World Journal of Microbiology & Biotechnology 11, 625–630

Interaction between lactic acid bacteria and yeasts in sour-dough using a rheofermentometer

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Rheofermentometer assays were used to characterize the leavening of sour-doughs produced using species of lactic acid bacteria (LAB) and yeasts, alone or in combination. *Saccharomyces cerevisiae* 141 produced the most CO_2 and ethanol whereas *S. exiguus* M14 and *Lactobacillus brevis* subsp. *lindneri* CB1 contributed poorly to leavening and gave sour-doughs without porosity. In comparison with that seen in sour-dough produced with yeast alone, yeast fermentation with heterofermentative LAB present was faster whereas that with homofermentative LAB (*L. plantarum* DC400, *L. farciminis CF3*) present was slower and produced more CO_2. Combining *L. brevis* subsp. *lindneri* CB1 with *S. cerevisiae* 141 decreased bacterial cell numbers and souring activity. However, addition of fructose to the sour-dough overcame these problems as well as activating *S. cerevisiae* 141.

Key words: Lactic acid bacteria, rheofermentometer, sour-dough, yeasts.

Associations between lactic acid bacteria (LAB) and yeasts are widely used in the production of beverages and fermented foods, including wheat and rye sour-dough breads (Devoyod & Desmazeaud 1971; Obelman 1985; Wood & Hodge 1985; Obelman 1985; Gobbetti 1992). The constitutive microflora of wheat sour-dough has been investigated in some detail and various species of homo- and heterofermentative LAB and yeasts have been identified (Kline & Sugihara 1971; Sugihara *et al.* 1971; Barber *et al.* 1983; Spicher 1987; Galli *et al.* 1988; Sarra *et al.* 1992; Gobbetti *et al.* 1994). Homofermentative LAB are responsible for development of sour-dough bread with a good grain and elastic crumb (Spicher 1983), whereas the heterofermentative LAB improve its taste (Spicher 1983; Salovaara 1993) and contribute to the leavening process. However, the leavening of sour-dough is fundamentally determined by the CO_2 produced through the fermentative activity of the yeasts present (Spicher 1983). The CO_2 is retained by the wheat gluten which is stretched into a visco-elastic film (Akdogan & Ozilgen 1992) which influences loaf volume and crumb density (Spicher 1983; Spicher & Pomeranz 1985). Sour-dough leavening is greatly influenced by the composition of the flour used and by the metabolic interactions between LAB and yeasts. Gobbetti *et al.* (1994a, b, c) investigated some aspects of carbohydrate and amino-acid metabolism in sour-doughs. Martinez-Anaya *et al.* (1990) showed the additive effect of some LAB on the gassing power of *Saccharomyces cerevisiae* and Corsetti *et al.* (1994) evaluated the effects of increasing soluble carbohydrates in enhancing the LAB fermentation.

In the present study, a rheofermentometer is used to characterize the leavening of wheat sour-dough by LAB and yeast starters, alone or combined.

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Materials and Methods

Microorganisms and Media

The *Lactobacillus brevis* subsp. *lindneri* CB1 (heterofermentative), *L. plantarum* DC400 (homofermentative), *L. farciminis* CF3 (homofermentative), *Saccharomyces cerevisiae* 141 (maltose-positive) and *S. exiguus* M14 (maltose-negative) used had all been isolated from wheat sour-dough (Gobbetti & Rossi 1991; Gobbetti *et al.* 1994). The lactic acid bacteria (LAB) and yeasts were pre-cultured in SDB broth (Kline & Sugihara 1971) and Sabouraud broth (Difco), respectively, for 24 h at 28°C. The cells were harvested by centrifugation at 10,000 × *g* for 10 min and washed twice with sterile distilled water. Baker's yeast (68% moisture) was purchased from local bakeries.

