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Effect of high pressure homogenisation on the capacity of *Lactobacillus plantarum* A6 to ferment rice/soybean slurries to prepare high energy density complementary food

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Abstract

New bioprocesses to prepare high energy density (HED) gruels for complementary young child feeding are being developed based on the ability of amylolytic lactic acid bacteria (ALAB) to modify the rheological characteristics of cereal-based slurries, provided appropriate pretreatment are applied. Gelatinisation is a common pre-treatment which could be implemented to enhance the action of amylases, and has been successfully used in a former study (Nguyen, T. T. T., Loiseau, G., Icard-Vernière, C., Rochette, I., Trèche, S., & Guyot, J.-P. (2007). Effect of fermentation by amylolytic lactic acid bacteria in process combinations on characteristics of rice/soybean slurries: a new method to prepare high energy density complementary foods for young children. *Food Chemistry, 100*, 623–631.) in combination with ALAB to prepare from a blend of rice/soybean flours semi-liquid fermented HED gruels with a high dry matter (DM) content (23–32%). In this study, it is shown that a mild pre-heating treatment which consists in suspending a rice/soybean flour blend in hot water (70 °C) combined with high pressure homogenisation (HPH) can substitute gelatinisation before fermentation by the ALAB *Lactobacillus plantarum* A6 to prepare HDE gruels after cooking of the fermented slurry. As an alternative, allowing better condition of handling and storage, spray drying can be applied to such pre-heated HPH treated fermented slurries to obtain fermented flours which can be used further to prepare HDE gruels.

Keywords: Gruel; Rice; Soybean; Energy density; High pressure homogenisation; Fermentation; Amylolytic bacteria; Spray drying

1. Introduction

In many developing countries, gruels used as complementary foods for young children are generally prepared from cereals or are mixed with legumes in water (Trèche & Mbome, 1999). The content of starch in the gruels is the main determinant of their energy density. Unfortunately, the dry matter concentration of traditional gruels prepared by mothers or at small-scale in traditional production units is not generally sufficient to provide the energy and the nutrients necessary to meet the nutritional requirements of young children (Sanni, Onilude, & Ibidapo, 1999; WHO/NUT, 1998). Gruels prepared from starch-based foods have a viscosity that increases very quickly according to their dry matter concentration. Mothers who prepare these gruels are faced with the following dilemma: to increase the concentration of flour and thus obtain gruel of very high viscosity difficult for children to swallow, or to dilute the slurry to obtain gruels of suitable consistency, but of low energy density.

Different methods can be used to prepare high energy density (HED) gruels with suitable consistency, such as extrusion cooking (Mouquet, Salvignol, Van Hoan, Mon-

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vois, & Trèche, 2003), or enzymatic starch hydrolysis, which can be performed after gelatinisation by adding either germinated flour (de Benoist, 1999; Lorri & Svanberg, 1993) or industrial amylases (Trèche, 1995). A limited 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).

In Asia, given the availability of rice and soy, as well as the traditional consumption of fermented foods, it seemed interesting to develop new bio-processes to produce HED-fermented complementary foods.

Recent studies have shown that the amylolytic lactic acid bacterium (ALAB) *Lactobacillus plantarum* A6 is incapable of efficient starch fermentation in a raw rice/soybean mixture, whereas a gelatinisation pre-treatment of the rice/soybean mixture allows the preparation of a fermented gruel with high energy density with suitable rheological properties (Nguyen et al., 2007). *L. plantarum* A6 was selected because of its ability to hydrolyse starch. It is also a well-documented strain on which molecular and physiological studies are available (Giraud, Champailler, & Raimbault, 1994; Giraud & Cuny, 1997; Florencio et al., 2000).

