organisms. *MIRCEN Journal, 5*, 169–179.

Olympia, M., Fukuda, H., Ono, H., Kaneko, Y., & Takano, M. (1995). Characterization of starch-hydrolysing lactic acid bacteria isolated from a fermented fish and rice food, "Burong Isda" and its amylolytic enzyme. *Journal of Fermentation and Bioengineering, 80*, 124–130.

Onyango, C., Henle, T., Hofmann, T., & Bley, T. (2004). Production of high energy density fermented *uji* using a commercial alpha-amylase or by single-screw extrusion. *Lebensmittel-Wissenschaft und-Technologie, 37*, 401–407.

Pintado, J., Raimbault, M., & Guyot, J. P. (2005). Influence of polysaccharides on oxygen dependent lactate utilization by an amylolytic *Lactobacillus plantarum* strain. *International Journal of Food Microbiology, 98*, 81–88.

Rodriguez-Sanoja, R., Morlon-Guyot, J., Jore, J., Pintado, J., Juge, J., & Guyot, J. P. (2000). Comparative characterization of complete and truncated forms of *Lactobacillus amylovorus* α-amylase and the role of the C-terminal direct repeats in raw starch binding. *Applied and Environmental Microbiology, 66*, 3350–3356.

Sanni, A., Morlon-Guyot, J., & Guyot, J. P. (2001). New efficient amylase producing strains of *Lactobacillus plantarum* and *Lactobacillus fermentum* isolated from different Nigerian traditional fermented foods. *International Journal of Food Microbiology, 72*, 53–62.

Svanberg, U. (1995). Lactic acid fermented foods for feeding infants. In K. H. Steinkraus (Ed.), *Handbook of indigenous fermented foods* (pp. 310–347). New York: Marcel Dekker.

Trèche, S. (1995). Techniques pour augmenter la densité énergétique des bouillies. In S. Trèche, B. de Benoist, D. Benbouzid, A. Verster, & F. Delpeuch (Eds.), *L'alimentation de complément du jeune enfant* (pp. 123–146). Paris: ORSTOM.

Trèche, S. (1999). Technique for increasing the energy density of gruel. In M. C. Dop, D. Benbouzid, S. Trèche, B. de Benoist, A. Verster, & F. Delpeuch (Eds.), *Complementary feeding of young children in Africa and the Middle East* (pp. 101–119). Geneva: WHO.

Van Soest, P. S. (1963). Use of detergents in the analysis of fibrous feeds II. A rapid method for the determination of fiber and lignin. *Journal of Association of Official Analytical Chemistry, 46*, 829–835.

Wanink, J. F., Van Viet, T., & Nout, M. J. R. (1994). Effect of roasting and fermentation on viscosity of cereal-legume based food formulas. *Plant Foods for Human Nutrition, 46*, 117–126.