Microflora of the Sourdoughs of Wheat Flour Bread. X. Interactions Between Yeasts and Lactic Acid Bacteria in Wheat Doughs and Their Effects on Bread Quality

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ABSTRACT

The action of five yeasts (*Saccharomyces cerevisiae, S. fructuum, Candida boidinii, C. guilliermondii,* and *Hansenula subpelliculosa*) and six lactic acid bacteria (*Lactobacillus plantarum, L. plantarum* ssp. *arabinosus, L. brevis, Streptococcus faecium,* and *Leuconostoc mesenteroides*) were studied in wheat doughs, individually and in combinations varying in type, number, and proportions of microorganisms. Fermentative and rheological properties of the doughs and baking quality of the resulting bread were evaluated. Except for *S. cerevisiae*, an increase in yeast or lactic acid bacteria level did not improve the performance of the microorganisms used. All combinations containing *S. cerevisiae* gave bread of high quality. Mixtures of *S. cerevisiae* and other yeasts improved baking performance when lactic acid bacteria were included. *C. boidinii* or *S. fructuum* mixed with *S. cerevisiae* gave better results than *H. subpelliculosa* or *C. guilliermondii*. *L. plantarum* followed by *L. brevis* and *Streptococcus faecium* resulted in better bread quality than the other lactic acid bacteria used.

Interactions between yeasts and lactic acid bacteria and their respective role in wheat doughs have not been well established (Pederson 1979). Fermentative activity (pH, total titratable acidity [TTA], acetic and lactic acid production, fermentation quotient) (Spicher et al 1981, 1982), proteolytic activity (Spicher and Nierle 1984a,b), and baking performance (pH, TTA, sensory characteristics of bread) have been shown to depend on process conditions (temperature, dough yield) (Spicher and Rabe 1980, 1983) and on type and proportions of yeasts when yeast is added to rye sourdoughs inoculated with homo- and heterofermentative lactobacilli (Spicher et al 1981, 1982; Spicher and Nierle 1984 a,b).

The behavior of yeasts and lactic acid bacteria individually and in selected combinations in Spanish wheat doughs has been studied previously (Barber et al 1983; 1985a,b,c; 1987; 1988a,b; Martinez-Anaya et al 1988). Fragmentary information has been published concerning the microflora from the San Francisco French bread sourdough (*Saccharomyces exiguus* and *Lactobacillus sanfrancisco*) (Sugihara et al 1971, Kline and Sugihara 1971, Saunders et al 1972) and saltine cracker processes (Fields et al 1982), but relationships to the quality of their respective baked products have not been determined.

This paper makes a systematic study of the interactions in wheat flour doughs between five yeasts and six lactic acid bacteria isolated from Spanish wheat doughs and their effects on bread quality.

MATERIALS AND METHODS

Five strains of pure yeasts—*Saccharomyces cerevisiae* (strain P-5), *S. fructuum* (Le 23), *Hansenula subpelliculosa* (Le 28), *Candida guilliermondii* (P-40), and *C. boidinii* (P-1)—and six lactic acid bacteria—*Lactobacillus plantarum* (B-39), *L. plantarum* ssp. *arabinosus* (B-19), *L. brevis* (B-33), *L. cellobiosus* (B-41), *Streptococcus faecium* (B-40), and *Leuconostoc mesenteroides* (B-41)—isolated from Spanish wheat doughs (Barber et al 1983, Barber and Baguena 1988, Baguena 1988) were used to make up 107 different microbial starters by varying the number, type, and proportions of constituent microorganisms (Table I). Details referring to microbial mass production and preservation techniques have been described previously (Barber et al 1988a, b; Martinez-Anaya et al 1989).

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Wheat doughs were made from each starter. The flour used had the following characteristics: alveograph specific deformation work, W ($\times 10^3$ ergs) = 193.8, P/L = 0.87; farinograph water absorption = 58.2%. Dough formulation and test baking were performed according to Barber et al (1988). Thirty-two parameters were determined in doughs and breads by following previously established procedures (Barber et al 1983, 1987). Fermentative characteristics were determined on doughs fermented for varied times: gassing power, 1-3 hr; pH, 0-3 hr; pH increase, 1-3 hr; TTA, 0-3 hr; and TTA increase, 1-3 hr. Dough plasticity was determined by the spread test on doughs fermented 0-3 hr. Bread quality was evaluated by volume, density, pH, TTA, and sensory analysis of the following qualities: loaf shape (scored from 0 = symmetrical, to 5 = too flat), aroma (0 = odd, 3 = characteristic), taste (0 = odd, 3 = characteristic), edibility (0 = correct, 2 = leathery, gummy), crust (0 = correct, 2 = coarse), crumb texture (0 = rough, inelastic; 2 = soft, elastic), and grain (0 = correct, 3 = very uneven).

Data were analyzed statistically using an IBM-AT computer and applying general analysis of variance (BMDP, 2V). Three replicate samples of each combination were used for analysis.

RESULTS AND DISCUSSION

Results are summarized in Figures 1-4 and Table II. Except for sample groups I-III, in which only the dose and proportion of microorganisms giving better results are plotted, the figures contain all the combinations described in Table I. Because of the large number of samples evaluated, only selected variables that resulted in higher specific weight or better overall bread quality are discussed.

Single Yeasts (group 1)

Yeasts were added to the doughs at two levels, 2 and 4% for *S. cerevisiae* ($4.2-9.0 \times 10^9$ viable cells/g, dw), and 4 and 6% for the other yeasts ($2.9 \times 10^9-3.1 \times 10^{10}$ viable cells/g, dw). The level giving the better performance was chosen for the study of mixtures of microorganisms.

