# Sourdough Lactic Acid Bacteria Effects on Bread Firmness and Staling

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ABSTRACT

***Lactobacillus sanfrancisco* CB1 and *Lactobacillus plantarum* DC400 were the most proteolytic and amylolytic strains studied. Breads started with LAB and yeasts had higher volumes than the baker's yeast-started bread. One bread with the highest initial firmness (*Saccharomyces cerevisiae* 141-*L. plantarum* DC400 starter) had the lowest final firmness. Breads produced with LAB showed the lowest enthalpy throughout 144 h. After 24 h storage the associations of *S. cerevisiae* 141 and *L. sanfrancisco* CB1 or *L. plantarum* DC400 gave a very low percentage increase of enthalpy compared to that from yeast alone. The enthalpy increased markedly when other LAB, neither proteolytic nor amylolytic, were used.**

**Key Words: bread, sourdough, lactic acid bacteria, firmness, staling**

## INTRODUCTION

PHYSICOCHEMICAL CHANGES (E.G. STALING, FIRMING) AND MICRObiological spoilage (e.g. ropiness, mold growth) markedly reduce the shelf-life of bread. Attempts have been made to improve the keeping quality of bread either by varying the product formulation (Ludewig, 1988; Ortolá et al., 1989), processing (Salovaara and Valjakka, 1987) or packaging conditions (Knorr and Tomlins, 1985; Ortolá et al., 1989). The addition to the dough of compounds occurring naturally in wheat flour (fructose and organic acids) has been proposed to direct the fermentation since they produce specific compounds associated with desired characteristics and good bread keeping quality (Barber et al., 1992; Gobbetti et al., 1995a).

The limited knowledge of the physicochemical changes involved in the mechanisms of bread staling and the economic importance of a longer keeping quality make this subject of great interest. Gluten and its ratio with starch were decisive factors in bread firmness with great influence on elastic changes during bread storage (Banecki, 1982). Variations of water activity and moisture contents in the near-crust area also influence bread staling and firmness (Czuchajowska and Pomeranz, 1989). Spontaneous sourdoughs with low pH and a high ratio of lactic and acetic acids produced breads with the highest volumes and lowest rates of staling during storage (Barber et al., 1992). The level of native lipids in intact flour was enhanced by shortening and was effective in decreasing firmness (Rogers et al., 1988). Changes in the ratio of amylose to amylopectin by adding waxy barley starch to a high-protein wheat flour resulted in softer bread one day after baking or after reheating (Ghiasi et al., 1984). Low-level treatments with α-amylases from different sources increased the water-binding capacity and gelatinization temperatures of starch as well as the bread-baking quality (Kuracina et al., 1987). Physical methods like crumb firmness and elasticity evaluation (Berglund and Shelton, 1993) and calorimetry analysis of starch changes during staling (Zeleznak and Hoseney, 1987) have also classically been used to study storage changes.

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Use of sourdough produces breads or baked goods indicated to have more flavor, and improved rheology and storage characteristics over products obtained using baker's yeast (Spicher, 1983; Röcken, 1996). Sourdough lactic acid bacteria (LAB) and yeasts have been shown to compete for carbon sources which influence acid production by bacteria (Gobbetti et al., 1994a). Homo- and hetero-fermentative LAB have been extensively evaluated for acidification properties, production of volatile compounds and proteolysis and have shown great differences among and within species (Gobbetti et al., 1995b, c, 1996a; Damiani et al., 1996). Mixed freeze-dried starter formulations have been prepared to overcome some difficulties of sourdough handling (Martinez-Anaya et al., 1993; Cossignani et al., 1996). Sourdough LAB have been shown to inhibit or delay microbial spoilage thus improving bread shelf-life (Spicher, 1983).

Acidification of the dough, proteolysis of gluten and moderate hydrolysis of starch are LAB activities which vary among sourdough strains and which may affect the physicochemical changes throughout bread shelf-life. No studies have been reported on the effect of different sourdough starters on bread staling. Our objective was to study various associations of LAB and yeast starters and their influence on the kinetics of sourdough bread firmness and staling.

