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Study of the behaviour of *Lactobacillus plantarum* and *Leuconostoc* starters during a complete wheat sourdough breadmaking process

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Abstract

The acidification properties, metabolic activity and technological performance of four individual *Lactobacillus plantarum* or *Leuconostoc* freeze-dried starters were investigated during a complete wheat sourdough breadmaking process including 0.2 g/100 g baker's yeast. Microbiological contents (lactic acid bacteria and yeasts), acidification characteristics (pH and total titratable acidity), soluble carbohydrates (maltose, glucose and fructose) and fermentative end-products (lactic and acetic acids, ethanol) contents were evaluated during both sourdough and corresponding bread dough fermentation. Biochemical and technological analysis of the resulting bread products are also presented. Some differences among strains in acidification properties and soluble carbohydrates availability were outlined both in sourdough and bread dough. Each individual *Leuconostoc* or *Lb. plantarum* starter was able to produce a characteristic fermentation and was found to ensure the production of breads with overall satisfactory acceptance.

Keywords: Sourdough; Fermentative end-products; Flour soluble carbohydrates; *Lactobacillus plantarum*; *Leuconostoc* sp.

1. Introduction

The use of sourdough in wheat bread production clearly improves dough properties, bread texture and flavour, delays the staling process and prevents bread from mould and bacterial spoilage (Gobbetti, 1998; Hammes & Gänzle, 1998; Martinez-Anaya, 2003). These benefits result from an appropriate balance between the metabolism of yeast and hetero- and homo-fermentative lactic acid bacteria (LAB) strains that represent the predominant microorganisms in natural sourdoughs. LAB metabolism is responsible for the production of organic acids and contributes, along with yeasts, to the production of aromatic compounds (Damiani et al., 1996; Martinez-Anaya, 1996a; Meignen et al., 2001). Sourdough microflora generally contain a complex mixture of yeasts (mainly *Saccharomyces cerevisiae*) and hetero- and homo-fermentative LAB. A large number of *Lactobacillus* species have been isolated including *Lactobacillus sanfranciscensis*, *Lb. pontis, Lb. brevis* and *Lb. plantarum* strains the most frequently described (Ottogali, Galli, & Foschino, 1996; Gobbetti, 1998; De Vuyst et al., 2002). Despite a predominance of lactobacilli, LAB cocci, belonging to *Leuconostoc* and *Pediococcus* genera, were also identified in traditional wheat sourdough from European countries (Infantes & Tourneur, 1991; Gabriel, Lefebvre, Vayssier, & Faucher, 1999; Corsetti et al., 2001). Starters composed of specific individual LAB, or mixed LAB and yeasts, became available a few years ago allowing the production of a full sourdough in a one-stage process. Such commercial starters improve the control of the sourdough production while ensuring reliable quality in bread production. The design of starters requires prior

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knowledge of the biochemical characteristics and baking potential of the microorganisms. Performance of LAB isolates has mainly been studied by characterization of the acidification properties such as pH, total titratable acidity (TTA) and lactic and acetic acids production during sourdough fermentation (Collar, 1996; Corsetti et al., 1998; Hammes & Gänzle, 1998). Furthermore, acetate production by heterofermentative metabolism is of major importance for the development of flavour. The molar ratio between lactic to acetic acid in bread (fermentation quotient, FQ) is considered optimum in the range between 2.0 and 2.7 (Hammes & Gänzle, 1998). Production of suitable end-products during dough fermentation depends on the availability of soluble carbohydrates. In wheat flour, the total concentration of maltose, sucrose, glucose and fructose is rather low and varies from 1.5 to 2 g/100 g, maltose being the most abundant fermentable carbohydrate. Important changes in carbohydrate fractions occurred during sourdough fermentation resulting from both enzymatic activities of the flour and metabolic conversions by microbial enzymes (Collar, 1996; Martinez-Anaya, 1996b; Gobbetti, 1998). Metabolism of carbohydrates varies depending on the LAB species, and even strains, the type of sugars, the co-presence of yeasts and the processing conditions (Rouzaud & Martinez-Anaya, 1993; Gobbetti, Corsetti, & Rossi, 1994; Martinez-Anaya & Rouzaud, 1996). The soluble carbohydrates remaining after microbial fermentation participate in browning reactions during baking, contributing to organoleptic characteristics of the bread (Collar, 1996). Furthermore, in most industrial applications, a variable amount of baker's yeast is added to bread doughs as leavening agent together with the sourdough preparation (Hammes & Gänzle, 1998). Therefore, baker's yeast enzymes may interfere with the metabolic activities of the sourdough microflora during the breadmaking process.