Fermentative activity. Doughs containing 2% of *S. cerevisiae* reached higher gassing power values (353.3 ml of CO_2/100 g of dough $\times$ 3 hr) than those doughs made with 6% of the yeasts *S. fructuum, H. subpelliculosa, C. boidinii,* and *C. guilliermondii* (75.5-257.3 ml). Gas production showed variable increases when the yeast level was increased; differences were only significant for *S. cerevisiae* (353.3-449.3 ml). *S. fructuum* produced 257 and 255 ml, *H. subpelliculosa* 242 and 236 ml, *C. guilliermondii* 117.7 and 134.3 ml, and *C. boidinii* 75.5 and 83 ml, when added at the 4% (Figs. 1 and 2) and 6% levels, respectively. Because better results were obtained with *S. cerevisiae*, and increasing the levels did not improve gassing power of the other yeasts, a concentration of 4% was selected for further studies with mixtures of yeasts and lactic acid bacteria.

The pH and TTA did not vary during 3 hr of fermentation when the yeast dose was increased. The pH after mixing ranged from 5.8 to 5.5 and decreased to 5.7–5.4 after 3 hr. TTA after mixing varied between 2.2 and 3.3 (ml 0.1*N* NaOH/10 g of dough, mb) and between 2.9 and 4.5 after 3 hr; the total increase of TTA after fermentation varied from 0.3 to 1.7 (Figs. 1 and 2).

Baking performance. Because of its greater gas-producing ability *S. cerevisiae* produced loaves of larger volume and lower density than the other yeasts used. Differences in yeast concentration did not result in differences in these parameters. Bread

TABLE I
Classification of Samples by Microorganism Composition

Group/ Case No.	Yeasts: Species[a]	Yeasts: Proportion	Yeasts: % in Dough[b]	Lactic Acid Bacteria: Species[c]	Lactic Acid Bacteria: Proportion	Lactic Acid Bacteria: Dose[d] (ml/100 ml flour)
Group I, single yeasts						
37	Sc	...	2	...	...	...
39	Sc	...	4	...	...	...
31	Sf	...	4	...	...	...
32	Sf	...	6	...	...	...
33	Cg	...	4	...	...	...
34	Cg	...	6	...	...	...
35	Cb	...	4	...	...	...
36	Cb	...	6	...	...	...
40	Hs	...	4	...	...	...
41	Hs	...	6	...	...	...
Group II, 2 yeasts						
42	Sc/Sf	1:3	4	...	...	...
38	Sc/Sf	3:1	4	...	...	...
54	Sc/Hs	1:3	4	...	...	...
55	Sc/Hs	3:1	4	...	...	...
43	Sc/Cg	1:3	4	...	...	...
44	Sc/Cg	3:1	4	...	...	...
45	Sc/Cb	1:3	4	...	...	...
46	Sc/Cb	3:1	4	...	...	...
47	Sf/Cg	1:3	4	...	...	...
48	Sf/Cg	3:1	4	...	...	...
49	Sf/Cb	1:3	4	...	...	...
50	Sf/Cb	3:1	4	...	...	...
Group III, 3 yeasts						
51	Sc/Sf/Cb	3:1:1	4	...	...	...
52	Sc/Sf/Cb	1:3:1	4	...	...	...
53	Sc/Sf/Cb	1:1:3	4	...	...	...
Group IV, single lactic acid bacteria						
96	...	...	...	Lp	...	1.5
97	...	...	...	Lp	...	3
98	...	...	...	Lpa	...	1.5
99	...	...	...	Lpa	...	3
100	...	...	...	Lb	...	1.5
101	...	...	...	Lb	...	3
102	...	...	...	Lc	...	1.5
103	...	...	...	Lc	...	3
104	...	...	...	Stf	...	1.5
105	...	...	...	Stf	...	3
106	...	...	...	Leum	...	1.5
107	...	...	...	Leum	...	3
Group V, 1 yeast + 1 bacterium						
3	Sc	...	4	Lp	...	1.5
4	Sc	...	4	Lpa	...	1.5
5	Sc	...	4	Lb	...	1.5
6	Sc	...	4	Lc	...	1.5
7	Sc	...	4	Stf	...	1.5
8	Sc	...	4	Leum	...	1.5
9	Sf	...	4	Lp	...	1.5
10	Sf	...	4	Lb	...	1.5
11	Sf	...	4	Stf	...	1.5
12	Sf	...	4	Leum	...	1.5
1	Hs	...	4	Lp	...	1.5
2	Hs	...	4	Lb	...	1.5
27	Cb	...	4	Lp	...	1.5
28	Cb	...	4	Lb	...	1.5
29	Cb	...	4	Stf	...	1.5
30	Cb	...	4	Leum	...	1.5

(continued next page)