Dough Kneading and Addition of Soluble Carbohydrates

The characteristics of the type O wheat flour used were: moisture, 15.4%; protein (N × 5.70), 10.7% dry matter (d.m.); fat, 1.8% d.m.; ash, 0.6% d.m. The Chopin Alveograph P value (M. Chopin, Boulogne, France) was 82.95. Wheat flour (250 g) and tap water (130 ml) containing 7 g prepared starter cells (14 g in the case of the combinations of microorganisms) or baker's yeast were used to produce the dough. Depending on the dry weight of the cells, the amount of tap water was modified in order to give a constant final dough yield of 155. When fructose was added, its final concentration was 6 g/kg dough. Doughs were produced with a continuous high speed mixer (60 × *g*) (M. Chopin). The total dough mixing time was 5 min (1 min mixing, 2 min stop, 2 min mixing).

Rheofermentometer Trials

The Chopin Rheofermentometer F_2 (Groupe Tripette et Renaud Villeneuve-La-Garenne, Cedex, France) is equipped with a thermostat-controlled, 1-l fermentation chamber containing a perforated (0.4-mm pore) aluminum vessel connected to a recorder. After mixing with the starter, 315 g dough were placed in the vessel, pressed with a 2-kg, cylindrical weight and the chamber hermetically closed. Fermentation was at 28°C for 3 h and the volume of CO_2 liberated through the perforated vessel was recorded by an electronic microprocessor. The leavening process is described in terms of: V_t, the area delimited by the upper line of the gas release curve, corresponding to the total volume of CO_2 (ml) produced during 3 h of fermentation; $V_t/2$, the total volume of CO_2 (ml) produced after 1.5 h of fermentation; V_r, the area delimited by the lower line of the gas release curve, corresponding to the total volume of CO_2 (ml) retained in the sour-dough; R_c, the CO_2 retention coefficient V_r/V_t, the area between the upper and lower lines being a measure of the amount of CO_2 liberated from the dough and therefore also the porosity of the dough; H_m, the maximum height (mm) of the upper curve; T_1, the time (min) to reach the maximum curve height; X, the maximum gas flow (mm/min) (H_m/T_1); and T_x, the time (min) when the porosity of the dough develops (point of separation between the upper and lower lines).

The rheofermentometer was calibrated according to the manufacturer's instructions except that NaCl was excluded and the effective mixing time was reduced to 3 min. Cell concentrations in the dough (7 g) and the temperature of fermentation (28°C) were selected to give a leavening time (3 h) compatible with the performance of the rheofermentometer.

Cell Counts

LAB and yeast cell numbers were estimated on SDB (Kline & Sugihara 1971) and Sabouraud (Difco) agars, respectively, after 72 h incubation at 28°C.

Determination of Organic Acids and Ethanol

A 10-g sample of leavened sour-dough (3 h) was diluted with distilled water (90 ml), homogenized with a Classic Blender (PBI International, Milan, Italy), mixed at 100 × *g* for 30 min and centrifuged at 12,000 × *g* for 30 min. D- and L-lactic acid, acetic acid and ethanol contents were determined in the supernatant using commercial, enzymatic kits (Boeringher-Mannheim, Milan, Italy). Each value given in the Results is the mean for three replicate sour-dough fermentations, each analysed twice. Standard deviations (SD) and coefficients of variation (CV) were calculated (Stanton 1988).

Results and Discussion

The reproducibility of each of the parameters measured with the rheofermentometer was determined on six replicates using *S. cerevisiae 141*; all showed good reproducibility, with V_t, $V_{t/2}$, V_r, R_c, H_m and T_x having CV < 2.1% (Table 1).

Sour-dough Fermentation with Individual Starters

Sour-doughs were initially produced with commercial baker's yeast and CO_2-producing starters (yeasts and heterofermentative LAB). The fermentation with commercial baker's yeast was characterized by a V_t of 1513 ml (Table 2). The cell count after the 3 h fermentation was similar to the initial value and ethanol production was 179 mmol/kg (Table 3). The ratio between the molar concentrations of CO_2 and ethanol produced was 1:1.