## MATERIALS & METHODS

### Microorganisms

*Lactobacillus sanfrancisco* CB1 (*L. brevis* subsp. *lindneri*) and *Lactobacillus fructivorans* DD10 (heterofermentative strains), *Lactobacillus plantarum* DC400 and *Lactobacillus farciminis* A80 (homofermentative strains), *Saccharomyces cerevisiae* 141 and *Saccharomyces exiguus* M14 were used from the culture collection of the Institute of Dairy Microbiology, Agricultural Faculty of Perugia, Italy and were previously identified from Italian sourdoughs (Gobbetti et al., 1994b). LAB and yeasts were cultured on sour dough bacteria (SDB; maltose 20 g/L, tryptone 6 g/L, yeast extract 3 g/L, Tween 80 0.3 g/L, fresh yeast extractives 500 mL/L, final pH 5.6) (Kline and Sugihara, 1971) and Sabouraud (Difco Laboratories, Detroit, MI) media, respectively, at 28°C for 24h. Cells were harvested at 10,000 × *g* for 10 min, washed twice with sterile, distilled water and then resuspended in sterile water which, diluted 1:10, gave an optical density (620 nm) of 1.25, yielding about $10^9$ and $10^7$ CFU/mL for LAB and yeasts, respectively.

### Assessments for proteolysis and starch hydrolysis

The selected LAB were previously assayed for proteolytic activity and capacity to hydrolyze starch. Proteinase activity was measured by the method of Twinning (1984) using fluorescent casein as substrate. The mixture reaction contained 20 μL of the described bacterial suspension and 180 μL of 1% (w/v) fluorescent casein in phosphate buffer pH 7.0, 0.1M. After 3 h incubation at 30°C, a unit (U) of proteinase activity was defined as the variation of 0.1 unit of fluorescence per 10 min.

Starch hydrolysis was determined in the presence of 20 μL of bacterial suspension and 180 μL of phosphate buffer pH 7.0, 0.1M, containing 10 g/L (w/v) of soluble starch. After 3 h incubation at 30°C the residual concentration of starch was enzymatically determined (Boehringer Mannheim).

### Dough kneading, fermentation and baking

The wheat flour contained: moisture 12.8%, protein (N × 5.70)

10.6% of dry matter (d.m.), fat 1.79% of d.m., and ash 0.60% of d.m.. Wheat flour (250 g), 110 mL tap water and 40 mL of cellular suspension, containing one or more microorganisms at the described cellular concentrations, were used to produce 400 g of dough (dough yield = 160) with a continuous high speed mixer (60 × *g*; optimal dough mixing time = 5 min) (Chopin & Co., Boulogne, Seine, France). When commercial baker's yeast was used, 2.5 g of cells were resuspended in 40 mL of sterile distilled water. Depending on the dry weight of the cells, the amount of tap water was slightly modified in order to provide the same final dough yield. The value of 160 was the usual dough yield for sourdoughs produced by wheat flour (Spicher, 1983).

Doughs (400 g) were individually placed in aluminum pans (25 cm × 10 cm, × ht 8 cm) and incubated at 28°C for 150 (yeast and LAB starters) or 190 min (yeasts alone). Fermentation times were selected on the basis of assays conducted by a Chopin Rheofermentometer $F_2$ (Groupe Tripette et Renaud, Villeneuve-La-Garenne, Cedex, France) which showed a maximum volume of $CO_2$ produced by baker's yeast and *S. cerevisiae* 141 in 190 min and an initial activation of yeast metabolism by homo- and heterofermentative LAB (Gobbetti et al., 1995d). After fermentation the sourdoughs were baked in a batch oven (Mondial Forni, Verona, Italy) at 220°C for 30 min. Sourdough breads were then cooled at room temperature (ca 20°C) for 90 min, vacuum packed in polyethylene bags, 95 μm thickness (Tillmanns S.p.a., Milan, Italy) and stored at 20°C for 192 h (Czuchajowska and Pomeranz, 1989; Barber et al, 1992). The presence of molds in a few stored loaves marked the end of the sourdough bread shelf-life. The parameters applied for producing sourdough breads corresponded to the conventional Italian breadmaking processes (Quaglia, 1984).

### Sourdough and bread characteristics

LAB and yeasts were determined before and after dough fermentation by plating on SDB or Wallerstein Laboratory (WL) Nutrient (Difco) media at 28°C for 72 h, respectively.