TABLE I *(continued from preceding page)*

Group/ Case No.	Yeasts			Lactic Acid Bacteria		
	Species[a]	Proportion	% in Dough[b]	Species[c]	Proportion	Dose[d] (ml/100 ml flour)
Group VI, 2 yeasts + 1 bacterium						
13	Sc/Sf	3:1	4	Lp	. . .	1.5
14	Sc/Sf	3:1	4	Lpa	. . .	1.5
15	Sc/Sf	3:1	4	Lb	. . .	1.5
16	Sc/Sf	3:1	4	Lc	. . .	1.5
17	Sc/Sf	3:1	4	Stf	. . .	1.5
18	Sc/Sf	3:1	4	Leum	. . .	1.5
56	Sc/Cb	3:1	4	Lp	. . .	1.5
57	Sc/Cb	3:1	4	Lpa	. . .	1.5
58	Sc/Cb	3:1	4	Lb	. . .	1.5
59	Sc/Cb	3:1	4	Lc	. . .	1.5
70	Sc/Cb	3:1	4	Stf	. . .	1.5
71	Sc/Cb	3:1	4	Leum	. . .	
64	Sf/Cb	1:3	4	Lp	. . .	1.5
65	Sf/Cb	1:3	4	Lpa	. . .	1.5
66	Sf/Cb	1:3	4	Lb	. . .	1.5
67	Sf/Cb	1:3	4	Lc	. . .	1.5
68	Sf/Cb	1:3	4	Stf	. . .	1.5
69	Sc/Cb	1:3	4	Leum	. . .	1.5
Group VII, 3 yeasts + 1 bacterium						
80	Sc/Sf/Cb	3:1:1	4	Lp	. . .	1.5
81	Sc/Sf/Cb	1:1:3	4	Lp	. . .	1.5
82	Sc/Sf/Cb	3:1:1	4	Lpa	. . .	1.5
83	Sc/Sf/Cb	1:1:3	4	Lpa	. . .	1.5
84	Sc/Sf/Cb	3:1:1	4	Lb	. . .	1.5
85	Sc/Sf/Cb	1:1:3	4	Lb	. . .	1.5
86	Sc/Sf/Cb	3:1:1	4	Lc	. . .	1.5
87	Sc/Sf/Cb	1:1:3	4	Lc	. . .	1.5
88	Sc/Sf/Cb	3:1:1	4	Stf	. . .	1.5
89	Sc/Sf/Cb	1:1:3	4	Stf	. . .	1.5
90	Sc/Sf/Cb	3:1:1	4	Leum	. . .	1.5
91	Sc/Sf/Cb	1:1:3	4	Leum	. . .	1.5
Group VIII, 1 yeast + 2 bacteria						
23	Sc	. . .	4	Lp/Lb	1:1	1.5
24	Sc	. . .	4	Lb/Stf	1:1	1.5
25	Sc	. . .	4	Lp/Stf	1:1	1.5
26	Sf	. . .	4	Lp/Lb	1:1	1.5
19	Sf	. . .	4	Lb/Stf	1:1	1.5
20	Sf	. . .	4	Lp/Stf	1:1	1.5
Group IX, 1 yeast + 3 bacteria						
21	Sc	. . .	4	Lp/Lb/Stf	1:1:1	1.5
22	Sf	. . .	4	Lp/Lb/Stf	1:1:1	1.5
Group X, 2 yeasts + 2 bacteria						
60	Sc/Sf	3:1	4	Lp/Lb	1:1	1.5
61	Sc/Sf	3:1	4	Lb/Stf	1:1	1.5
62	Sc/Sf	3:1	4	Lp/Stf	1:1	1.5
72	Sc/Cb	3:1	4	Lp/Lb	1:1	1.5
73	Sc/Cb	3:1	4	Lb/Stf	1:1	1.5
74	Sc/Cb	3:1	4	Lp/Stf	1:1	1.5
76	Sf/Cb	1:3	4	Lp/Lb	1:1	1.5
77	Sf/Cb	1:3	4	Lb/Stf	1:1	1.5
78	Sf/Cb	1:3	4	Lp/Stf	1:1	1.5
Group XI, 2 yeasts + 3 bacteria						
63	Sc/Sf	3:1	4	Lp/Lb/Stf	1:1:1	1.5
75	Sc/Cb	3:1	4	Lp/Lb/Stf	1:1:1	1.5
79	Sf/Cb	1:3	4	Lp/Lb/Stf	1:1:1	1.5
Group XII, 3 yeasts + 2 bacteria						
92	Sc/Sf/Cb	3:1:1	4	Lp/Lb	1:1	1.5
93	Sc/Sf/Cb	3:1:1	4	Lb/Stf	1:1	1.5
94	Sc/Sf/Cb	3:1:1	4	Lp/Stf	1:1	1.5
Group XIII, 3 yeasts + 3 bacteria						
95	Sc/Sf/Cb	3:1:1	4	Lp/Lb/Stf	1:1:1	1.5

[a] Sc = *Saccharomyces cerevisiae,* Sf = *S. fructum,* Hs = *Hansenula subpelliculosa,* Cg = *Candida guilliermondii,* and Cb = *C. boidinii.*
[b] Percentage of yeast (flour basis) (yeast viable counts = 3.10^{9}–3.10^{10} cells/g of yeast, dw).).
[c] Lp = *Lactobacillus plantarum,* Lpa = *L. Plantarum* ssp. *arabinosus,* Lb = *L. brevis,* Lc = *L. cellobiosus,* Stf = *Streptococcus faecium,* and Leum = *Leuconostoc mesenteroides.*
[d] Bacteria in milliliters per 100 grams of flour (viable counts = 1 – 14.10^{10} cells/ml).