Organic acid production and carbohydrate metabolism during sourdough fermentation depend to various extents on microbial starter composition and on process parameters such as flour ash content, dough yield, fermentation time and temperature and NaCl concentration (Martinez-Anaya, Pitarch, Bayarri, & Benedito de Barber, 1989; Martinez-Anaya, Grana, & Torner, 1993; Martinez-Anaya, Benedito de Barber, & Collar Esteve, 1994; Gobbetti et al., 1995; Gianotti et al., 1997; Rouzaud & Martinez-Anaya, 1997; Meignen et al., 2001). All of the above studies have focused on the metabolic activities of individual isolates or mixed starters using species such as *Lb. sanfranciscensis*, *Lb. plantarum*, and *Lb. brevis*. In addition, a previous study in our laboratory on the characterization of the LAB microflora of traditional wheat sourdoughs from the Midi-Pyrénées region (France) has revealed the importance of *Leuconostoc* genera since such heterofermentative cocci were detected associated with *Lb. plantarum* in all the nine sourdoughs tested (Gabriel et al., 1999). As far as we know, no other study which included *Leuconostoc* strains has been reported concerning wheat bread fermentation. The acidification potential of *Leuconostoc mesenteroides* strains was only checked during rye sourdough fermentation (Lönner & Preve-Akesson, 1988) and pizza dough fermentation (Coppola, Pepe, & Mauriello, 1998).

The aim of this study was to evaluate the behaviour of four individual freeze-dried starters including *Lb. plantarum* (homofermentative), *Leuconostoc citreum* and *Leuc. mesenteroides* (heterofermentative) during a complete wheat sourdough breadmaking process including baker's yeast addition. Microbiological, biochemical, physico-chemical and technological analyses have been performed on sourdoughs, and on bread doughs over the fermentative period and on final bread products.

2. Materials and methods

2.1. Microorganisms

Four LAB strains, previously isolated from traditional French wheat sourdoughs, were used in this study: *Lactobacillus plantarum* AELLI12 and EMRS4, *Leuconostoc citreum* BELLI7 and *Leuc. mesenteroides cremoris* AMSE2 (Gabriel et al., 1999). These strains have been found to be able to ferment maltose, glucose and fructose and were identified by 16S ribosomal DNA (rDNA) sequencing (Molecular typing centre, Institut Pasteur, Lille, France). These strains were used in a lyophilized form with approximately 1×10^{10} cfu/g (Bioprox, France). Commercial compressed baker's yeast (*Saccharomyces cerevisiae*) was used during the breadmaking process (Lesaffre, Marcq-en-Baroeul, France).

2.2. Sourdough preparation and fermentation

Wheat flour for traditional breadmaking (T65 as according to the French classification on ash content, malt flour <0.1 g/100 g, without ascorbic acid) was used (Gers Farine Minoterie, France). The main characteristics of the flour were: moisture 15.6 g/100 g, protein 11.5 g/100 g, damage starch fraction 7.5 g/100 g and falling number 275 s.

Sourdough preparation in laboratory conditions included 250 g wheat flour, 150 ml tap water, 3.8 g salt and 10 ml starter suspension in order to produce a firm dough with an initial viable counts of about 1×10^7 LAB cfu/g (dough yield DY = 160). After kneading with continuous speed mixer (60*g*) for 10 min at room temperature, dough samples were divided into 10 g

portions and placed in an incubator at 28 °C over 25 h. An uninoculated dough was prepared under the same conditions as a control.

2.3. Breadmaking conditions

An initial dough was made with 1500 g of wheat flour, 900 g of tap water containing the LAB starter and 22.5 g salt and was mixed for 11 min at 40 rpm (Mahot LAB 25 mixer). The individual LAB starter was dissolved in tap water (2.5 g in 50 ml) and immediately incorporated into the dough in order to obtain an inoculation rate of about 1×10^7 LAB cfu/g. The dough was incubated at 27 °C for 20 h (65% relative humidity, RH).

Final bread dough formulation was: 2000 g of flour, 1280 g tap water, 400 g sourdough prepared as described above, 4 g of compressed baker's yeast and 44 g salt. Ingredients were mixed for 5 min at 40 rpm and 12 min at 80 rpm with a Mahot Lab 25 mixer. Water temperature was determined to yield a final dough temperature of 25 °C at the end of mixing. The dough was fermented for 2 h at 22 °C (65–75% RH), then divided into 350 dough pieces. After a dough resting period of 20 min, bread doughs were mechanically moulded and incubated for 5 h at 27 °C. Baking was carried out at 250 °C ± 10 °C for 25 min in a Bongard oven. Bread products were cooled at room temperature (~20 °C) for 45 min.

The breadmaking process were performed at the technical centre of the "Centre Technique de la Conversation des Produits Agricoles" (CTCPA, Auch, France). Wheat sourdough bread productions were performed in duplicate.

2.4. Sourdough and bread characteristics

Ten gram samples were collected at various times during the process (sourdough and dough fermentation period, final bread products) and were immediately refrigerated at 4 °C before microbiological analyses (maximum 2 h) or frozen at −30 °C for biochemical analyses. When required, the samples were thawed overnight at 4 °C. The pH values and TTA were determined using a SchottCG840 pHMeter on a 10 g dough or bread crumb sample blended with 90 ml of distilled water. The TTA value was expressed as the amount (ml) of 0.1 mol/l NaOH needed to achieve a final pH of 8.5.

LAB and yeasts cell numbers were determined by plating appropriate dilutions (TS) on MRS (deMan, Rogosa and Sharpe) with 200 mg/ml cycloheximide and malt agar with 0.5 g/l chloramphenicol media, respectively. The plates were incubated for 48 h at 30 °C for LAB and 25 °C for yeasts.