High pressure homogenisation (HPH) is another treatment able to modify starches. HPH has been shown to affect high molecular weight polymers causing denaturation of proteins (Messens, Van Camp, & Huyghebaert, 1997) and gelatinisation of starch (Douzals, Marechal, Coquille, & Gervais, 1996; Douzals, Perrier Cornet, Gervais, & Coquille, 1998). Rubens, Snauwaert, Heremans, and Stute (1999) proposed a two-step mechanism for pressure-induced starch gelatinisation similar to the heat gelatinisation process. In the first step, amorphous regions are hydrated causing a swelling of the granules and a distortion of crystalline regions. In the second step, the crystalline regions become more accessible to water. In other respects, pressure-induced gelatinisation and heat gelatinisation differed. During heat gelatinisation of starch, many changes take place simultaneously or successively, including granule swelling, the loss of birefringence, an increase in viscosity and fragmentation of the granule. Limited swelling of the melted granule (up to twofold in diameter) and the maintenance of the granular character (Stute, Klingler, Boguslawski, Eshtiaghi, & Knorr, 1996) are typical of pressure-gelatinised starches.

Each starch has a typical range of pressure in which gelatinisation occurs (Stute et al., 1996). Muhr and Blanshard (1982) showed that induced high pressure gelatinisation of wheat starch occurred in an excess of water at 450 MPa at ambient temperature. Bauer and Knorr (2005) showed that the rate of damaged starch increased with an increase of pressure for various starches at 5% concentration (potato, wheat, tapioca). At 57 °C, the effect of temperature was much more distinct and the pressure-induced gelatinisation took place over a far smaller pressure range.

To investigate an alternative to the gelatinisation treatment described by Nguyen et al. (2007), this work aims to explore combined effects of high pressure homogenisation and amylolytic acid fermentation by *L. plantarum* A6 on the characteristics of low energy density (12% dry matter) and high energy density (22% dry matter) rice/soybean slurries.

2. Materials and methods

2.1. Raw material preparation

Rice (*Oryza sativa* L.) and soybean (*Glycine max* (L.) Merill) were purchased in a market in Hanoi, Vietnam. Rice was dehulled and milled with a 150 μm pore mesh.

Soybeans were processed into full fat soy flour. The beans were first soaked for 15 min and then sterilized 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% full fat soy flour by weight was used throughout this study to prepare slurries at different dry matter content (DM). The proximate composition is (% DM) starch: 69.18 ± 1.38; crude protein: 14.34 ± 0.01; crude lipids: 5.43 ± 0.03; ashes: 1.27 ± 0.01; acid detergent fibre: 1.55 ± 0.18 (proximate composition was determined as described by Nguyen et al. (2007)).

This formulation, which corresponds to the energy and macronutrient requirements of young children in complement to intakes by breast feeding (Lutter & Dewey, 2003), was developed using the Alicom non-commercial software created by IRD.

2.2. Experimental design

The different process combinations investigated are summarized in Fig. 1. Before fermentation, slurries at 12 % DM (low energy density) were homogenised without previous heat treatment, whereas slurries at 22% DM (high energy density) were homogenised with or without heat treatment. For heat treatment, 5 kg of rice/soybean (80/20 w/w) blend (room temperature) was mixed with a suitable volume of water at 70 °C during 10 min. After 10 min, the resulting temperature of the mixture was 57 °C.

High pressure homogenisation (HPH) of the rice/soybean flour mixture was performed with a two stage APV homogeniser (model 15M, Baker, Evreux, France). The apparatus is commonly used for milk homogenisation. The first stage ensures the fractionation of droplets and the second stage disaggregated the droplets. Suspensions of flour blends were treated with a pressure of 25 MPa in the first step and of 5 MPa in the second step. HPH slurries were used to prepare fermented gruels by cooking after fermentation. An alternative pathway consisting in preparing fermented flours, obtained after the spray-drying of the HPH fermented slurries, was investigated. These ‘‘pre-trea-