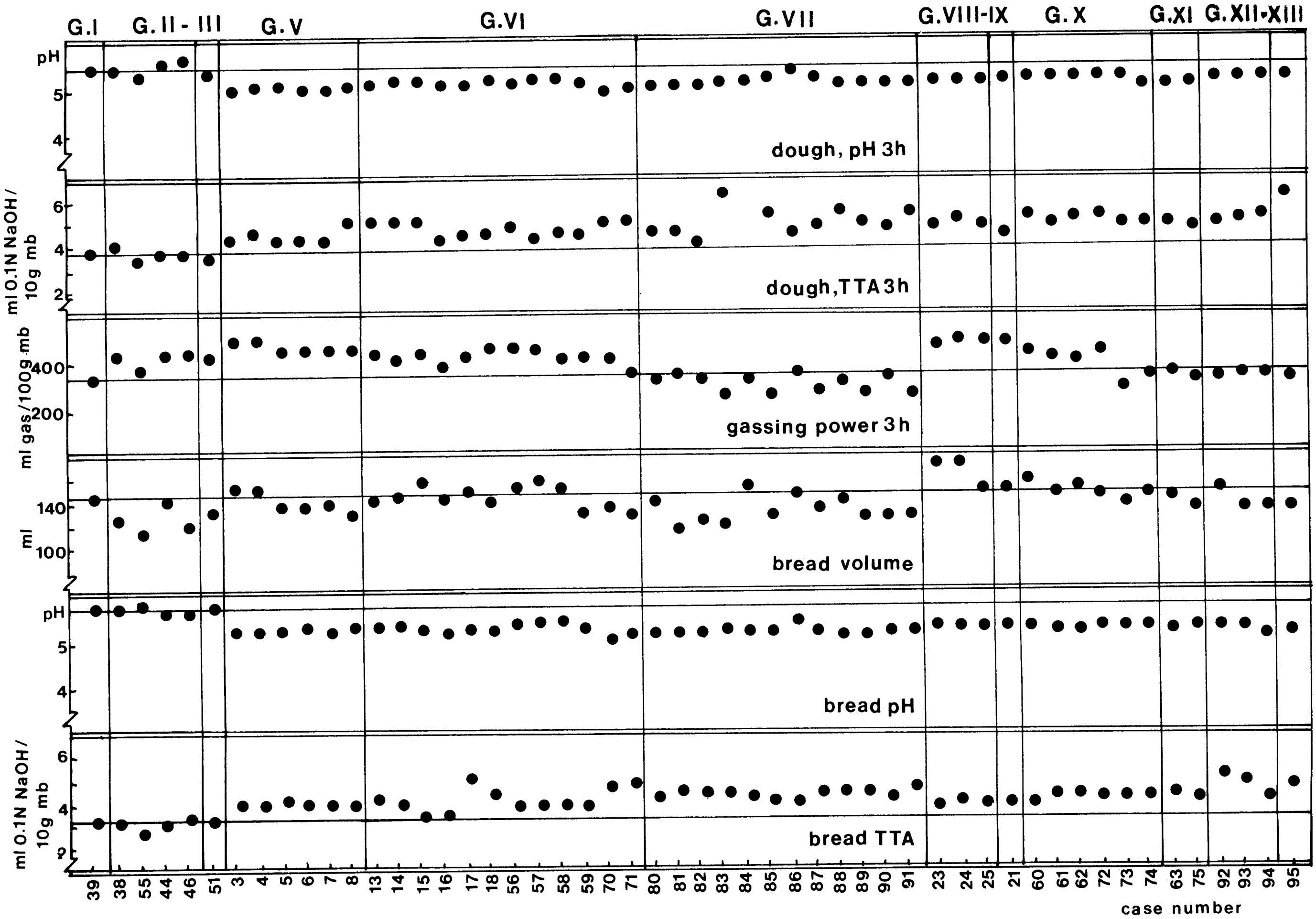

Fig. 1. Fermentative and baking properties of combinations of yeasts, including *Saccharomyces cerevisiae*, and lactic acid bacteria in wheat flour doughs. Case numbers and group Roman numerals correspond with those in Table I. The horizontal lines indicate values for *S. cerevisiae* (4% dose) alone.

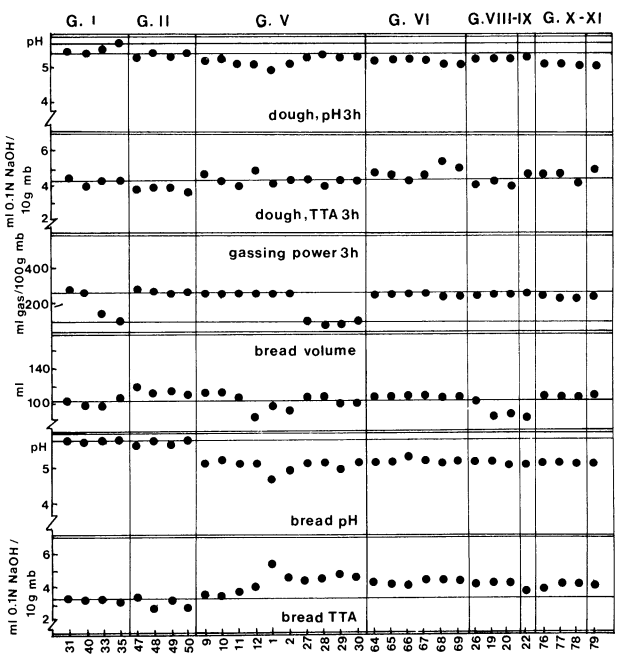

Fig. 2. Fermentative and baking properties of combinations of yeasts, excluding *Saccharomyces cerevisiae*, and lactic acid bacteria in wheat flour doughs. Case numbers and group Roman numerals correspond with those in Table I. The horizontal lines point out values for the best and the worse performing yeast (4% dose) (which sometimes coincide).

volume was about 150 cm^3 and density 0.33 g/cm^3 (Fig. 1). The other yeasts gave noticeably lower volumes, and increasing levels of the yeasts did not result in improvement. Differences in loaf volume for the four other yeasts were small, ranging from 79 to 106 cm^3 (Fig. 2), and the corresponding densities ranged from 0.46 to 0.64 g/cm^3.

Breads made from single yeasts had a coarse crust, gummy crumb, rough crumb texture, and uneven structure. *S. cerevisiae* produced the breads with the best flavor and shape (Figs. 3 and 4). No clear differences that might be attributable to yeast dose could be detected.

Yeast Mixtures (groups II and III)

Fifteen combinations containing different proportions of two (group II) and three (group III) yeasts (Table I) were used for making wheat flour doughs. The total amount of yeast inoculated was 4%.