The residual sugar levels (maltose, glucose, fructose) and the fermentation end products (lactic and acetic acids, ethanol) were determined and quantified by HPLC. Dough extracts prior analysis were treated as described previously (Lefebvre, Gabriel, Vayssier, & Fontagné-Faucher, 2002) with weak modifications in order to reduce the dilution rate during the extraction procedure and so to improve the sensitivity of the detection. Ten grams of sample were homogenized with 60 ml of cold distilled water. The sample was adjusted to 100 g by weighing and was centrifuged for 5 min at 4000*g* at 15 °C. Twenty millilitres aliquot of supernatant were stirred with 5 ml of Carrez I solution (potassium II hexaferrocyanate, 0.085 mol/l) and 5 ml of Carrez II solution (zinc sulfate, 0.25 mol/l) and then neutralized (pH 8.0 ± 0.5) with NaOH 0.5 mol/l. The volume was adjusted to 50 ml with distilled water and the solution was twice filtered in order to eliminate proteins. HPLC analysis was carried out using a Beckman chromatograph (Beckman equipped with a 126 programmable solvent module, a 406 analog interface and a 156 refractive index detector). The HPLC apparatus was connected to a computer with the Beckman system Gold Software and fitted with an Alcott 708 autosampler. The column was an ion-exclusion ICE-ORH-801 model (300 mm × 6.5 mm, Transgenomic, USA). The mobile phase was 0.001 N sulphuric acid at a flow rate 0.75 ml/min, and the oven temperature was held at 45 °C. All determinations were performed in duplicate and results were expressed as mean values.

The bread volume was measured using the rapeseed displacement method. Bread weight was also recorded. Four loaves were used for each evaluation. Breads characteristics were evaluated using a modified French breadmaking test procedure (BIPEA, Bureau Interprofessionnel d'Etudes Analytiques).

3. Results and discussion

3.1. Acidification properties during sourdough fermentation

A preliminary experiment was conducted in lab-scale in order to evaluate the acidification properties—dynamics of pH decrease and final pH—of the four individual freeze-dried LAB starters: *Leuconostoc* (heterofermentative) AMSE2 and BELLI7 and *Lb. plantarum* (homofermentative) AELLI12 and EMRS4 strains. The effect of the LAB starter on pH values was monitored over a 24 h sourdough fermentation and was compared with an uninoculated dough prepared under the same conditions (Fig. 1). As expected, the uninoculated flour–water–salt mixture (dotted line) presented no acidification, the pH value remaining nearly constant (~6.1). During the same period the endogenous LAB microflora of the flour, initially <100 cfu/g dough, reached 1.2×10^7 cfu/g dough

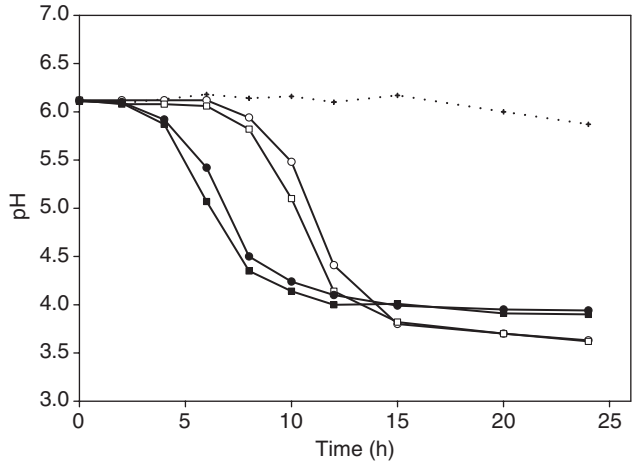

Fig. 1. pH changes during sourdough fermentation with four different LAB starter: (○) *Lactobacillus plantarum* AELLI12, (□) *Lactobacillus plantarum* EMRS4, (●) *Leuconostoc mesenteroides* AMSE2 and (■) *Leuconostoc citreum* BELLI7. (----): uninoculated control dough.

leading to a slight pH decrease at the end of the incubation period. The pH value of the samples inoculated with each individual LAB starter decreased over the incubation period from 6.1 to about <4.0. A stable final pH was reached after 15–20 h. The final pH was slightly higher (3.9 versus 3.6) for the two heterofermentative strains. Utilization of a *Leuconostoc* starter has resulted clearly in a more rapid decrease of the pH value than when the inoculation was performed with homofermentative *Lb. plantarum* strains. These observations could not be simply assigned to a difference in LAB population since initial LAB cell numbers varied from 3.5×10^6 cfu/g (AELLI12-E MRS4) to 1.0×10^7 cfu/g (AMSE2-BELLI7) and reached about 1.0×10^9 cfu/g (9×10^8–2.0×10^9 cfu/g) for all the strains after 20 h. Moreover, similar results on microbial growth and acidification rate have been obtained previously using liquid culture preparations (Lefebvre et al., 2002 and data not shown). Production of CO_2 was apparent as indicated by an increase of the dough volume (about 50% after 20 h fermentation) only for the two *Leuconostoc* starters. Finally, these overall experiments have shown that freeze-drying does not affect the viability nor functionality of these four LAB strains.