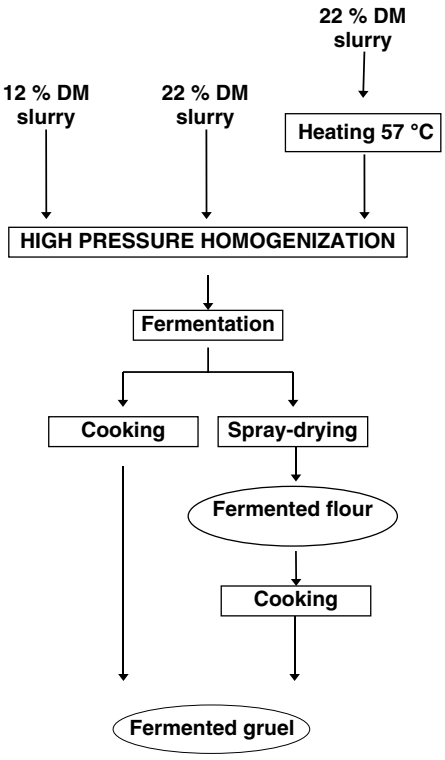

Fig. 1. Flow chart showing the different process combinations tested to produce fermented gruels from rice/soybean slurry.

ted'' flours were then used to prepare HED gruels by cooking in water (Fig. 1).

Fermented slurries were dried in an APV spray-dryer (Baker, model LAB No3, Evreux, France). A short residence time with the rapid drying of small droplets was enhanced by a high inlet temperature (160–200 °C) followed by a low outlet temperature (70–90 °C) and with the flow of 8 l h^{-1}.

2.3. Micro-organisms and cultivation methods

The amylolytic strain *L. plantarum* A6 (LMG 18053, BCCM, Gent, Belgium) was used and prepared under the same conditions as described by Nguyen et al. (2007). Strain A6 produces 64% D(−) lactic acid (Guyot, Calderon, & Morlon-Guyot, 2000), however, for the purpose of this work, it was chosen as a model strain because it has been particularly well documented and that the gene coding for its α-amylase is stable.

A600 was measured with a Spectronic 401 spectrophotometer (Milton Roy, Paris, France). Before measurements, cell cultures were appropriately diluted in sterile medium at an A600 inferior to 0.4 for appropriate readings in the linear range of the relation [A600 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.

2.4. Fermentation conditions

Fermentations were performed in batches using 10 l fermenters filled to 8 l. Temperature was maintained constant at 30 °C. The pH was recorded on-line using steam sterilisable pH combination electrodes (Broadley James Corporation, California) connected to a TR20A pH transmitter (Demca, France). Inoculation was done with washed cells, the initial cell concentration being estimated as described above.

Samples were regularly taken from the fermenter for chemical and physical analyses. All experiments were done in triplicate. Statistical analyses (ANOVA) was performed using the software STATGRAPHICS Plus 5.1.

2.5. Analytical methods

2.5.1. Chemical analysis

Total starch content was estimated by the determination of 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) according to Batey (1982), Holmes, Bjöck, Drews, and Asp (1986) and using a conversion factor of 0.9. The results obtained also included mono and disaccharides, which were disregarded as they are only present in only small quantities in raw cereal grains.

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

2.5.2. Organic acids and sugar content

Gruel sample (2 g) 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. Suspension (1.3 ml) was mixed with either 0.2 ml 2 N H_2SO_4 in microtubes (for lactic acid analysis) or 0.2 ml 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, Ivry-sur-Seine, France), and sugars by HPIC using a Dionex Carbopac PA1 column as previously described (Calderon, Loiseau, & Guyot, 2001).

2.5.3. 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 using the Rheowin v.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^{-1}.

2.5.4. Particle size analysis

Particle size distributions of the samples were measured by low angle laser light scattering (Mastersizer-S, Malvern Instruments, UK). The scattered light data from 2000 snapshots of 2 ms were recorded within one measurement and transformed to a distribution of particle-size information according to the Mie theory by the accompanying software Mastersizer-S v2.18. A sample was dispersed in ethanol and was added to the circulating liquid until an obscuration of 15–20% was recorded. The results obtained were diameters of equivalent spheres expressed in volume.