Fermentative activity of yeast mixtures. In binary combinations of yeasts, gassing power was dependent on the individual fermentative ability of each yeast and its proportion in the mixtures. As expected, samples with higher amounts of *S. cerevisiae* (75%) (Fig. 1) produced more gas; furthermore, these mixtures showed a synergistic effect on gas production and gave values close to that observed with *S. cerevisiae* alone. With the remaining combinations containing less *S. cerevisiae* (25%), gas production varied in relation to the other species of yeasts. Both *C. boidinii* and *C. guilliermondii* produced more gas than expected from their single contribution, whereas *S. fructuum* and *H. subpelliculosa* only showed an additive effect (Fig. 1). Mixtures of three yeasts (group III), including *S. cerevisiae, S. fructuum,* and *C. boldinii*

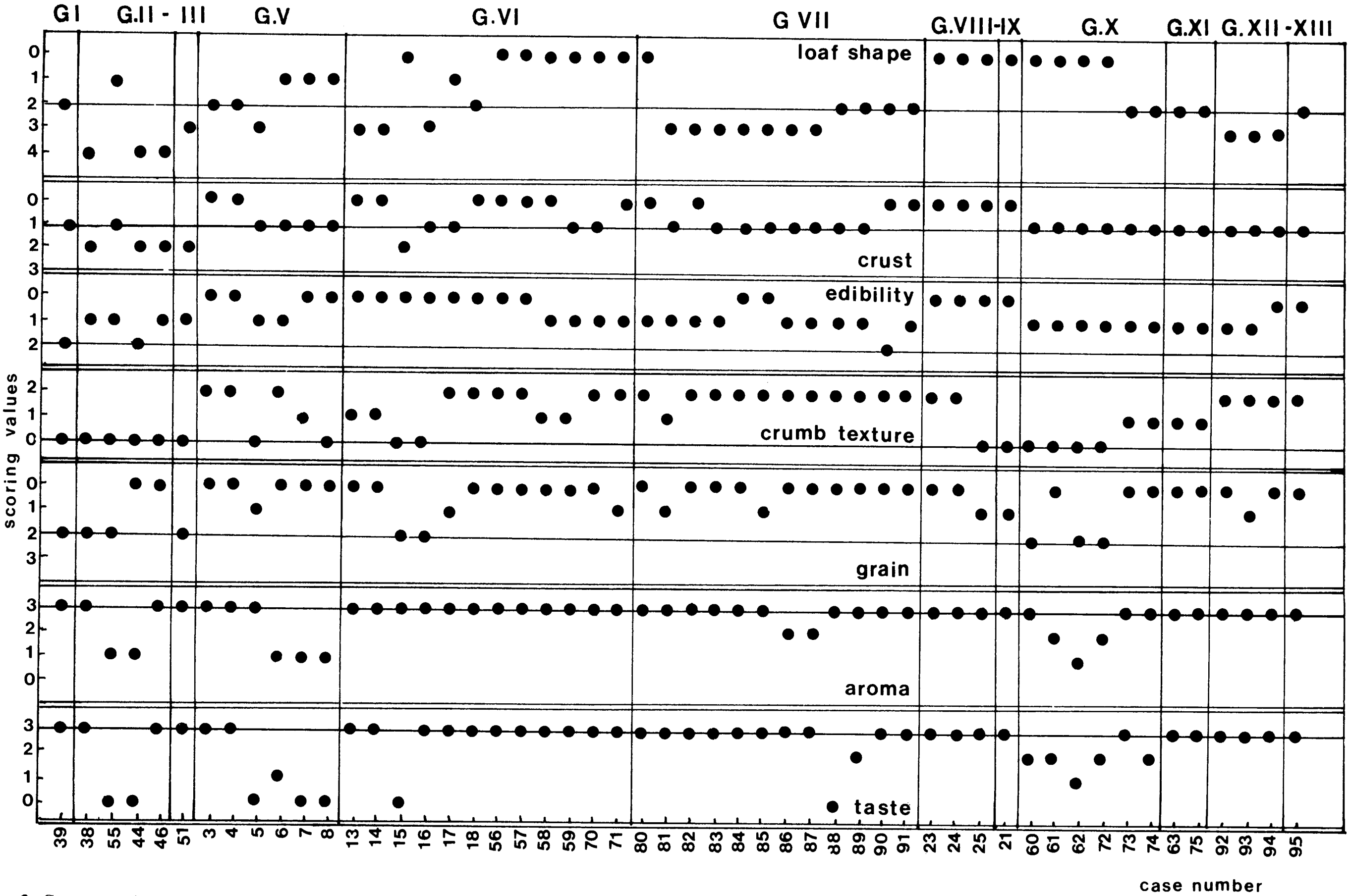

Fig. 3. Sensory characteristics of wheat flour breads made with combinations of yeasts, including *Saccharomyces cerevisiae*, and lactic acid bacteria. Case numbers and group roman numerals correspond with those in Table I. The horizontal lines indicate values for *S. cerevisiae* (4% dose) alone.

in three different proportions produced more gas during fermentation than the corresponding contribution from each individual yeast. In the sample with 60% *S. cerevisiae*, this value was close to that recorded for 100% *S. cerevisiae* (Fig. 1).

The combinations of yeasts without *S. cerevisiae* (Table I) consisted of *S. fructuum* and *C. bodinii* or *C. guilliermondii* in two proportions. After 3 hr, gassing power reached values similar to that obtained with *S. fructuum* alone (Fig. 2), indicating a synergistic effect on gas production attributable to the combined presence of both yeasts.

Nevertheless, no significant ($\alpha = 0.05$) differences were observed for pH and TTA that developed in doughs with mixtures of yeasts during fermentation compared with doughs made from each single yeast (Figs. 1 and 2).

Baking performance of yeast mixtures. Bread made from combinations of two or three yeasts including *S. cerevisiae* gave volumes between 105 and 140 cm^3 (Fig. 1). The higher value is of similar magnitude to that of bread prepared with *S. cerevisiae* alone and corresponded to the samples containing 25% *C. guilliermondii.*

Loaf volumes of bread obtained with *S. fructuum* and *C. boidinii* or *C. guilliermondii* varied from 118 to 136 cm^3, higher values than those obtained from each yeast separately (Fig. 2).