3.2. Characteristics of sourdough and bread dough fermentation

Sourdoughs were prepared from each starter using the same conditions as mentioned above, fermented for 20 h and incorporated at 20 g/100 g level (base flour) in corresponding wheat bread doughs in a semi-pilot scale breadmaking process. Leavening was ensured by addition of a small amount of baker's yeast during the bread dough preparation. In order to limit the competition for carbohydrate sources between LAB and yeasts and so to promote the lactic fermentation, over alcoholic fermentation, the incorporation rate was only 0.2 g/100 g that is 10-fold lower than usual amounts of baker's yeast addition in bread production. Bread dough containing baker's yeast alone in the same amount was included in the test series as a control. Acidification (pH, TTA), carbohydrates (maltose, glucose and fructose), organic acids (acetic and lactic acids) and ethanol contents were monitored on sourdoughs before and at the end of the fermentation period (Table 1), on bread doughs just after mixing and after 2, 4:30 and 7 h fermentation (Fig. 2A–2C). LAB and yeast counts were determined by appropriate plating of sourdough and bread dough samples.

3.3. Microbiological analysis

The initial cell number of LAB was very similar in each case with an average value of 4.0×10^6 cfu/g (ranging from 3.0×10^6 to 6.7×10^6 cfu/g, Table 1). At the end of the sourdough fermentation, the LAB population reached 1.6×10^9 cfu/g, ranging from 0.9×10^9 to 2.3×10^9 cfu/g. Owing to the 20% level of sourdough addition, the initial LAB population in the bread dough had an average value of 1.7×10^8 cfu/g and the LAB population increased up to 8.7×10^8 cfu/g. No noticeable differences in LAB cell counts were observed within the various experiments. Total yeast cell counts were $<10^4$ cfu/g dough throughout the fermentation period.

Numeration of viable yeast cells in the different sets of compressed baker's yeast added in the bread dough for leavening purpose gave homogeneous values ranging from 6.0×10^9 to 1.5×10^{10} cfu/g. Therefore the initial baker's yeast population in the bread doughs was near 1×10^7 cfu/g and the endogenous yeast microflora of the flour could be considered as negligible. The yeast population remained unchanged until the end of the process with values ranging from 1.1×10^7 to 2.4×10^7 cfu/g. Compressed baker's yeast contained 1×10^4 to 1×10^5 cfu/g LAB cells as contaminants that could be considered as negligible in the LAB inoculated doughs. However, the initial LAB cells in the yeasted control could reached 5×10^6 cfu/g bread dough at the end of the fermentation period.

3.4. Soluble carbohydrates

Immediately after mixing, the initial amounts of soluble carbohydrates in sourdough were quite similar in all the samples, with respectively 1.50, 0.14 and 0.08 g/100 g for maltose, glucose and fructose (Table 1). After 20 h fermentation, maltose encountered a slight increase (1.5–2.5 g/100 g) and no differences were

Table 1
Microbial contents, pH and TTA, soluble carbohydrates (maltose, glucose and fructose) and fermentative end-products (lactic and acetic acids, ethanol) concentration at the beginning and after 20 h sourdough fermentation

	Time (h)	*Lb. plantarum*		*Leuconostoc* sp.	
		EMRS4	AELLI12	BELLI7	AMSE2
LAB (cfu/g dough)	0	$3.4 \pm 0.3 \times 10^6$	$4.4 \pm 1.1 \times 10^6$	$3.0 \pm 0.4 \times 10^6$	$6.7 \pm 1.6 \times 10^6$
	20	$2.3 \pm 0.4 \times 10^9$	$1.5 \pm 0.2 \times 10^9$	$0.9 \pm 0.0 \times 10^9$	$1.6 \pm 0.7 \times 10^9$
pH	0	5.99 ± 0.30	5.77 ± 0.36	6.01 ± 0.25	6.01 ± 0.23
	20	3.87 ± 0.06	3.82 ± 0.06	4.05 ± 0.06	4.08 ± 0.10
TTA	0	1.8 ± 0.2	2.1 ± 0.5	2.1 ± 0.3	2.0 ± 0.2
	20	7.3 ± 0.4	7.3 ± 1.0	7.1 ± 0.2	7.0 ± 0.2
Maltose (g/100 g)	0	1.48 ± 0.05	1.43 ± 0.18	1.60 ± 0.06	1.51 ± 0.05
	20	2.62 ± 0.11	2.44 ± 0.03	2.38 ± 0.12	2.56 ± 0.08
Glucose (g/100 g)	0	0.14 ± 0.03	0.17 ± 0.05	0.14 ± 0.03	0.14 ± 0.03
	20	0.12 ± 0.02	0.28 ± 0.04	0.23 ± 0.04	0.10 ± 0.03
Fructose (g/100 g)	0	0.08 ± 0.01	0.08 ± 0.02	0.09 ± 0.02	0.07 ± 0.00
	20	0.12 ± 0.01	0.04 ± 0.01	0.37 ± 0.02	0.39 ± 0.00
Lactic acid (g/100 g)	0	—	—	—	—
	20	0.55 ± 0.05	0.57 ± 0.01	0.40 ± 0.02	0.32 ± 0.01
Acetic acid (g/100 g)	0	—	—	—	—
	20	—	0.03 ± 0.02	0.08 ± 0.01	0.08 ± 0.01
Ethanol (g/100 g)	0	—	—	—	—
	20	—	—	0.06 ± 0.00	0.05 ± 0.05