The pH, total titratable acidity (TTA) and lactic acid concentration (Boehringer Mannheim) were determined in samples as described by Corsetti et al. (1994). The loaf volume was measured by rapeseed displacement (Barber et al., 1992). After differential scanning calorimetry measurements, the dry matter of each individual bread was determined by puncturing and drying the pan at 105°C for 12 h.

The total concentration of amino acids in the sourdoughs was determined by gas chromatography (Chrompack 9000, Middelburg, The Netherlands) on a 25 m × 0.53 mm fused silica column (Chrompack) with CP-Sil-8 CB as stationary phase and flame ionization detection (Gobbetti et al., 1994c).

### Crumb firmness measurements

The crumb firmness of samples during storage was determined using an Instron Universal Testing Machine (UTM). Analyses were conducted according to the AACC method 74-09 (1986) on 25 mm thick slices which were compressed to 40% of their original height. The force (Newton = N) reading, measured at 25% of compression, expressed the resistance of the crumb to the penetrating plunger and represented the crumb firmness.

### Differential scanning calorimetry

For differential scanning calorimetry (DSC) we used a Perkin Elmer DSC-4 (Perkin Elmer, Norwalk, Connecticut, USA) fitted with a Thermal Analysis Data Station and a Graphics Plotter HP7470A. Three loaves were analyzed for each sourdough bread production. Before analysis, one slice was cut from the middle of the loaf and pieces were taken in triplicates from the center (3.5 cm dia) of the slice and subsequently blended. Aliquots of crumb (ca 20 mg) were then placed in a preweighed sample pan, which was sealed and reweighed. The sample pan was placed in the calorimeter at 30°C, allowed to equilibrate 10 min and heated to 85°C at 10°C/min. A pan containing aluminum, of about the same weight, was used as reference. Endotherms were analyzed (Thermal Analysis Data Station) to calculate enthalpy values ($\Delta Hg$), peak onset temperature (To) and peak maximum temperature (Tp). The temperature axis of the instrument was calibrated using indium.

The variations of enthalpy during bread storage were calculated as percentage increases with respect to values determined after 2h storage. This was assumed to indicate fresh breads which contained practically no retrograded amylopectin (Siljeström et al., 1988). Enthalpy values for fresh crumb reported by others have ranged from 0.10 (Czuchajowska and Pomeranz, 1989) to > 1.9–3.0 J/g (Barber et al., 1992).

### Statistical analysis

Proteinase and amylolytic activities, sourdough and bread characteristics, and DSC values after 2 h storage were analyzed by one-way analysis of variance based on 3 replicates. Mean separation, in the presence of a significant F value, was based on the least significant difference. UTM and DSC values were analyzed as a factorial experiment where each combination of starter and time of storage was replicated three times. A correlation analysis between the volumes of breads, firmness and DSC values was also carried out.

All statistical analysis were carried out by S.A.S. package (SAS Institute, Inc., 1985), available at the Computer Centre of the University of Perugia. Significance of differences was defined at $P < 0.05$.

## RESULTS & DISCUSSION

### Tests for proteolysis and starch hydrolysis

The LAB used to produce sourdoughs differed markedly in proteolysis (Table 1). *L. sanfrancisco* CB1 showed a proteinase activity 10 times higher than *L. fructivorans* DD10 and *L. farciminis* A80, while *L. plantarum* DC400 gave an intermediate hydrolysis of the fluorescent casein. Further incubation markedly lessened the differences between strains CB1 and DC400 (data not shown). *L. sanfrancisco* has been reported to be the most proteolytic sourdough LAB (Gobbetti et al., 1996a) and its proteolytic enzymes have been characterized (Gobbetti et al., 1996b). Except for *L. plantarum* DC400, no starch hydrolysis was detected. Amylolytic strains of *L. plantarum* have been identified from plant environments (Giraud et al., 1991).