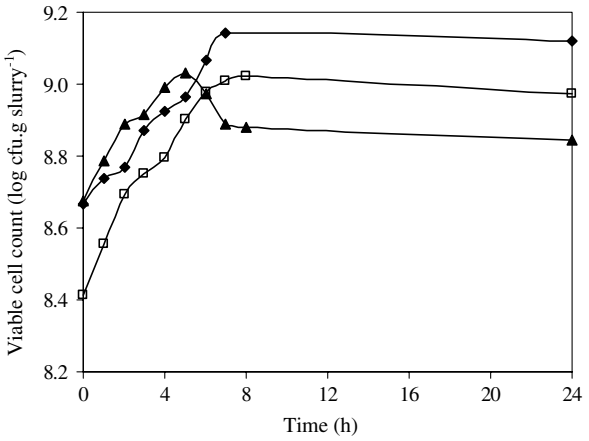

Fig. 3. Growth of *L. plantarum* A6 in rice/soybean (▲) HPH slurry 12% DM; (♦) HPH slurry 22% DM and (□) HPH slurry 22% DM heated at 57 °C.

3. Results

3.1. Effects of high pressure homogenisation (HPH) on the fermentation of rice/soybean slurries

3.1.1. Pressure homogenisation and particle size

Fig. 2 depicts the effect of HPH on the particle size distribution of slurry suspensions at 12% and 22% DM at ambient temperature, and at 22% DM at 57 °C. Measurements were also done after fermentation. The unimodal particle size distribution before HPH became bimodal after. The HPH treatment of 12% DM and 22% DM slurries at ambient temperature decreased the particle size. Before HPH, the size of 90% of particles was lower than 115 μm and after HPH lower than 61 μm and 67 μm, respectively, for the 12% DM and 22% DM slurries. No difference was observed between fermented and non-fermented HPH treated slurries (Fig. 2(A) and (B)).

Heating the slurry at 57 °C followed by the HPH treatment of 22% DM slurries modified the size distribution of particles. After HPH, the size had increased to 140 μm.