Values are expressed as the mean of two experiments which were twice analysed. Values <0.01 g/100 g dough were reported as dimensionless.

observed within the four LAB starters. This could be attributed to the hydrolytic activity of cereal amylases on the starch fraction damaged during the milling process (Mathewson, 2000). A nearly constant level of maltose was obtained during the bread dough fermentation within all the samples, even the control (Fig. 2A-a), as the result of the balance between the microbial consumption and the starch hydrolysis by the enzymatic activity of the flour.

The evolution of the glucose concentration during the 20 h sourdough fermentation was variable within the LAB strains and was not related to an homo- or heterofermentative metabolism (Table 1). A nearly constant concentration for EMRS4 and AMSE2 (0.10–0.12 g/100 g) while a slight increase for AELLI12 and BELLI7 (0.28–0.23 g/100 g) strains were found. Glucose consumption by the LAB strains could explain a regular decrease in this sugar content during the bread dough fermentation (Fig. 2A-b). The change of the glucose content was quite different for the control as the glucose concentration increase up to 0.5 g/100 g.

The amount of fructose during the sourdough fermentation remained nearly constant for the two *Lb. plantarum* strains whereas a significant increase was observed when inoculation was performed with the two *Leuconostoc* strains (0.38 versus 0.14 g/100 g, Table 1). Such an increase in fructose content during sourdough fermentation due to *Leuconostoc* metabolism was previously reported (Lefebvre et al., 2002). A difference in behaviour between homo and heterofermentative starter was also observed during the bread dough fermentation (Fig. 2A-c). The fructose content decrease down to 0.19 g/100 g in the control and the bread doughs inoculated with the *Lb. plantarum* strains (down to 0.18–0.21 g/100 g) while it remained unchanged for doughs inoculated with each *Leuconostoc* strains (0.39 g/100 g).

Presence of sucrose in the dough was also determined. Sucrose content in an uninoculated dough was found to be 0.27 g/100 g. Sucrose determination was performed separately from the others soluble carbohydrates on a C18 column since an ion-exclusion column would not allow efficient separation of the two monosaccharides maltose and sucrose. We also observed that sucrose hydrolysis to glucose and fructose by yeast invertase occurred during bread dough mixing and was achieved within 10 min (data not shown). This rapid sucrose hydrolysis could explain the significant differences observed in the initial amounts of carbohydrates between sourdough and bread dough just after mixing: 2 and 4-fold higher, respectively for glucose and fructose. Sucrose hydrolysis, coupled with the inefficient

separation of maltose/sucrose, could be also responsible for the lower initial maltose concentration in the control than in the LAB inoculated bread doughs (1.2 g/100 g versus 1.5–1.7 g/100 g). The production of fructose during the dough fermentation could not be assigned to the yeast invertase activity upon sucrose since it could not be detected after mixing. The increase in fructose production during *Leuc.* fermentation could be attributed to an hydrolytic activity on the fructosans fraction from the flour (Escriva & Martinez-Anaya, 2000). *Leuc. citreum* was reported to produce oligofructosyl and inulin-like exopolysacharides (Olivares-Illana, Wacher-Rodarte, Le Borgne, & Lopez-Munguia, 2002) and its production/hydrolysis could also be accountable in the resulting fructose availability.