### Characteristics of sourdoughs

Starting with a cellular concentration of $10^6$ yeast and $10^8$ bacteria CFU/g dough, the yeast (1.0 to 5.3 × $10^5$ CFU/g) and LAB (4.5 to 8.1 × $10^7$ CFU/g) cell numbers decreased at the end of sourdough fermentations (Table 2). These results were similar to those reported by Yondem et al. (1992) and Gobbetti et al. (1995d) which showed an initial cell death phase followed by a second period of growth when fresh cells were used as starters in a short time fermentation. In the mixed starters, the final ratio between LAB and yeasts was confirmed to be 100:1 (Gobbetti et al., 1994b). Starting from common values of pH 5.5 and TTA 4.0 (mL of 0.1N NaOH/10 g dough), the sourdoughs produced with homofermentative LAB such as *L. plantarum* DC400 and *L. farciminis* A80 had the lowest pH and highest TTA. The lactic acid production followed the same trend, being the highest (4.2 g/kg) in all sourdoughs fermented with *L. plantarum* DC400. Heterofermentative LAB showed a lesser acidification activity. In agreement with the proteolysis results, the concentration of free amino acids (995

Table 1—Proteinase activity (Units) on fluorescent casein and starch hydrolysis (g/L) by different *Lactobacillus* strains

| Strains | Proteinase activity (U) | Hydrolyzed starch (g/L) |
|---|---|---|
| *L. sanfrancisco* CB1 | 33.5a (4.7)[a] | 0.17b (0.06) |
| *L. fructivorans* DD10 | 2.8c (0.3) | 0.12b (0.03) |
| *L. plantarum* DC400 | 12.4b (1.9) | 1.83a (0.30) |
| *L. farciminis* A80 | 3.4c (0.3) | 0.11b (0.02) |

[a]Mean values in the same column followed by the same letters are not different at P>0.05. Numbers reported in parentheses are standard deviation of means.

to 875 mg/kg) was two times higher in all the sourdoughs started with *L. sanfrancisco* CB1 than in the others. Doughs fermented with yeasts alone showed decreased initial concentrations of free amino acids. All these characteristics were within ranges of values determined for Italian sourdoughs (Gobbetti et al., 1994d).

### Characteristics of breads

The main differences of pH and TTA of sourdoughs produced with different starters were maintained in the breads (Table 2). A moderate decrease of acidity occurred due to the effect of baking.

Volumes of sourdough breads produced with LAB were higher than when baker's yeast or *S. cerevisiae* 141 were used alone. In these values no substantial differences were observed using baker's yeast or the pure culture of strain 141. Two main groupings (795 –805 mL and 820–838 mL) were found when LAB were associated with yeasts and the highest volumes (835–838 mL) were obtained when homofermentative strains (DC400 and A80) were individually associated with *S. cerevisiae* 141. The four breads in the highest volume group were most highly acidified. Barber et al. (1992) reported that breads produced with spontaneous sourdoughs had higher volumes and better crumb grain than those started with conventional baker's yeast. Gobbetti et al. (1995d) showed a greater fermentative capacity of yeasts when associated with homo- rather than heterofermentative LAB. The volume of individual loaves did not change notably during storage in polyethylene bags (data not shown).

The moisture content of the bread crumbs ranged from 44.5 to 45.5%, decreased to an average 43.0% by the end of storage (8 days) and no appreciable differences were related to the type of starter. No effects of strains on the variation of moisture during baking and storage were reported by Barber et al. (1992). Other analyses (Barber et al., 1992; Rogers et al., 1988) on freeze-dried sourdough bread samples which were standardized for moisture content showed slightly higher values of enthalpy than fresh stored breads but no difference in percentage increases.