Combinations of *S. cerevisiae* with *S. fructuum* or *C. boidinii*, or both, produced bread having sensory characteristics (mainly typical aroma and taste) close to those of bread made with the *S. cerevisiae* alone (Fig. 3). The presence of other yeasts in addition to *S. cerevisiae*, such as *H. subpelliculosa* or *C. guilliermondii*, resulted in odd flavors. Bread made with mixtures of *S. fructuum* and *C. boidinii* or *C. guilliermondii* resulted in similar scores for sensory analysis, except for aroma, which was more characteristic for the *S. cerevisiae-C. boidinii* combination (Fig. 4).

Lactic Acid Bacteria

Wheat flour doughs were inoculated with lactic acid bacteria

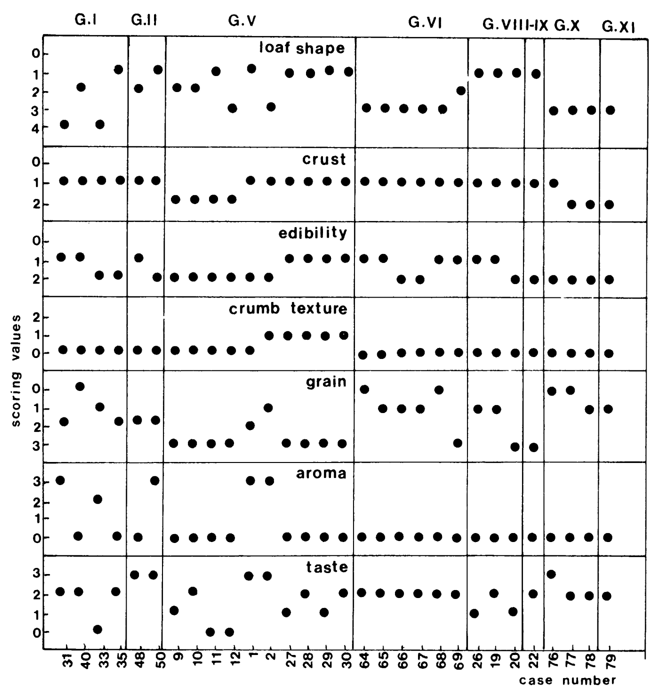

Fig. 4. Sensory characteristics of wheat flour breads made with combinations of yeasts, excluding *Saccharomyces cerevisiae*, and lactic acid bacteria. Case numbers and group Roman numerals correspond with those in Table I.

at two levels, 1.5 and 3 ml/100 g of flour (2.2–13.9 × 10^{10} viable cells/ml) (group IV). The level showing better performance was chosen to prepare combinations with yeasts.

Fermentative activity of single lactic acid bacteria (group IV). Gas production of lactic acid bacteria was not dependent on the

TABLE II
Fermentative Characteristics and Baking Performance of Lactic Acid Bacteria

Group IV Sample[a]	Dose (ml)	Dough Properties Before and After Fermentation: pH 0 hr	pH 3 hr	TTA[b] 0 hr	TTA[b] 3 hr	Gas Production[c] (ml) 3 hr	Loaf Volume (cm^3)	Bread pH	Bread TTA[b]	Sensory Analysis[d]: Loaf Shape	Crust	Edibility	Crumb Texture	Grain	Aroma	Taste
Lp	1.5	5.62	5.21	2.30	3.84	28.67	71.7	4.77	4.56	1	1	2	0	3	0	0
Lp	3	5.53	4.92	2.25	4.35	22.3	68.8	4.22	5.23	1	1	2	0	3	0	0
Lpa	1.5	5.62	5.04	2.36	3.64	31.67	67.5	4.98	3.99	1	1	2	0	3	0	0
Lpa	3	5.57	4.85	2.46	4.35	26.67	72.5	4.50	5.02	1	1	2	0	3	0	0
Lb	1.5	5.62	5.35	2.71	3.74	38.00	66.3	...	...	1	1	2	0	3	3	1
Lb	3	5.55	5.10	2.82	4.35	37.67	76.7	...	...	1	1	2	0	3	0	2
Lc	1.5	5.60	5.17	2.36	3.99	45.17	86.3	4.88	4.02	1	1	2	0	3	0	3
Lc	3	5.56	4.80	2.56	4.51	54.00	58.8	4.54	5.07	1	1	2	0	3	0	3
Stf	1.5	5.62	4.90	2.66	4.20	53.30	83.3	4.59	3.48	1	1	2	0	3	1	1
Stf	3	5.61	4.50	2.66	5.12	36.00	67.5	4.55	4.07	3	2	2	0	3	1	1
Leum	1.5	5.66	5.05	1.95	4.30	50.67	76.3	5.14	3.46	1	1	2	0	3	0	0
Leum	3	5.60	4.63	2.56	4.71	50.67	91.3	4.66	4.51	1	1	2	0	3	0	0

[a] Lp = *Lactobacillus plantarum*, Lpa = *L. plantarum* ssp. *arabinosus*, Lb = *L. brevis*, Lc = *L. cellobiosus*, Stf = *Streptococcus faecium*, and Leum = *Leuconostoc mesenteroides*.
[b] Total titratable acidity expressed in milliliters of 0.1*N* NaOH per 100 g of dough or 10 g of bread.
[c] Expressed in milliliters of gas produced by 100 g of dough.
[d] Loaf shape (scored from 0 = symmetrical, to 5 = too flat), crust (0 = correct, 2 = coarse), edibility (0 = correct, 2 = leathery, gummy), crumb texture (0 = rough, inelastic; 2 = soft, elastic), grain (0 = correct, 3 = very uneven), aroma (0 = odd, 3 = characteristic), and taste (0 = odd, 3 = characteristic).

level of microorganisms added to the dough (Table II). It varied between 22.3 and 53.3 ml of CO_2/100 g of dough × 3 hr. The pH of doughs decreased faster and reached lower values when using the highest level of lactic acid bacteria. After 3 hr, the pH varied between 5.4 and 5.1 with *L. brevis* and 4.9 and 4.5 with *Streptococcus faecium* when using a level of 1.5 to 3 ml. TTA values were closely correlated with those of pH. After a 3-hr fermentation, the TTA ranged between 3.64 ml 0.1*N* NaOH/10 g of dough, mb (*L. plantarum* ssp. *arabinosus*) and 4.3 (*Leuconostoc mesenteroides*) for the 1.5-ml dose level, and between 4.35 (*L. plantarum, L. plantarum* ssp. *arabinosus*, and *L. brevis*) and 5.1 (*Streptococcus faecium*) for the 3-ml level.