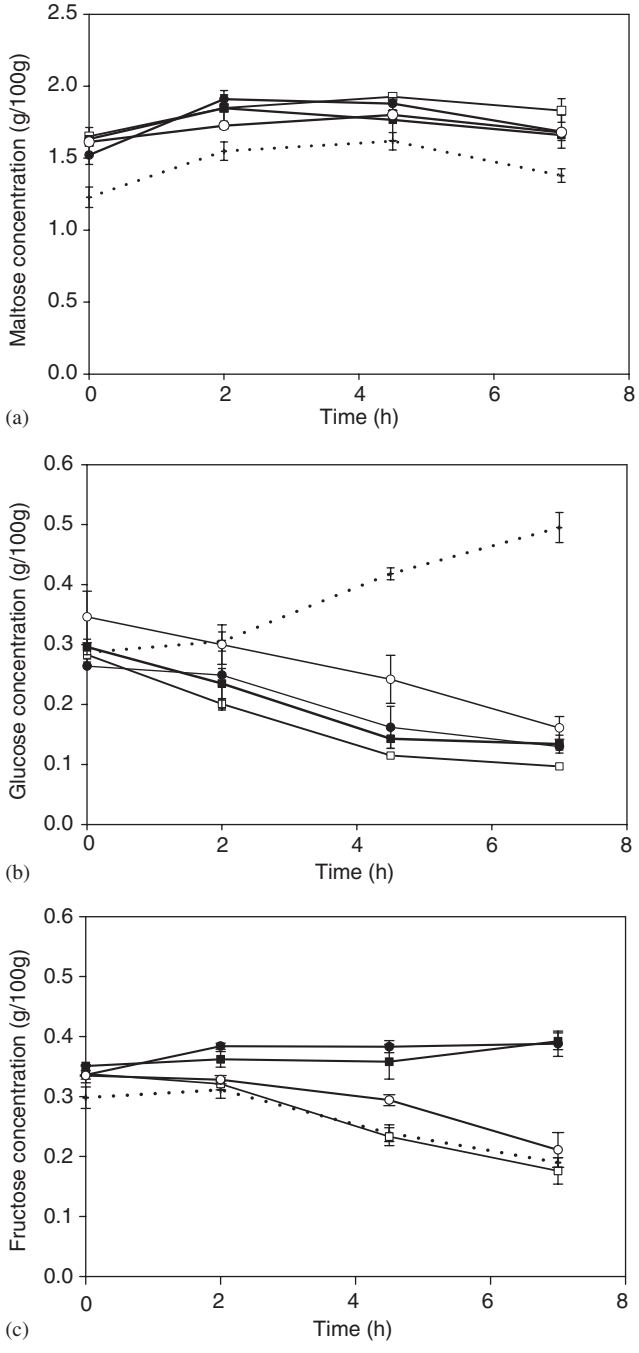

Fig. 2A. Acidification and biochemical characteristics during bread dough fermentation. Evolution of the soluble carbohydrates (a) maltose, (b) glucose and (c) fructose content. Evolution of the fermentative end-products (a) lactic acid, (b) acetic acid and (c) ethanol content. Symbol legends as in Fig. 1. Values are the means of two breadmaking process and two HPLC analyses.

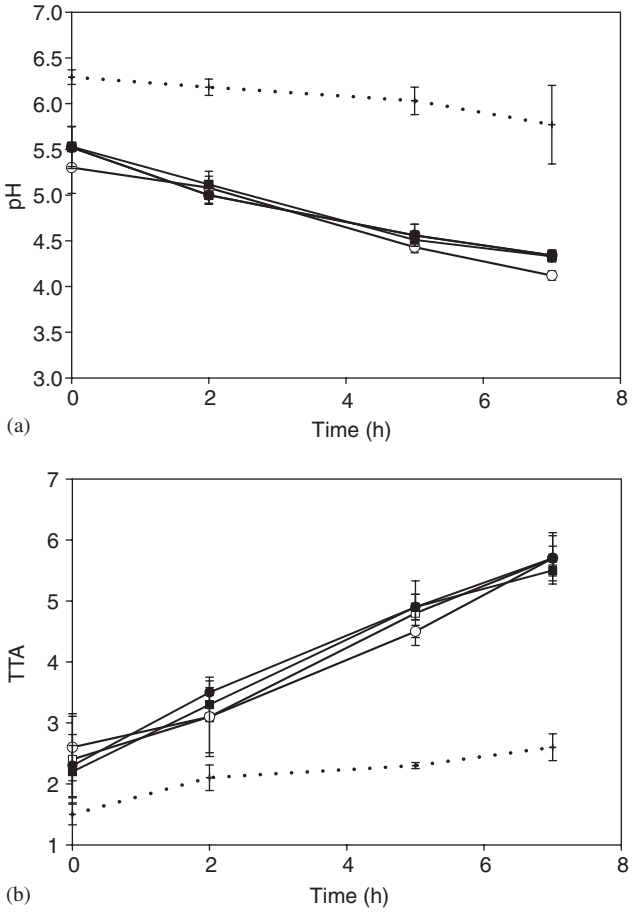

Fig. 2B. Acidification and biochemical characteristics during bread dough fermentation. Evolution of pH (a) and TTA (b). Symbol legends as in Fig. 1. Values are the means of two breadmaking process and two HPLC analyses.

3.5. Acidification characteristics

Initial sourdough pH (~6) and TTA (~2) values were quite similar for each LAB strain and lactic and acetic acids and ethanol were absent at the beginning of the process (<0.001 g/100 g) (Table 1). At the end of the sourdough fermentation, no noticeable differences in the pH decrease (up to 3.8), TTA increase (up to 7) and the lactic acid production (0.55 g/100 g) were observed between the two strains of *Lb. plantarum*. Acetic acid and ethanol were not detected. Inoculation