The sour-dough fermentation with *S. cerevisiae* 141 showed only slight differences to that with commercial baker's yeast (Figure 1A) with V_t and ethanol concentration of 1437 ml and 170 mmol/kg, respectively (Tables 2 and 3). Due to the longer time (63 min) necessary to reach maximum gas production, a reduction in the flow speed from 1.51 to 1.06 mm/min was observed. *Saccharomyces cerevisiae* 141 had already produced 66% of its maximum CO_2 (Table 2) after 1.5 h but gas production continued throughout the 3 h of fermentation. The pH decreased from 5.95 to 5.75 but yeast-cell numbers at the end of leavening were about the same as initial values (Table 3). According to Yondem *et al.* (1992), the growth of *S. cerevisiae* 141 during sour-dough fermentation is characterized by an initial death phase (from 2×10^6 to 2×10^5 c.f.u./g), followed by a second period of growth in which cell numbers return to their initial value. In the present study, gas and ethanol production by *S. cerevisiae* 141 approached those of commercial baker's yeast even though the latter was used at higher cell concentrations (3×10^7 c.f.u./g compared with 10^5 to 10^6 c.f.u./g).

The sour-dough started with the maltose-negative *S. exiguus* M14 was characterized by very different kinetics (Figure 2A). The V_t value was much lower than that of *S. cerevisiae* 141 and porosity did not occur (upper and lower lines were superimposed). The very small difference between V_t and V_r values showed that the CO_2 produced was almost totally retained by the sour-dough (Table 2). This absence of porosity explains the unsatisfactory bread-crumb texture obtained. $V_{t/2}$ was 450 ml (83% of the total CO_2), indicating a short fermentative activity, probably due to the inability of *S. exiguus* M14 to utilize maltose (Sugihara *et al.* 1970). *Saccharomyces cerevisiae* 141, after depleting the soluble carbohydrates initially present in the flour, further extends the fermentation by using the maltose released from the damaged starch by the action of α- and β-amylases in the flour. The low fermentative power of *S. exiguus* was confirmed by the poor ethanol production of 67 mmol/kg (Table 3).

Table 1. Rheofermentometer parameters evaluated in six replicated sour-doughs using *Saccharomyces cerevisiae* 141 as starter.

	V_t (ml)	$V_{t/2}$ (ml)	V_r (ml)	R_c	H_m (mm)	T_i (min)	X (mm/min)	T_x (min)
Mean	1449	951	1197	0.82	66	65	1.03	58.33
Standard deviation	30.80	12.34	17.21	0.0076	0.96	9.16	0.15	1.15
Coefficient of variation (%)	2.1	1.3	1.4	0.9	1.4	14.1	14.6	1.97

Table 2. Rheofermentometer parameters of sour-doughs started with individual and associated lactic acid bacteria and yeasts.

Starter microorganism*	V_t (ml)	$V_{t/2}$ (ml)	V_r (ml)	R_c	H_m (mm)	T_i (min)	X (mm/min)	T_x (min)
Baker's yeast	1513	956	1225	0.81	67.9	45	1.51	23
S. cerevisiae 141	1437	948	1185	0.82	66.7	63	1.06	57
S. exiguus M14	543	450	535	0.99	47.6	33	1.44	–
L. brevis ssp. *lindneri* CB1	311	196	308	0.99	19.3	45	0.43	–
S. cerevisiae 141 + *L. brevis* ssp *lindneri* CB1	1427	1070	1182	0.83	76.1	21	3.62	37
S. exiguus M14 + *L. brevis* ssp. *lindneri* CB1	860	602	806	0.94	58.4	15	3.89	68
S. cerevisiae 141 + *L. plantarum* DC400	1591	1130	1257	0.79	78.6	63	1.25	37
S. cerevisiae 141 + *L. farciminis* CF3	1647	1120	1324	0.80	78.2	63	1.24	49
L. brevis ssp. *lindneri* CB1†	345	231	351	1.00	22.8	27	0.84	–
S. cerevisiae 141†	1789	1163	1397	0.78	73.2	39	1.88	49
S. cerevisiae 141 + *L. brevis* ssp. *lindneri* CB1†	1449	1087	1195	0.82	87.2	21	4.15	43

* The rheofermentometer parameters were not detectable in the sour-dough produced with the homofermentative *L. plantarum* DC400 strain.

† Sour-dough fermented with added fructose (6 g/kg).