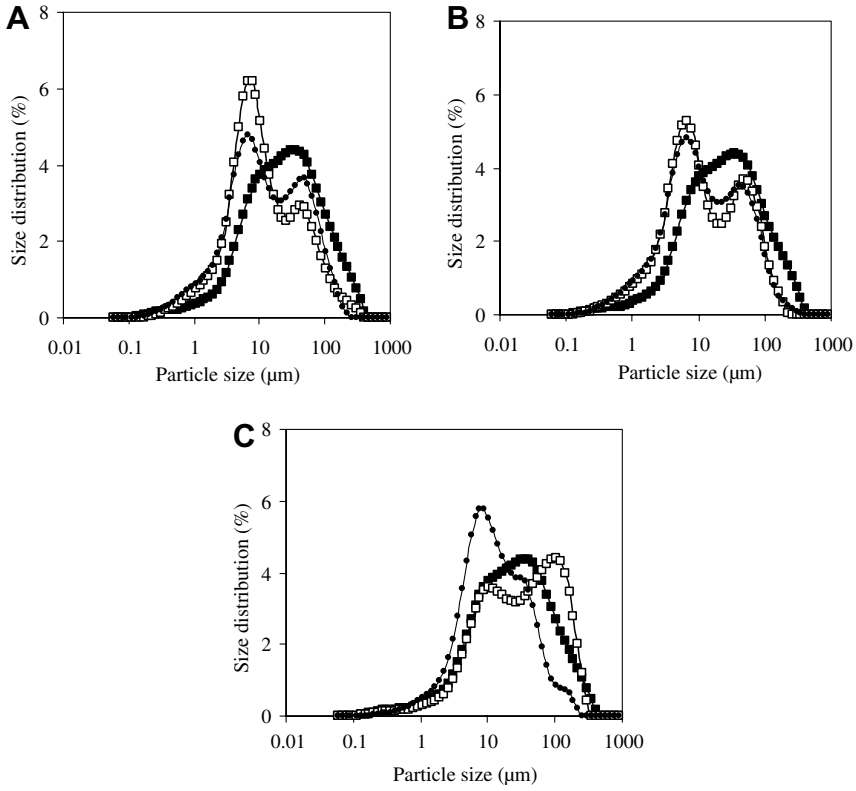

Fig. 2. Particle size distribution of slurries before HPH (■), after HPH (□) and (●) after HPH and fermentation by *L. plantarum* A6. (A) 12% DM slurry, (B) 22% DM slurry, (C) 22% DM slurry heated at 57 °C.

Fermentation drastically modified the particle size distribution of the heated 22% DM slurry and HPH treated: after 24 h of fermentation, the size of 90% of particles was smaller than 49 μm.

3.1.2. Fermentation kinetics of L. plantarum A6 on rice/soybean HPH slurries

A limited growth of the strain A6 occurred on HPH rice/soybean slurries. The maximum population concentration of 8.9–9.1 log cfu g^{-1} was reached after 5 h of fermentation for the 12% DM slurry, and 7 h for the 22% DM slurries specific growth rates for HPH treated slurries of 12%, 22% and 22% heated at 57 °C DM were 0.16, 0.15 and 0.19, respectively (Fig. 3).

In all fermentations, a decrease in pH was observed without any lag from the beginning of the fermentation (Fig. 4). For all fermentations (Fig. 4(A)–(C)) initial and final pH were very similar (around values of 6.7–3.5, respectively). The time required to reach a pH of 4.5, below which the inhibition of food-borne pathogens could be expected, was 5 h for 12% DM and 22% DM slurries and 7 h for the 22% DM slurry heated at 57 °C.

Lactic and acetic acid production was detected during the fermentation of all HPH slurries (Fig. 4), acetic acid