Baking performance of lactic acid bacteria. Loaf volumes were low, ranging from 59 to 91 cm^3. An increase in lactic acid bacteria concentration did not result in higher values. The pH and TTA of bread reached different values according to the dose level used. For the lowest level (1.5 ml) pH and TTA were 4.59–5.12 and 3.46–4.56, respectively, and for the highest level (3 ml) 4.42–4.66 and 4.07–5.23 (Table II). The sensory characteristics of bread made with single strains of lactic acid bacteria were not satisfactory. The loaves were flat, with a coarse crust, leathery and rough crumb, very uneven grain, and sour flavor. No significant differences could be observed in these characteristics when using two different levels of the microorganisms (Table II). Because the lower level resulted in a better baking performance, it was selected for the preparation of combinations of lactic acid bacteria and yeasts.

Mixtures of Yeasts and Lactic Acid Bacteria

Fermentative activity of yeast and lactic acid bacteria combinations (groups V–XIII). The addition of lactic acid bacteria to doughs containing a single yeast species (groups V, VIII, and IX) resulted in a decrease of pH during fermentation. The final pH after 3 hr was around 5.0 for doughs with all yeasts except *C. boidinii*, which gave higher values of 5.2–5.4 depending on the type of lactic acid bacteria used (Fig. 1). The TTA increased in the presence of lactic acid bacteria, and after 3 hr ranged from 3.8 to 5.2 (Fig. 1), corresponding generally to the highest values of doughs containing *S. cerevisiae*. Some lactic acid bacteria, e.g., *L. plantarum, L.plantarum* ssp. *arabinosus,* and *L. brevis*, or their combinations, *L. plantarum* and *L. brevis* and/or *Streptococcus faecium,* had an additive effect on gassing power in doughs with *S. cerevisiae* (Fig. 1) that was not observed with other yeasts or lactic acid bacteria.

In mixtures containing two or three different species of yeasts with a single lactic acid bacterium (groups VI and VII), the pH and TTA of doughs during fermentation was of the same order as that with combinations belonging to group V. On the other hand, no additional effect on gassing power was found attributable to lactic acid bacteria compared with that observed for combinations of yeasts (groups II and III, Figs. 1 and 2).

Mixtures of groups X–XIII, containing several yeasts and lactic acid bacteria (Figs. 1 and 2), showed no differences in fermentative activity when compared with groups VI and VII, containing several yeasts and a single lactic acid bacterium.

Baking performance of combinations of yeasts and lactic acid bacteria. In the presence of each strain of lactic acid bacteria, bread volume showed significant ($\alpha = 0.05$) differences in comparison with breads made from doughs containing only *S. cerevisiae, C. boidinii,* or *C. guilliermondii.* In contrast, bread volumes obtained with *S. fructuum* and *L. plantarum* or *L. brevis* were significantly higher (25–35%)(Fig. 2). Combinations of *S. cerevisiae* with *L. plantarum* or *L. brevis* and *Streptococcus faecium* had a synergistic effect on bread volume, which was about 15% greater than that observed with the yeast alone (Fig. 1). With the other lactic acid bacteria and yeasts, no additional effect could be detected. A similar action took place when using *S. cerevisiae* and *S. fructuum* or *C. bodinii* and lactic acid bacteria (group X). With these mixtures, the bread volume was 140–150 cm^3, of the same order as that produced by *S. cerevisiae* alone, even though those mixtures contained only 75% *S. cerevisiae*. With other binary and ternary combinations of yeasts (groups VI, VII, and X–XIII), adding lactic acid bacteria did not produce further effects on loaf volume.

In the presence of lactic acid bacteria, the pH of the resulting bread was, as expected, lower and the TTA higher than for mixtures with yeasts alone; the pH values ranged from 4.92 to 5.33, and TTA from 3.38 to 5.13 (Figs. 1 and 2).

Lactic acid bacteria added to doughs with yeasts including *S. cerevisiae* noticeably improved the sensory properties of bread (Fig. 3), mainly in relation to loaf shape, crust, edibility, and grain. Bread flavor, however, maintained typical characteristics. *L. plantarum* was the best performing lactic acid bacteria. When using yeasts other than *S. cerevisiae*, no definitive trend on sensory quality of breads that might be attributable to lactic acid bacteria could be established (Fig. 4).