### Crumb firmness during bread storage

The dependent variable, firmness, increased throughout the time of storage, with values from 9.2N (at 24 h) up to 27.6N (at 192 h storage) (Table 3). Significant differences were found among starters where, as averages over time, the association of *S. cerevisiae* 141 with *L. plantarum* DC400 had the lowest firmness value ($P<0.05$). As shown by a significant starter by time interaction, changes in crumb firmness over time differed. A rapid initial increase (from 24 to 48 h) was found in all starters, but from 48 to 96 h only the firmness of 141-DC400 and that of the starter 141-M14-CB1-DC400 did not change. The firmness of bread produced by the former starter did not increase further up to 192 h showing, at that time, the lowest value among all samples ($P<0.05$). On the contrary, the initially soft bread, 141-CB1, increased in firmness and at 8 days had the highest compression force (33.6N) which differed ($P<0.05$) from all other sourdough breads. Some studies (Axford et al., 1968; Stöllman and Lundgren, 1987) have reported an increase in firmness with decreasing volume of breads. Although the starter *S. cerevisiae* 141-*L. plantarum* DC400 gave the highest volume and the lowest firmness, no correlation ($r \leq 0.22$) was found between these two parameters considering data from all the sourdough breads. Sourdough breads with pH 4.18 to 4.54 (e.g. when *S. cerevisiae* 141 was associated with *L. plantarum* DC400 or *L. farciminis* A80) and similar volumes (Table 2) had very different increases in firmness over time (Table 3). *L. plantarum* DC400 produced bread with the lowest pH and was the only amylolytic strain used. Barber et al. (1992) reported that breads baked with malted whole grain wheat flours containing high amounts of dextrins were characterized by a longer storage time than those from unmalted flour. Sourdough breads produced with the associations of two yeasts (141 and M14) and LAB (CB1 and DC400) showed an intermediate firmness which may reflect the opposing influences of individual bacterial strains. Due to limited variations of moisture during storage under vacuum packaging, we confirmed that, besides the type of starter used, bread firming was predominantly influenced by other physicochemical factors (Czuchajowska and Pomeranz, 1989).

At 10 days storage, the breads produced by yeast alone had some external molds. A delayed (7 days) molding was observed for breads produced with LAB (data not shown). A study on the inhibitory activity of sourdough LAB against molds is in progress at our laboratory.

### Differential scanning calorimetry

DSC values (Table 4) for the first endothermal peak (between 35 and 70°C) referred to as the staling endotherm (Russel, 1983) and assigned to melting of retrograded amylopectin (Eliasson, 1985) were considered (Piazza et al., 1994). According to other studies (Czuchajowska and Pomeranz, 1989; Barber et al., 1992), preliminary DSC measurements conducted in the range 100–130°C to dissociate the amylose-lipid complexes showed no differences among samples or during storage (data not shown). The enthalpy values of the staling endotherm of sourdough breads after 2h storage ($\Delta Hg$ = 0.30 - 0.52 J/g) were assumed to be indicative of fresh breads which contained practically no retrograded amylopectin (Siljeström et al., 1988). No differences were detected within the various sourdough breads or during the storage for the onset temperature or for the maximum peak temperature. As reported by Soulaka and Morrison (1985), Tp values are mainly influenced by the storage temperature rather than by other

Table 2—Characteristics of sourdough and corresponding bread samples

| | Sourdoughs | | | | | Breads | | |
|---|---|---|---|---|---|---|---|---|
| **Starters** | **CFU/g yeasts** | **CFU/g bacteria** | **pH** | **TTA mL NaOH** | **Amino acids[a] mg/kg** | **pH** | **TTA mL NaOH** | **Volume (mL)** |
| Baker's yeast | 2.0 x $10^5$a (0.6)[b] | | 5.32a (0.00) | 3.50c (0.25) | 354e (31.2) | 5.63a (0.05) | 2.00c (0.10) | 770.41e (9.53) |
| *S. cerevisiae* 141 | 1.0 x $10^5$a (0.4) | | 5.35a (0.02) | 3.30c (0.05) | 382ed (28.3) | 5.68a (0.03) | 2.10c (0.05) | 769.86e (6.67) |
| *S. cerevisiae* 141 + *L. sanfrancisco* CB1 | 1.0 x $10^5$a (0.4) | 5.2 x $10^7$a (0.4) | 4.72b (0.02) | 4.50b (0.25) | 995a (39.0) | 4.98b (0.03) | 3.20b (0.15) | 795.40d (4.21) |
| *S. cerevisiae* 141 + *L. sanfrancisco* CB1 + *L. plantarum* DC400 | 1.0 x $10^5$a (0.3) | 8.1 x $10^7$a (0.4) | 4.01f (0.01) | 6.65a (0.15) | 912b (26.9) | 4.23ef (0.04) | 4.10a (0.15) | 820.46b (5.40) |
| *S. cerevisiae* 141 + *L. plantarum* DC400 | 5.3 x $10^5$a (0.5) | 7.2 x $10^7$a (0.5) | 4.04f (0.03) | 6.40a (0.15) | 712c (23.3) | 4.18f (0.05) | 4.10a (0.20) | 835.71a (5.81) |
| *S. cerevisiae* 141 + *S. exiguus* M14 + *L. sanfrancisco* CB1 + *L. plantarum* DC400 | 1.7 x $10^5$a (0.3) | 6.5 x $10^7$a (0.6) | 4.11e (0.01) | 6.35a (0.15) | 875b (27.5) | 4.30e (0.03) | 4.10a (0.10) | 822.13b (6.81) |
| *S. cerevisiae* 141 + *L. farciminis* A80 | 5.2 x $10^5$a (0.2) | 4.5 x $10^7$a (0.5) | 4.34d (0.04) | 6.30a (0.20) | 410d (25.4) | 4.54d (0.05) | 4.20a (0.30) | 838.73a (7.18) |
| *S. cerevisiae* 141 + *L. fructivorans* DD10 | 1.4 x $10^5$a (0.3) | 5.1 x $10^7$a (0.4) | 4.60c (0.05) | 4.60b (0.25) | 395ed (26.0) | 4.80c (0.05) | 3.80b (0.20) | 805.12c (7.03) |