Table 3. pH, cell numbers, lactic acid, acetic acid and ethanol in sour-doughs started with individual and associated lactic acid bacteria and yeasts, after 3 h at 28°C.‡

Starter microorganism	pH*	Cell number† (c.f.u./g)	Lactic acid (mmol/kg)	Acetic acid (mmol/kg)	Ethanol (mmol/kg)
Baker's yeast	5.72	3×10^7	2^a	1.5^a	179^a
S. cerevisiae 141	5.75	3×10^6	2^a	2.3^{be}	170^{ae}
S. exiguus M14	5.90	4×10^6	2^a	0.8^c	67^b
L. brevis ssp. *lindneri* CB1	4.50	3×10^8	24^b	3.3^d	31^c
L. plantarum DC400	4.36	2×10^8	35^c	ND	ND
S. cerevisiae 141 + *L. brevis* ssp. *lindneri* CB1	4.72	$2 \times 10^6 + 8 \times 10^7$	20^d	2.3^{be}	173^a
S. exiguus M14 + *L. brevis* ssp. *lindneri* CB1	4.54	$5 \times 10^6 + 2 \times 10^8$	24^b	3.0^d	94^d
S. cerevisiae 141 + *L. plantarum* DC400	4.38	$4 \times 10^6 + 1 \times 10^8$	36^c	2.5^b	175^a
S. cerevisiae 141 + *L. farciminis* CF3	4.40	$3 \times 10^6 + 9 \times 10^7$	23^b	2.0^e	164^e
L. brevis ssp. *lindneri* CB1§	4.39	2×10^9	25^b	15.0^f	12^f
S. cerevisiae 141§	5.53	3×10^6	2^a	3.0^d	228^g
S. cerevisiae 141 + *L. brevis* ssp. *lindneri* CB1§	4.53	$4 \times 10^6 + 9 \times 10^8$	23^b	8.5^g	234^g

* The initial pH of the sour-doughs was 5.95.

† The initial cell numbers of lactic acid bacteria and individual yeasts were 1×10^7 and 4×10^7 c.f.u./g (Baker's yeast) or 4×10^6 c.f.u./g (other yeasts), respectively.

‡ Values in the same column with different superscript letters differ significantly ($P < 0.05$).

§ Sour-doughs fermented with added fructose (6 g/kg).

ND—not detected.

The rheofermentometer data for *L. brevis* subsp. *lindneri* CB1 produced a low gas release curve (Figure 1B) with a V_t that was only 22% of that of *S. cerevisiae* 141 (Table 2). During the 3-h fermentation, cell numbers increased from 1×10^7 to 3×10^8 c.f.u./g. The final pH was 4.50, and lactic and acetic acid concentrations were 24 and 3.3 mmol/kg, respectively (Table 3), showing that *L. brevis* subsp. *lindneri* CB1 was responsible for most of the souring