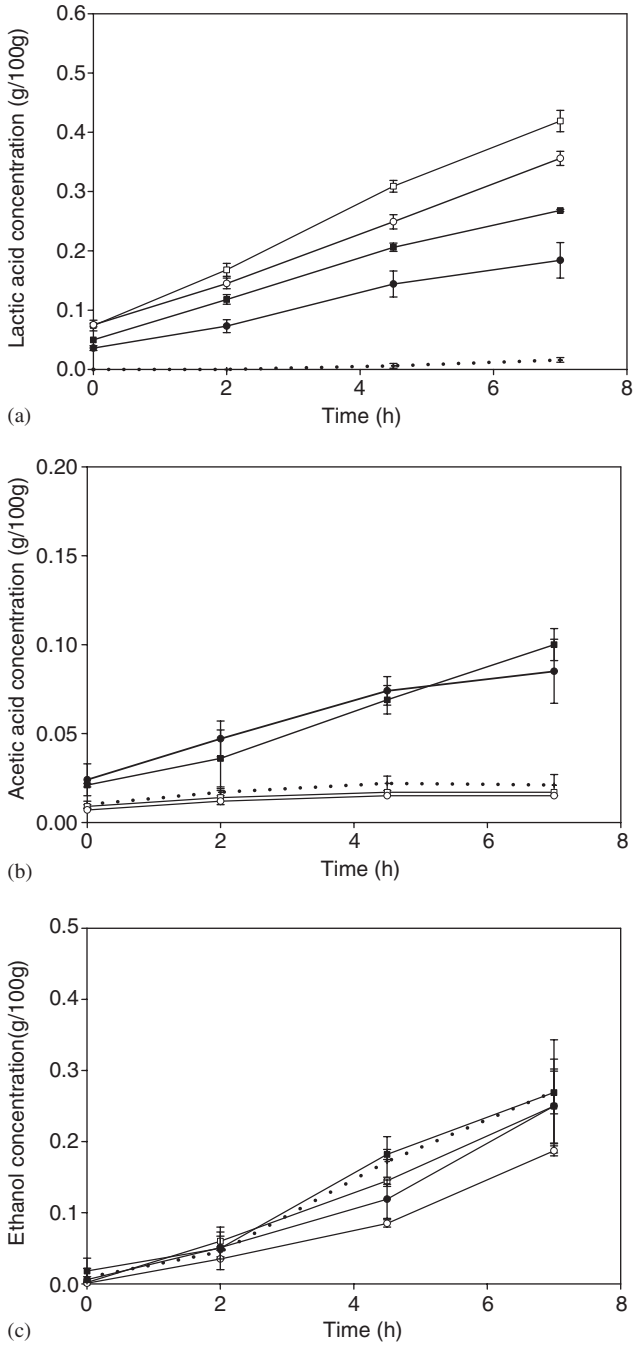

Fig. 2C. Acidification and biochemical characteristics during bread dough fermentation. Evolution of the fermentative end-products (a) lactic acid, (b) acetic acid and (c) ethanol content. Symbol legends as in Fig. 1. Values are the means of two breadmaking process and two HPLC analyses.

with each *Leuconostoc* strains have led to a pH decrease slightly lower (4.1) as previously observed and could be related to a lower production of lactic acid (0.32–0.40 g/100 g). Initial pH values (pH 5.3–5.5; Fig. 2B) and lactic acid concentration (0.07–0.05 g/100 g, Fig. 2C-a) of the bread doughs were higher than in sourdoughs just after mixing or in the control dough according to the 20% level sourdough addition. As expected, pH and TTA values of the control dough containing yeast alone varied only slightly during the fermentation period (pH 6.3–5.8, TTA 1.5–2.6). No organic acid production was observed thus confirming that LAB originating from flour or from compressed baker's yeast should be negligible. Lactic acid production (Fig. 2C-a) increased regularly during the bread dough fermentation with final values from 0.18 (AMSE2) to 0.42 g/100 g (EMRS4). Lactic acid content in final fermented dough was about 60% lower than in the corresponding 20 h fermented sourdough. Homofermentative *Lb. plantarum* starters allowed higher acid production than heterofermentative starters. Obviously, lactic acid production in bread dough was variable according the LAB starter with significant variations between each LAB strains. The two *Lb. plantarum* strains were not found equivalent in lactic acid production as previously observed during sourdough fermentation despite similar pH and TTA changes. *Leuc. citreum* BELLI7 allowed a higher production of lactic acid than *Leuc. mesenteroides* AMSE2.

Significant amount of acetic acid was only detected with heterofermentative starter, both *Leuconostoc* strains leading to a similar final content of 0.08 g/100 g in fermented sourdough (Table 1) and 0.10 g/100 g in bread dough (Fig. 2C-b). Quite similar values in both 20 h fermented sourdoughs and 7 h fermented bread doughs could be correlated to the relative high fructose content in the initial bread dough. Fructose can affect the acetate branch of the heterolactic pathway of LAB and fructose supply was found effective in increasing acetic acid production with heterofermentive strains (Röcken, Rick, & Reinkemeier, 1992; Stolz, Vogel, & Hammes, 1995; Collar, 1996).

Production of ethanol by the heterofermentative metabolism of *Leuconostoc* strains was shown in sourdough fermentation (Table 1) whereas production of ethanol during the bread dough fermentation was mainly due to the baker's yeast metabolism (Fig. 2C-c). Final ethanol production (0.25 g/100 g) were similar for the control dough as the LAB started doughs (0.25–0.27 g/100 g).

No noticeable differences in gassing power were observed between the LAB inoculated and the control doughs as judged by dough expansion indicating that the leavening process is mainly performed by baker's yeast and that heterofermentative LAB metabolism is only of minor importance in this case.