[a]The initial concentration of amino acids in the dough was 423 mg/kg.
[b] Mean values in the same column followed by the same letters are not different at $P > 0.05$. Values in parentheses are standard deviation of means.

Table 3—Firmness values (N) of sourdough bread crumb during storage

| Starters | 24h | 48h | 96h | 144h | 192h | Average over time[a] |
|---|---|---|---|---|---|---|
| Baker's yeast | 9.2mn (0.3)[b] | 17.4kl (0.9) | 22.1ghi (1.5) | 24.8defg (2.6) | 27.3bcde (1.5) | 20.2[c] |
| *S. cerevisiae* 141 | 8.7n (1.1) | 19.1ijkl (1.7) | 23.8fgh (1.4) | 24.0fg (1.0) | 27.6bcd (2.2) | 20.7CB |
| *S. cerevisiae* 141 + *L. sanfrancisco* CB1 | 7.5n (2.0) | 20.2ijk (1.5) | 28.5bc (0.9) | 29.6b (1.9) | 33.6a (1.0) | 23.9A |
| *S. cerevisiae* 141 + *L. sanfrancisco* CB1 + *L. plantarum* DC400 | 8.6n (1.3) | 16.7l (1.3) | 27.4bcde (1.4) | 28.2bc (0.9) | 29.1bc (7.6) | 22.0B |
| *S. cerevisiae* 141 + *L. plantarum* DC400 | 11.8m (0.8) | 18.7jkl (1.8) | 19.7ijkl (1.6) | 20.0ijk (1.6) | 22.0ghi (3.1) | 18.4D |
| *S. cerevisiae* 141 + *S. exiguus* M14 + *L. sanfrancisco* CB1 + *L. plantarum* DC400 | 10.4mn (1.9) | 18.7jkl (2.3) | 20.9hij (1.3) | 23.5fgh (2.0) | 26.4cdef (0.2) | 20.0C |
| *S. cerevisiae* 141 + *L. farciminis* A80 | 8.6n (0.8) | 18.8jkl (0.9) | 23.4fgh (0.8) | 24.4efg (1.0) | 27.8bcd (0.7) | 20.6CB |
| *S. cerevisiae* 141 + *L. fructivorans* DD10 | 8.8n (0.7) | 19.4ijkl (0.9) | 24.2fg (1.0) | 24.9cdefg(1.0) | 27.3bcde (0.5) | 20.9CB |
| Average over starter[c] | 9.2E | 18.6D | 23.8C | 24.9B | 27.6A | |

[a]Mean values over time followed by the same capital letters are not different at P > 0.05.
[b]Mean values followed by the same small letters are not different at P > 0.05. Values in parentheses are standard deviation of means.
[c]Mean values over starter followed by the same capital letters are not different at P > 0.05.