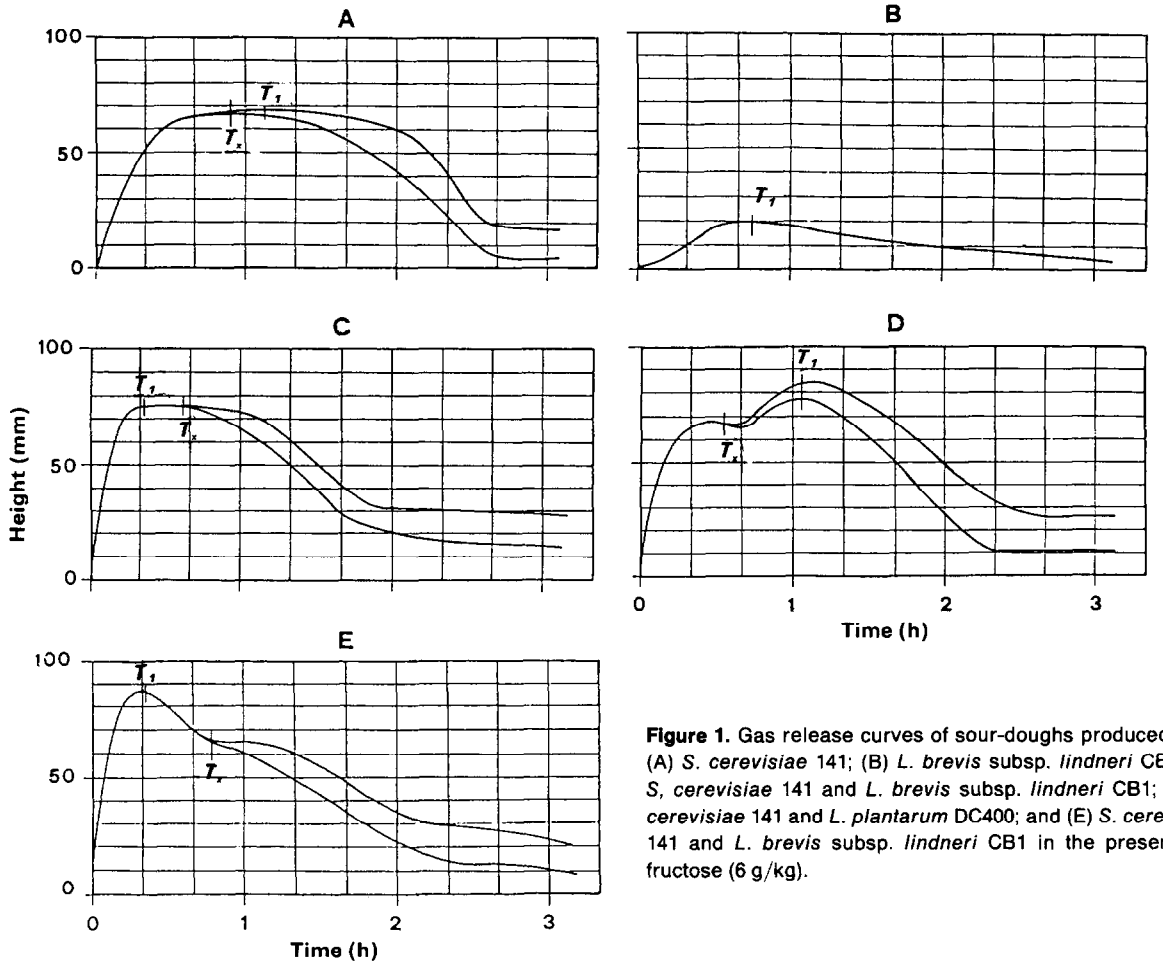

Figure 1. Gas release curves of sour-doughs produced with: (A) *S. cerevisiae* 141; (B) *L. brevis* subsp. *lindneri* CB1; (C) *S. cerevisiae* 141 and *L. brevis* subsp. *lindneri* CB1; (D) *S. cerevisiae* 141 and *L. plantarum* DC400; and (E) *S. cerevisiae* 141 and *L. brevis* subsp. *lindneri* CB1 in the presence of fructose (6 g/kg).

(Spicher 1983). As Martinez-Anaya *et al.* (1990) also found, the sour-doughs started with LAB alone gave unsatisfactory breads with flat loaves and coarse crusts and crumbs.

Sour-dough Fermentation by Combined Starters

Since leavening of yeasts is fundamental to obtain good bread production, the effects of combined yeast–LAB starters were compared with those of yeast starters used alone.