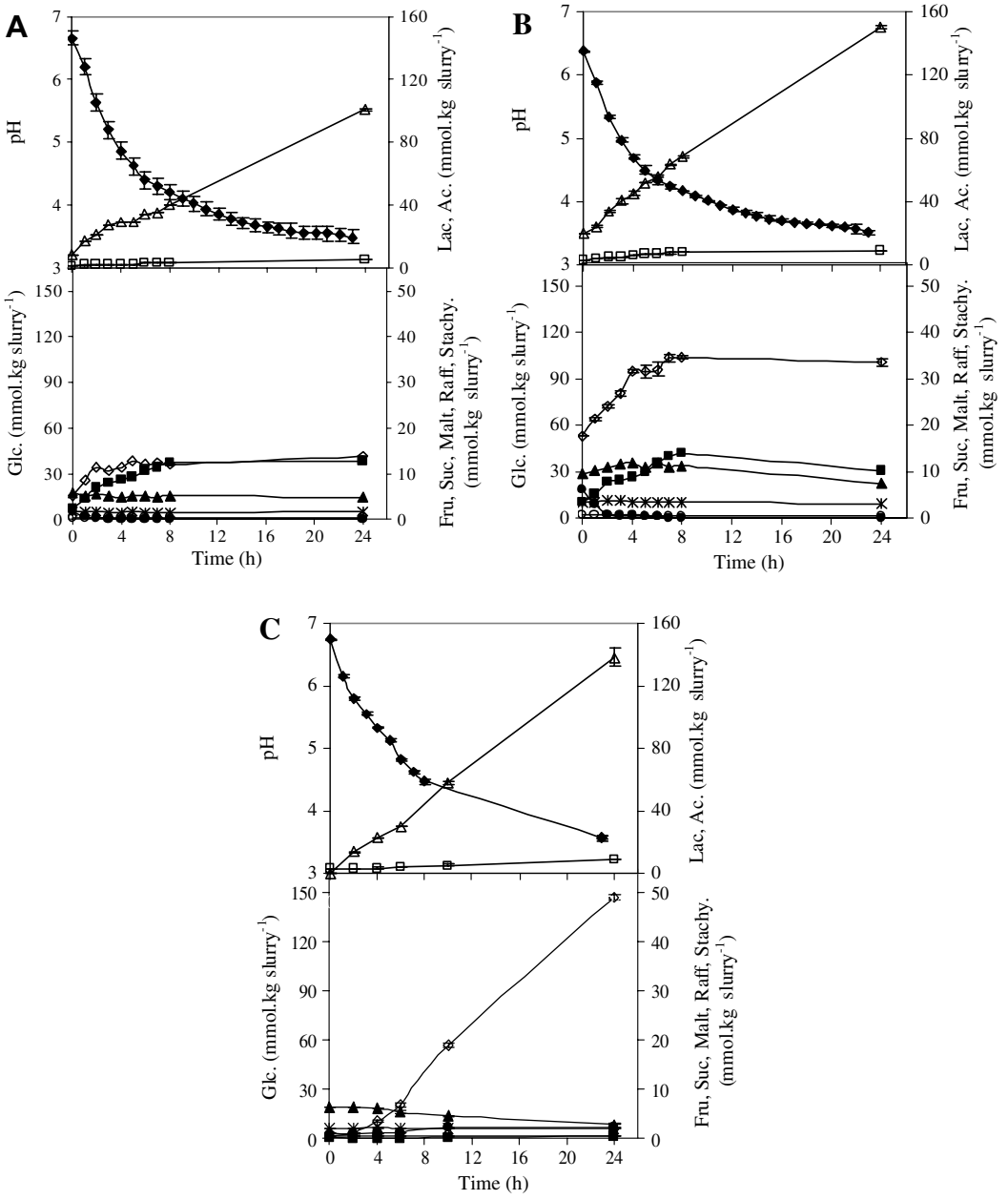

Fig. 4. Kinetics of fermentation of rice/soybean slurries: (A) HPH-12%-DM slurry; (B) HPH-22%-DM slurry; (C) HPH-22%-DM-heated (57 °C) slurry. Symbols: (♦) pH; (△) lactic acid; (□) acetic acid; (◇) glucose; (■) fructose; (▲) sucrose; (●) maltose; (○) raffinose; (∗) stachyose.

being produced in small amounts as expected from the metabolic characteristics of the *L. plantarum* A6 strain. The decrease in pH was therefore due mainly to lactic acid production. A higher rate of lactic acid production was obtained with 22% DM slurries than with the 12% DM slurry. Lactic acid was produced at higher concentration (150.1 ± 1.5 mmol kg^{-1}) with 22% DM slurry than with 22% DM heated slurry (138.3 ± 5.7 mmol kg^{-1}). The concentration of lactic acid in fermented 12% DM slurry was 100.5 ± 3.9 mmol kg^{-1}. All values were significantly different at $P < 0.05$.

During fermentation of the 12% DM slurry, lactic acid formation cannot be explained by the consumption of sucrose since its concentration was low and remained constant. The small quantity of maltose initially present was consumed quickly at the beginning of fermentation. Glucose and fructose concentrations increased slowly at the beginning of the fermentation and rapidly levelled off whereas lactic acid production continued. Glucose concentration at the end of the fermentation was 38 mmol kg^{-1}. For the 22% DM slurry, the changes in sugars were similar except for the glucose concentration, which increased quickly during the first 8 h to reach a concentration of 108 mmol kg^{-1} after 24 h fermentation.

In contrast, during the fermentation of 22% DM slurry heated at 57 °C, the fructose did not accumulate and glucose concentration was continuously increasing. A very high glucose production (148 mmol kg^{-1}) was observed at the end of the fermentation.

No changes in the concentration in stachyose and raffinose during all fermentations were observed.