Table 4 —Differential scanning calorimetric (DSC) data for melting amylopectin crystallites in the crumb of bread stored 2h and percentage increases of enthalpy (Δ*Hg*) during storage

| | DSC after 2h storage | | | Percent increase in *DHg* during storage[a] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Starters | To (°C)[b] | Tp (°C)[b] | *ΔHg* (J/g) | 24h | 48h | 96h | 144h | 192h | Average over time[c] |
| Baker's yeast | 51.26a (1.28)[d] | 58.61a (1.75) | 0.40ab (0.09) | 250ijk (44.1)[e] | 353h (24.0) | 466fg (29.5) | 584ab (21.8) | 595a (9.3) | 450A |
| *S. cerevisiae* 141 | 49.48a (1.74) | 61.87a (2.29) | 0.30b (0.01) | 245ijk (16.6) | 340h (28.9) | 495def (46.5) | 611a (28.2) | 614a (61.7) | 461A |
| *S. cerevisiae* 141 + *L. sanfrancisco* CB1 | 51.98a (1.78) | 58.26a (2.16) | 0.52a (0.07) | 104n (27.2) | 182lm (22.3) | 245ijk (5.6) | 235jk (9.7) | 244ijk (11.7) | 202E |
| *S. cerevisiae* 141 + *L. sanfrancisco* CB1 + *L. plantarum* DC400 | 48.30a (1.93) | 58.74a (2.61) | 0.38ab (0.08) | 204klm (9.0) | 237jk (52.4) | 373h (29.8) | 464fg (25.2) | 475efg (19.9) | 350C |
| *S. cerevisiae* 141 + *L. plantarum* DC400 | 49.09a (1.35) | 60.18a (2.12) | 0.44ab (0.01) | 164m (16.5) | 250ijk (18.0) | 274ij (17.3) | 364h (35.5) | 370h (14.6) | 284D |
| *S. cerevisiae* 141 + *S. exiguus* M14 + *L. sanfrancisco* CB1 + *L. plantarum* DC400 | 50.79a (1.41) | 60.65a (2.02) | 0.35b (0.06) | 229jkl (54.9) | 377h (69.0) | 441g (15.5) | 445g (49.0) | 452fg (25.7) | 389B |
| *S. cerevisiae* 141 + *L. farciminis* A80 | 48.79a (1.86) | 61.75a (2.34) | 0.38ab (0.02) | 211klm (2.9) | 289i (22.1) | 385h (32.4) | 516cde (26.5) | 531cd (26.5) | 387B |
| *S. cerevisiae* 141 + *L. fructivorans* DD10 | 49.12a (1.99) | 60.36a (2.26) | 0.45ab (0.08) | 182lm (8.2) | 277ij (20.9) | 361h (15.7) | 533cd (11.4) | 545bc (9.2) | 380B |
| Average over starter[f] | | | | 199D | 288C | 380B | 469A | 478A | |

[a] The percentage increases refer to the Δ*Hg* calculated after 2 h of storage.
[b]To = onset temperature; Tp = maximum peak temperature.
[c]Mean values over time followed by the same capital letters are not different at P > 0.05.
[d]Mean values in the same column followed by the same small letters are not different at P > 0.05. Values in parentheses are standard deviation of means.
[e]Mean values followed by the same small letters are not different at P > 0.05.
[f]Mean values over starter followed by the same capital letters are not different at P > 0.05.

breadmaking parameters.

During the first 144h storage, the enthalpy values (as a percentage of values determined after 2 h) sharply increased in almost all sourdough breads but no changes were observed in the subsequent 48h ($P<0.05$) (Table 4). According to Czuchajowska and Pomeranz (1989), these results, together with the slight change in moisture throughout storage, indicated that the retrogradation of the amylopectin may not be avoided by either packaging or by storage of the crumb under conditions which reduce moisture loss.

A significant interaction was found for starter by time of storage. Well-defined differences were found within the different sourdough breads. The starter 141-CB1 showed the lowest average increase (202%), followed by 141-DC400 (284%) and 141-CB1-DC400 (350%) ($P<0.05$) (Table 4). Highest values were in breads started with *S. cerevisiae* 141 or baker's yeast (461 and 450%, respectively).