Compared with *S. cerevisiae* 141 alone, the associative growth of *S. cerevisiae* 141 and *L. brevis* subsp. *lindneri* CB1 decreased the time taken for the gas flow to reach its maximum value (H_m = 76.1 mm) from 63 to 21 min (Figure 1c) (Table 2). X increased from 1.06 to 3.62 mm/min and 75% of the total amount of CO_2 was produced after 1.5 h of fermentation. According to the CO_2 production, > 80% of the total ethanol production was also reached by 1.5 h (data not shown). At the end of fermentation, yeast cell numbers in the dough produced with the mixed starter were similar to those in the dough produced using just the yeast. The decrease in pH caused by *L. brevis* subsp. *lindneri* CB1 (Table 3) was probably responsible for an initial activation of yeast metabolism. Nevertheless, the heterofermentative LAB did not increase the ethanol content and gassing-power of the sour-dough and V_t was similar to that in the sour-dough produced with *S. cerevisiae* 141 alone. Previous results (Gobbetti *et al.* 1994b) showed that *S. cerevisiae* 141 strongly influenced carbohydrate evolution, causing a reduction in the *L. brevis* subsp. *lindneri* CB1 fermentation. Compared with LAB-started sour-dough, lactic and acetic acid productions were lower, at 20 and 2.3 mmol/kg, respectively and bacterial cell numbers were reduced from 3×10^8 to 8×10^7 c.f.u./g (Table 3). Since *S. cerevisiae* 141 also produces acetic acid (2.3 mmol/kg), the apparent decrease in the bacterial synthesis of this acid in dough produced by the mixed starter was probably under-estimated.

The positive influence of *L. brevis* subsp. *lindneri* CB1 on the sour-dough was strengthened when this LAB was associated with maltose-negative *S. exiguus* M14. H_m, T_1 and X were 47.6 mm, 33 min and 1.44 mm/min, respectively, in dough produced with *S. exiguus* M14 alone, compared with 58.4 mm, 15 min and 3.89 mm/min, respectively, in the combined fermentation (Figure 2C) (Table 2). Probably due to the lack of competition for the wheat soluble carbohydrates, since maltose is the preferred energy source for *L. brevis* subsp. *lindneri*, V_t (860 ml) as well as ethanol concentration

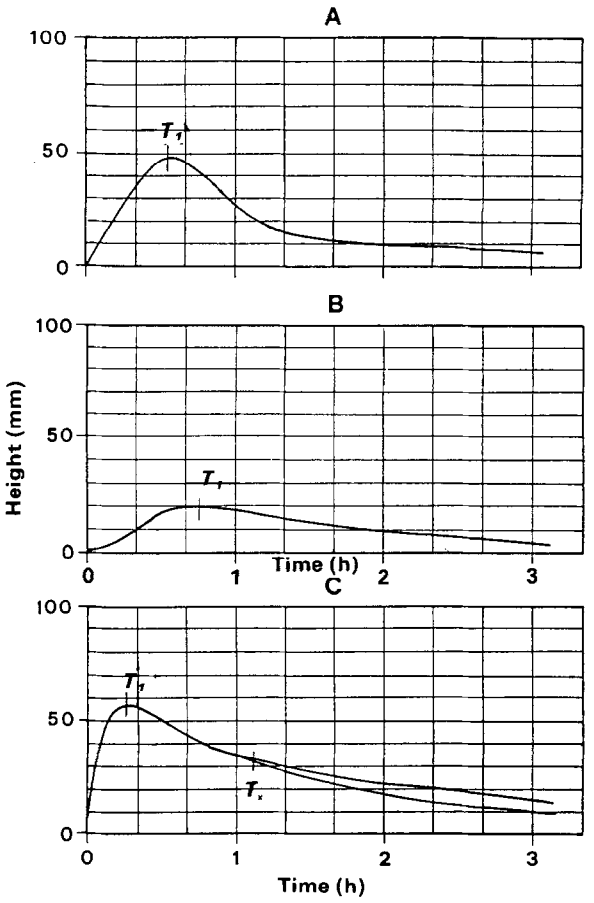

Figure 2. Gas release curves of sour-doughs produced with: (A) *S. exiguus* M14; (B) *L. brevis* subsp. *lindneri* CB1; and (C) *S. exiguus* M14 and *L. brevis* subsp. *lindneri* CB1.

(94 mmol/kg) (Table 3) were about the sum of those produced by each microorganism alone. Porosity appeared (T_x = 68 min) and the sour-dough achieved a satisfactory texture. Not only was fermentation accelerated, as seen when the combined *S. cerevisiae* 141–*L. brevis* subsp. *lindneri* CB1 starter was used, but this combination also benefited from the lack of competition between many of the metabolic demands of the two microorganisms. Bacterial cell numbers and organic acid production were the same as when *L. brevis* subsp. *lindneri* CB1 was used alone (Table 3).