LITERATURE CITED

BAGUENA, R. 1988. Microflora y propiedades funcionales de la masa madre y su relación con las características de la masa panaria y del

pan. Doctoral thesis. Universidad de Valencia, Spain.
BARBER, S., and BAGUENA, R. 1988. Microflora de la masa panaria. V. Aislamiento, identificación y evaluación de las propiedades funcionales de microorganismos de la masa madre. Rev. Agroquim. Tecnol. Aliment. 28:67.
BARBER, S., BAGUENA, R., MARTINEZ-ANAYA, M. A., and TORNER, M. J. 1983. Microflora de la masa panaria. I. Identificación y propiedades funcionales de microorganismos de masas madre industriales elaboradas con harina de trigo. Rev. Agroquim. Tecnol. Aliment. 23:552.
BARBER, S., BENEDITO DE BARBER, C., MARTINEZ-ANAYA, M. A., MARTINEZ, J., and ALBEROLA, J. 1985a. Cambios en los ácidos orgánicos volátiles C_2–C_5 durante la fermentación de masas panarias preparadas con masas madre comerciales y con microorganismos puros. Rev. Agroquim. Tecnol. Aliment. 25:223.
BARBER, S., MARTINEZ-ANAYA, M. A., and BAGUENA, R. 1985b. Microflora de la masa madre panaria. II. Propiedades funcionales en disolución de azúcares de cepas puras de *S. cerevisiae* y de las levaduras comerciales de las que proceden. Rev. Agroquim. Tecnol. Aliment. 25:436.
BARBER, S., MARTINEZ-ANAYA, M. A., and BAGUENA, R. 1985c. Microflora de la masa madre panaria. III. Propiedades funcionales en masas elaboradas con harina de trigo de cepas puras de *S. cerevisiae* y de las levaduras comerciales de las que proceden. Rev. Agroquim. Tecnol. Aliment. 25:447.
BARBER, S., MARTINEZ-ANAYA, M. A., BAGUENA, R., and TORNER, M. J. 1987. Microflora de la masa madre panaria. IV. Propiedades funcionales en masas elaboradas con harina de trigo de cultivos puros—levaduras y lactobacilos—aislados de masas madre industriales. Rev. Agroquim. Tecnol. Aliment. 27: 107.
BARBER, S., TORNER, M. J., MARTINEZ-ANAYA, M. A., and BENEDITO DE BARBER, C. 1988. Microflora de la masa madre panaria. VI. Efectos de la liofilización sobre la viabilidad y las propiedades funcionales de lactobacilos de la fermentación panaria. Rev. Agroquim. Tecnol. Aliment. 28:79.
BARBER, S., TORNER, M. J., MARTINEZ-ANAYA, M. A., and BENEDITO DE BARBER, C. 1989. Microflora of the sour dough of wheat flour bread. IX. Biochemical characteristics and baking performance of wheat doughs elaborated with mixtures of pure microorganisms. Z. Lebensm. Unters. Forsch. 189:6.
FIELDS, M. L., HOSENEY, R. C., and VARRIANO-MARSTON, E. 1982. Microbiology of cracker sponge fermentation. Cereal Chem. 59:23.
KLINE, L., SUGIHARA, T. F. 1971. Microorganisns of the San Francisco sour dough bread process. II. Isolation and characterization of undescribed bacterial species responsible for the souring activity. Appl. Microbiol. 21:459.
MARTINEZ-ANAYA, M. A., TORNER, M. J., URQUIDI, M. E., EJARQUE, J., and BENEDITO DE BARBER, C. 1988. Microflora de la masa madre panaria. VII. Efectos de las técnicas de conservación y del tiempo de alracenamiento sobre las propiedades funcionales en disolucíon de azúcar, de masa microbiana de *Lactobacillus brevis*. Rev. Agroquim. Tecnol. Aliment. 28:490.
MARTINEZ-ANAYA, M. A., PITARCH, B., BAYARRI, P., and BENEDITO DE BARBER, C. 1989. Microflora de la masa madre panaria. VIII. Cambios en azúcares y producción de ácidos durante la fermentación de masas de trigo elaboradas con cultivos puros de microorganismos. Rev. Agroquim. Tecnol. Aliment. 29:1.
PEDERSON, C. S. 1979. Microbiology of food fermentations, 2nd ed. Avi Publ. Co.: Westport, CT.
SAUNDERS, R. M., NG, H., and KLINE, L. 1972. The sugars of flour and their involvement in the San Francisco sour dough french bread process. Cereal Chem. 49:86.
SPICHER, G., and NIERLE, W. 1984a. Die Mikroflora des Sauerteiges. XIX. Der Einfluss von Temperatur und Teigausbeute auf die proteolytische Wirkung der Milchsäurebakterien des Sauerteiges. Z. Lebensm. Unters. Forsch. 179:36.
SPICHER, G., and NIERLE, W. 1984b. Die Mikroflora des Sauerteiges. XX. Der Einfluss der Hefe auf die proteolytichen Vorgänge im Verlaufe der Sauerteiggärung. Z. Lebensm. Unters. Forsch. 179:109.
SPICHER, G., and RABE, G. 1980. Die Mikroflora des Sauerteiges. XI. Der Einfluss der Temperatur auf die Lactac-/Acetatbildung in mit heterofermentativen Milchsäurebakterien angestellten Sauerteigen. Z. Lebensm. Unters. Forsch. 171:437.
SPICHER, G., and RABE, E. 1983. Die Mikroflora des Sauerteiges. XVI. Der Einfluss der Teigausbeute auf die Lactac-/Acetatbildung in mit Milchsaure-bakterien angestellten Sauerteigen. Z. Lebensm. Unters. Forsch. 176:190.
SPICHER, G., RABE, E., SOMMER, R., and STEPHAN, H. 1981. Die Mikroflora des Sauerteiges. XIV. Uber das Verhalten homofermentatlven Sauerteigbakterien und Hefen bei gemeinsamer Kultur. Z. Lebensm. Unters. Forsch. 173:271.
SPICHER, G., RABE, E. SOMMER, R. and STEPHAN, H. 1982. Die Mikroflora des Sauerteiges. XV. Uber das Verhalten heterofermentativen Sauerteigbakterien und Hefen bei gemeinsamer Kultur. Z. Lebensm. Unters. Forsch. 174:222.
SUGIHARA, T. F., KLINE, L., and MILLER, M. W. 1971. Microorganisms of the San Francisco sour dough bread process I. Yeast responsible for the leavening action. Appl. Microbiol. 21:456.

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