3.6. Bread characteristics

The main differences in acidity (pH and TTA values) mentioned above that characterized the doughs were maintained among the breads (Table 2). The lactic and acetic acid contents of bread showed the same trends as

Table 2
Bread crumb pH and TTA, soluble carbohydrates (g/100 g crumb) and organic acids and ethanol (g/100 g crumb) contents

	Control	*Lb. plantarum*		*Leuconostoc* sp.	
		EMRS4	AELLI12	BELLI7	AMSE2
PH	6.16±0.18	4.11±0.08	4.14±0.05	4.35±0.06	4.34±0.06
TTA	1.7±0.2	5.3±0.4	5.0±0.2	5.2±0.3	4.9±0.5
Maltose	2.02±0.15	1.70±0.04	1.58±0.10	1.55±0.08	1.60±0.21
Glucose	0.0±0.0	0.01±0.01	0.03±0.01	0.01±0.00	0.03±0.00
Fructose	0.16±0.005	0.15±0.00	0.18±0.02	0.34±0.01	0.35±0.03
Lactic acid	0.015±0.002	0.408± 0.024	0.342±0.026	0.252±0.012	0.188±0.038
Acetic acid	0.025±0.004	0.019±0.001	0.034±0.014	0.106±0.004	0.181±0.016
Ethanol	0.027±0.028	0.016±0.006	0.001±0.001	0.013±0.008	0.00±0.00

Values are expressed as the mean of two breadmaking experiments which were twice analysed.

Table 3
Physical properties and technological assessment of the resulting breads produced with each individual LAB starters

	Control	*Lb. plantarum*		*Leuconostoc* sp.	
		EMRS4	AELLI12	BELLI7	AMSE2
Volume (cm^3)	1424±57	1426±58	1298±151	1454±27	1328±134
Density (g/cm^3)	0.19±0.01	0.19±0.01	0.21±0.02	0.18±0.004	0.21±0.02
Technological assessment (score 0–10)					
Dough machinability	7.9±0.4	9.2±0.8	9.6±0.2	8.9±0.2	8.9±0.8
Bread appearance	7.2±0.4	9.7±0.3	9.8±0.3	8.9±0.4	9.6±0.3
Crumb appearance	8.5±0.0	9.5±0.5	9.5±0.0	9.5±0.0	9.6±0.2
Sensory properties	3.2±0.0	6.6±0.4	6.2±0.7	6.7±0.2	6.6±0.5

Values are expressed as the mean of two breadmaking experiments.

for the corresponding doughs. Such acids production led to a fermentative quotient (QF) that was more suitable for *Leuc. citreum* BELLI7 than for *Leuc. mesenteroides* AMSE (1.9 versus 1.3) since value in the range between 2.0 and 2.7 is considered optimum (Hammes & Gänzle, 1998). Ethanol was lost during baking. Residual glucose contents in breads started with LAB were very low (0.01–0.03 g/100 g) and could not be detected in the yeasted control, indicative of its active participation in browning reactions occurring during baking. No significant differences were observed in maltose and fructose contents between fermented doughs and the corresponding products whereas a slight increase was detected for the control breads.

Sourdough addition did not change the physical properties of the breads (volume, density), but dough machinability and functionality (dough consistency, resistance to extension, extensibility, elasticity, etc.) during the process and bread appearance were judged to be better with LAB started samples (Table 3). However, the main differences between control and sourdough breads were observed in sensory properties (smell and taste). Satisfactory flavour was produced only with the LAB started samples. The yeasted control had an alcoholic smell and taste. Overall acceptance was quite similar for all the starters and no differences could be detected between hetero- and homo-fermentative strains. Even though the latter do not produce acetic acid, sensory qualities could be attributed to other organic acids and volatile compounds (Damiani et al., 1996; Martinez-Anaya, 1996a).

4. Conclusions

Analyses performed in this study have shown that each of the individual LAB strains—even homofermentative—used as starter culture was able to produce a characteristic fermentation and could be used in a breadmaking process with baker's yeast addition in order to provided breads with suitable qualities. Complex co-interactions in carbohydrates metabolism were outlined within the fermented doughs containing individual LAB starter and baker's yeast. The residual contents of the various compounds are the result of the balance between enzymatic activities of the flour and microflora and the consumption by these microorganisms. Acidification properties and organic acids formation during dough fermentation were dependent on the microbial starter composition and determined the acid

profiles of the resulting breads. Nevertheless, under the conditions used in this study, the improving effect of microbial wheat sourdoughs on breadmaking performance was closely independent on the LAB starter. Comparative experiments with starter containing both *Lb. plantarum* and *Leuconostoc* sp. strains could be investigated in order to check the likely advantage of the association of homo and heterofermentative metabolism during wheat bread sourdough process. It also could be interesting to compare acidification properties and characteristics related to bread quality between *Leuconostoc* starters and others heterofermentative *Lactobacillus* strains such as *Lb. sanfranciscensis,* which is considered a key microorganism in sourdough production. Further experiments on the metabolic activity, potential EPS production of these LAB cocci could also provide evidence of their potentiality in sourdough fermentation.

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