Except for the starter which included two yeasts (141-M14-CB1-DC400) and that based on *S. cerevisiae* 141-*L. farciminis* A80, the *ΔHg* increases at 24h were lower in all breads started with LAB than in those which used *S. cerevisiae* 141 or baker's yeast. Especially no increase of enthalpy was observed after 96 h with *S. cerevisiae* 141-*L. sanfrancisco* CB1. This starter gave the bread with the lowest percentage increases of enthalpy throughout storage with a final value of 244% which was <40% of that from bread started with *S. cerevisiae* 141 alone. *L. sanfrancisco* CB1 was the most proteolytic strain used (Table 1); its proteolytic system has been previously characterized (Gobbetti et al., 1996a) and a proteinase, a dipeptidase and an aminopeptidase were purified (Gobbetti et al., 1996b). Contrary to the other strains used, the hydrolysis of the gluten subunits by *L. sanfrancisco* CB1 produced a marked increase of free amino acids at the end of fermentation (Table 2). Gluten was a deciding factor in bread staling (Banecki, 1982). The sourdough bread produced by *S. cerevisiae* 141 and *L. plantarum* DC400 behaved similarly (final percentage increase of enthalpy 370%). As reported by the UTM analysis (Table 3), this association was optimal for delaying bread firmness. The partial correlation between results from the two methods (UTM and DSC) also indicated by others (Barber et al., 1992), has been attributed to different sensitivities to changes which occur at a molecular level or to macroscopic properties of the bread. The molecular modifications produced in the gluten-starch network by the proteolysis of *L. sanfrancisco* CB1 retarded staling (as determined by DSC) but probably had negative effects on firmness. As reported by Fearn and Russel (1982) DSC values were irrespective of the volume of breads and

did not correlate well ($r \leq 0.32$). The final increases of enthalpy in sourdough breads produced by other homo- (*L. farciminis* A80) and heterofermentative (*L. fructivorans* DD10) strains were very high (>530%) and did not show the same positive effect on retarding the staling. These strains did not greatly differ from strains DC400 and CB1 for acidification properties (Table 2) but were neither proteolytic nor amylolytic (Table 1). Breads produced with various associations of yeasts (141 and M14) and bacteria (CB1 and DC400) strains showed higher enthalpy values than those started with *S. cerevisiae* 141 and individual LAB. As for other sourdough characteristics (e.g. souring, production of volatiles) (Gobbetti et al. 1995b, c; Damiani et al., 1996), the selection of strains as well as microbial associations should be considered for their effects on bread staling.

As reported previously (Czuchajowska and Pomeranz, 1989; Barber et al., 1992; Piazza et al., 1994), bread staling is very complex and concomitant causes may have either augmenting or opposing effects. Siljeström et al. (1988) have shown that sourdough and the souring activity reduced the starch hydrolysis by endogenous wheat flour α-amylases and thus limited the liberation of low molecular mass dextrins which have less tendency to retrograde or interfere with starch crystallization (Silverstein, 1964) and delay bread staling. In the presence of a lower initial rate, the starch hydrolysis in sourdough breads has been found higher than that of conventional production by baker's yeast (Cerletti et al., 1993). Barber et al. (1992) reported an acid starch hydrolysis which generated dextrins when sourdough was used. Sourdoughs with the lowest pH and highest TTA have been proven to be the most favorable for producing a bread with highest volume, good crumb grain and lowest rate of staling during storage (Barber et al., 1992). Our results confirmed the positive effects of souring; the bread produced by *S. cerevisiae* 141 and *L. plantarum* DC400 had one of the lowest pH values, 4.18. When compared with bread produced by yeast alone, souring delayed both bread firmness and staling. However, strains which did not differ greatly in acidification properties but which demonstrated (*L. sanfrancisco* CB1 and *L. plantarum* DC400) or did not demonstrate (*L. fructivorans* DD10 and *L. farciminis* A80) proteolytic and amylolytic activity had completely different effects on bread shelf-life. A strain-specific influence, which involves properties other than acidification, appeared to be predominant. The production of organic acids (Barber et al., 1992), the bacterial hydrolysis of starch and proteolysis of gluten subunits (Gobbetti et al., 1996a), are activities involved in bread staling (Banecki, 1982) which may explain the different effects of LAB starters.

## CONCLUSIONS

THE USE OF SOURDOUGHS IMPROVED THE OVERALL CHARACTERistics of breads. In addition the use of a specific strain of LAB in sourdough breadmaking may delay firmness and staling. UTM and DSC analyses indicated better storage of bread produced by LAB rather than conventional use of yeasts. Effects of souring, proteolysis and amylolysis on delaying firmness and staling have been presumed but future study is needed to elucidate the relationships among these bacterial activities, amylopectin retrogradation and bread rheology.

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