3.1.3. Damaged starch rate and rheological properties

The damaged starch rate in the untreated flour blend was 26% and the soluble matter content was 10 g/100 g of DM (Table 1). Low changes in damaged starch rate and in soluble matter of 12% DM and non pre-heated 22% DM slurries after HPH and fermentation were observed. Similarly, only a slight increase in damaged starch rate was observed with the 22% DM slurry heated at 57 °C but non-HPH treated (Table 1). In contrast, a dramatic increase in these two parameters was observed after HPH treatment and fermentation for the 22% DM slurry heated at 57 °C.

For 12% DM and 22% DM with or without the HPH treatment, gruels obtained after cooking were very thick and their viscosity could not be measured in our experimental conditions.

Measurements were possible only with the pre-heated slurries. In such a case, HPH treatment and fermentation affect both the viscosity and the Bostwick flow and a fluid gruel was obtained after fermentation by strain A6 (Table 1).

Furthermore, it was observed that HPH increased the physical stability of the slurry since no separation of phases was observed before and after the fermentation.

3.2. Rheological comparison of gruels prepared from sour spray-dried flours

As an alternative to the processing of the HPH-fermented slurries into gruels after cooking, the study investigated whether HED-fermented gruels could also be prepared from sour flours obtained from the spray-drying of the HPH-fermented slurries. Rheological measurements were made on gruels prepared at different DM contents of spray-dried flours.

It was only possible to spray-dry the fermented 12% DM slurry because at 22% DM slurry, a sedimentation of starch happened during the spray-drying process. However, the consistency of gruels prepared at DM contents between 12% and 19% from the spray-dried sour slurry was too stiff to be measurable.

In contrast, it was possible to spray-dry the 22%DM slurry heated at 57 °C, HPH treated and fermented. The gruels prepared at different DM contents from the resulting fermented flour gave very interesting results. The viscosity of gruels prepared at 12–18% DM was between 0.1 and 0.3 Pa s and the Bostwick flow was very high ($\geqslant 200$ mm 30 s^{-1}) since the gruel had a liquid consistency.

Table 1
Damaged starch rate, soluble matter, apparent viscosity and Bostwick flow of slurries of 12% DM, 22% DM and 22% DM heated at 57 °C after HPH treatment (HPH T0) and after 24 h fermentation (HPH-FER T24) by *Lactobacillus plantarum* strain A6

		Damaged starch rate (g/100 g of DM)	Soluble matter (g/100 g of DM)	Apparent viscosity at 83 s^{-1} (Pa s)[a]	Bostwick flow (mm 30 s^{-1})[a]
Flour blend		26.09 ± 0.02	10.16 ± 0.37	ND (1)	ND (1)
12% DM	HPH T0	30.90 ± 0.07	12.02 ± 0.65	ND (1)	ND (1)
	HPH-FER T24	32.65 ± 0.01	15.25 ± 0.19	ND (1)	ND (1)
22% DM	HPH T0	31.48 ± 0.08	11.49 ± 0.23	ND (1)	ND (1)
	HPH-FER T24	32.66 ± 0.05	18.17 ± 0.47	ND (1)	ND (1)
22% DM heated at 57 °C	heated 57 °C, before HPH treatment	37.72 ± 0.75	17.62 ± 0.24	ND (1)	ND (1)
	HPH T0	82.19 ± 0.53	45.16 ± 0.34	2.17 ± 0.10	25 ± 1.41
	HPH-FER T24	84.09 ± 0.66	58.12 ± 0.19	0.89 ± 0.02	108 ± 2.83

(1) No measurement was possible because of the stiff consistency of the gruel.
[a] Measured after cooking for 15 min.

For gruels at 22 % DM, the viscosity was 0.7 Pa s and the Bostwick flow was 148 mm 30 s^{-1}. At 32% DM, the gruel had a viscosity of 1.2 Pa s and a Bostwick flow of 108 mm 30 s^{-1}.