When *L. plantarum* DC400 was combined with *S. exiguus* M14 it produced a dough similar to that fermented by the yeast alone (data not shown). However, the presence of this LAB positively modified *S. cerevisiae* 141 fermentation; in agreement with Hansen *et al.* (1989), who demonstrated a greater adaptability of yeasts to grow in association with homofermentative rather than with heterofermentative LAB, H_m in the presence of *L. plantarum* DC400, increased from 66.7 to 78.6 mm at the same T_1 and V_f reached 1591 ml with porosity appearing at 37 min (Figure 1D) (Table 2). The gas release curve was characterized by an inflection point at about 45 min of fermentation, and the same kinetics was observed with the *S. cerevisiae* 141–*L. farciminis* CF3 association. The sour-dough produced with *S. cerevisiae* 141–*L. plantarum* DC400 contained more lactic acid (16 mmol/kg) than that produced by *L. brevis* subsp. *lindneri* CB1–*S. cerevisiae* 141 (Table 3). While acetic acid, which is mainly produced by heterofermentative LAB, is responsible for a shorter and harder gluten, lactic acid can gradually account for a more elastic gluten structure (Stegemann & Rohrlich 1958; Lorenz 1983). With the supplementary maltose from starch hydrolysis, this elastic structure retains the CO_2 produced during the last fermentation phase and characterizes the gas release curves of Figure 1D. Neither cell numbers of *L. plantarum* DC400 nor lactic acid production decreased when this LAB was grown in association with *S. cerevisiae* 141 (Table 3).

Sour-dough Fermentation in the Presence of Fructose

Since it was previously demonstrated (Gobbetti *et al.* 1995) that the addition of fructose to dough increased acetic acid production by *L. brevis* subsp. *lindneri* CB1 and enhanced the fermentation quotient (the molar ratio between lactic and acetic acids) the effect of fructose on the leavening kinetics of a starter of *L. brevis* subsp. *lindneri* CB1 and/or *S. cerevisiae* 141 was also evaluated. Addition of fructose to the sour-dough produced using *L. brevis* subsp. *lindneri* CB1 alone increased acetic acid production (from 3.3 to 15 mmol/kg) and cell numbers (from 3×10^8 to 2×10^9 c.f.u./g), decreased ethanol production from 31 to 12 mmol/kg (Table 3), slightly increased CO_2 production (V_f increasing from 311 to 345 ml) and almost doubled the gas production rate (X increasing from 0.43 to 0.84 mm/min) (Table 2). Although addition of fructose to dough started with just *S. cerevisiae* 141 had no effect on the cell counts, it increased the final amount of ethanol produced and the leavening resulted in the highest V_f value (1789 ml) for any sour-dough produced (Tables 2 and 3). Addition of sucrose, glucose and maltose to doughs also stimulated yeast fermentation but did not cause similar increases in bacterial activity (data not shown). *Lactobacillus brevis* subsp. *lindneri* CB1 has the capacity to co-ferment fructose in the presence of maltose (Gobbetti *et al.* 1995). Fructose, in part, pushes the metabolism toward the acetate kinase pathway, producing traces of mannitol, increasing acetic acid production and reducing ethanol concentration. In sour-doughs produced with the combined starter, addition of fructose accelerated starter activity (after 21 min of fermentation the maximum value of gaseous flow increased from 76.1 to 87.2 mm with a flow speed of 4.15 mm/min) (Figure 1E) (Table 2), produced the highest ethanol concentration observed and ensured the appropriate LAB fermentation in terms of pH, cell numbers and lactic and acetic acid production (Table 3).

This study is the first in which a rheofermentometer was used to characterize the leavening of sour-doughs by LAB

and yeast starters. This made it relatively easy to evaluate starter performance and this should facilitate production of better sour-dough bread.

Acknowledgements

The present research was supported by National Research Council of Italy, Special Project RAISA, Sub project 4, Paper n 2314. The authors gratefully acknowledge the technical assistance of D. Babou, S. Theodorakis and L. Mariotti.

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(Received in revised form 14 April 1995; accepted 3 June 1995)