In addition to these results, it is interesting to note that spray-drying did not affect drastically the viability of the A6 strain, since high counts ($2–7 \times 10^6$ cfu/g of sour flour) were obtained after treatment.

4. Discussion

Similarly to previous observations with gelatinised rice/soybean slurries (Nguyen et al., 2007), HPH slurries enabled only a limited bacterial growth, however the expected pattern of lactic acid fermentation by *L. plantarum* A6 was obtained, i.e. lactic acid being the dominant end-product formed with very low amount of acetic acid. However, a low final pH value was reached due to a good acidification.

The high pressure homogenisation of the flour blend slurry at ambient temperature decreased the particle size of flour blends but this did not result in increasing the sensitivity of the starch to amylase enzymes produced by the *L. plantarum* A6 strain. The fact that lactic acid continued increasing whereas mono and disaccharides concentration levelled off, suggests that an additional carbon source is being used such as starch. However, in spite of the chemical changes indicated by fermentation patterns, there were no measurable physical effects on the consistency of the gruels which remained very thick. Thus, this process combination failed to substitute to gelatinisation as a pre-treatment to produce HED gruels at 22% DM having the appropriate consistency as obtained by Nguyen et al. (2007).

The combination of mil thermal treatment with HPH treatment of the 22% DM slurry resulted in an increased particle size and damaged starch rate. Swelling of starch granules could explain this phenomenon (Muhr & Blanshard, 1982; Stute et al., 1996). The strong decrease of the viscosity and increase of Bostwick flow of the gruel obtained after the cooking of the pre-heated HPH-fermented slurry indicate that the treatments applied to the slurry enabled the amylase from *L. plantarum* A6 to efficiently hydrolyse the starch fraction. There is also another major difference in the fermentation pattern compared to that of the fermented HPH slurry which has not been pre-heated: whereas growth behaviour, lactic acid production and pH drop were very similar between both types of slurries, only in the case of the pre-heated slurry a continuous increase in glucose concentration over the 24 h fermentation period was observed, more striking, most of the glucose was produced after the growth stopped. This is consistent with an improved accessibility of the food matrix to enzymatic hydrolysis, and suggest also that the amylase produced during the growth phase remained active after the cells entered into the stationary phase and at low pH values (between 4.5 and 3.5).

The change of viscosity due to the ALAB allowed the preparation of fermented gruel with a high dry matter content (22% DM) and a calculated energy density of 94 kcal/100 g of gruel. This value is higher than the recommended value of 84 kcal/100 g of gruel for children of 9–11 months old at a rate of 2 meals/day added to average breast milk intake (Dewey & Brown, 2003).

Spray drying was used successfully to prepare fermented flours from the pre-heated HPH-fermented 22% DM slurry. Nguyen et al. (2007) showed that the use of spray drying enabled the preparation of flours from gelatinised fermented slurries made of a blend of rice soybean, enabling the preparation of HED gruels with an elevated DM content (up to 32%). In our study it is shown that spray drying can also be applied to the HPH pre-heated slurry. These fermented flours will become easier to handle, to pack and to store. Drying the HPH-fermented slurries provides additional protection against spoilage and pathogenic bacteria (Lardinois et al., 2003). ALAB survival after spray drying confirms previous results (Nguyen et al., 2007) and provides the flour with a microflora which might help in preventing microbial contamination.

5. Conclusion

Pre-heating of the slurry combined to HPH treatment is an alternative to conventional thermal gelatinisation to prepare new starch-based HED gruels allowing implementation of the ability of ALABs to hydrolyse starch. The feasibility to use in situ amylase production by ALABs was demonstrated. However, to produce HED fermented gruels at industrial scale, it will be recommendable to select ALAB strains which only produce L(+) lactic acid. Furthermore, it would be interesting to investigate if this new bioprocess would allow to preserve the vitamins and improve the bioavailability of mineral micronutrients which could be added to improve the nutritional quality of the gruels.

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