

# Effect of leavening microflora on pizza dough properties

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S. COPPOLA, O. PEPE AND G. MAURIELLO. 1998. Fourteen different starter cultures containing one strain of *Saccharomyces cerevisiae* with and without individual or combinations of lactic acid bacteria (*Lactobacillus plantarum*, *Lact. sanfrancisco*, *Enterococcus faecium*, *Leuconostoc mesenteroides*) were employed to investigate the role of leavening microflora on the properties of pizza doughs. Microbiological, chemical and physical characteristics of doughs prepared with the same flour and under the same processing conditions were determined. Leavening times and acidification properties depended on the microbial association used. The proportions of lactic and acetic acid produced by lactic acid bacteria were consistent with the metabolic properties of the strains employed. The bacteria/yeast ratios arising from microbial counts at the end of the leavening process were always lower in comparison to sour- or bread-doughs. The size of the yeast population did not change much, while bacteria showed from one to four duplications. Rheologically, the fermented doughs could only be significantly distinguished from the control dough with regard to the elastic modulus. Principal Component Analysis was applied to the acidimetric data. The scattergram of the two principal components effectively discriminated 13 of the 14 pizza dough types.

## INTRODUCTION

Pizza, the invention of which must be credited to Naples (Ensminger *et al.* 1995), is one of the most widespread convenience or fast foods in the world. At present, in addition to the very large quantities prepared artisanally and cooked and promptly consumed all around the world, firms have shown increasing interest in pizza dough production, also garnished and ready to be baked, and mostly sold after freezing. In Italy, in July 1996 for example, sales of frozen pizzas increased by 40·2% over the previous year (De Biase and Zacchetti 1996). However, in spite of such commercial success, this kind of product is widely characterized by highly variable and often unsatisfactory quality.

The overall quality of a pizza depends chiefly on the dough whose properties are undoubtedly affected by the leavening process, in addition to the flour type and preparation procedure.

There have been no studies on the leavening process of doughs for pizza. Doughs are usually prepared for the straight process by using compressed bakery yeast as a starter.

However, previous investigations on doughs sampled at 10 typical pizza restaurants in the Naples area (Coppola *et al.* 1996) demonstrated the concurrence of a wide range of species of lactic acid bacteria, probably arising both from contamination of yeast and from the environment.

Microbial composition of the starter culture is known to influence the quality of bakery products (Spicher 1983; Lönner and Preve-Åkesson 1989; Collare Esteve *et al.* 1994; Röcken and Voysey 1995). Therefore, in this study, a yeast culture was variously combined with four species of both homofermentative and heterofermentative lactic acid bacteria in order to obtain 14 different starters to determine their influence on microbiological, chemical and physical characteristics of doughs prepared with the same flour and under the same processing conditions.

## MATERIALS AND METHODS

### Micro-organisms, growth conditions and inocula preparation

*Lactobacillus plantarum* E5, *Lactobacillus sanfrancisco* M207, *Leuconostoc mesenteroides* A27, *Enterococcus faecium* A86 and *Saccharomyces cerevisiae* T22 were used. All the strains were

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isolated from pizza doughs as reported in a previous study (Coppola *et al.* 1996); lactobacilli and enterococci were identified according to Balows *et al.* (1992), leuconostocs according to Villani *et al.* (1997) and yeasts by the key of Barnett *et al.* (1990).

Lactobacilli and leuconostocs were grown in MRS broth (Oxoid CM 359), enterococci in M 17 broth (Oxoid CM 817) and yeasts in Malt extract (Oxoid CM 057). Bacterial cultures were incubated overnight at 30 °C; the yeast was cultured for 2 d at the same temperature under aerobic conditions with rotatory shaking. Cells were collected by centrifugation (5000 g), washed with sterile distilled water and resuspended to obtain  $5 \pm 0.5 \times 10^9$  micro-organisms ml<sup>-1</sup> (direct microscopic counts).

### Microbial composition of starters

*Saccharomyces cerevisiae* T22 was used alone and in different combinations with the strains of lactic acid bacteria as reported in Tables 1 and 2. An uninoculated dough was included as control.

### Pizza dough formation and leavening conditions

The doughs were prepared by kneading in a mixer (model KPM50 Professional by KitchenAid, St Joseph, MI, USA) for 5 min at room temperature and at medium speed, 500 g wheat flour, 280 g top water, 8 g salt and starter suspension to achieve viable counts of about  $5 \times 10^7$  cfu g<sup>-1</sup> of both yeasts and lactic acid bacteria in the final dough.

Use was made of wheat flour labelled '00' according to the Italian classification with a protein content of 10.3% (dry basis), ash content of 0.43% (dry basis) and moisture content of 13%.

The dough yield (DY = dough weight  $\times$  100 divided by flour weight) ranked 160. Doughs were shaped into loaves of about 400 g and incubated in graduated glass containers at 23 °C, a temperature usually found in the work environment.

### Dough characterization

The leavening ability of each dough was defined as time of leavening necessary to raise the initial volume 2.5-fold.

Total titratable acidity (TTA) and pH were determined by standard methods (Anon. 1978). Lactic acid (LA) and acetic acid (AA) were quantified by enzymatic methods (Boehringer Mannheim).

Microbial counts were performed on modified Chalmers agar plates (Vanos and Cox 1986) after weekly incubation at 30 °C.

Rheological characterization was kindly carried out by Prof. P. Masi, Department of Food Science at this University, by compressive tests performed by means of an Instron Uni-

versal Testing Machine model 4201, utilizing Instron software series 4301 for data acquisition, as previously reported (Coppola *et al.* 1996).

### Statistics

Statistical treatment of data (standard deviation, analysis of variance and Principal Component Analysis) were performed using Systat software for Macintosh. Rheological data were analysed by the test of Dunnett for multiple comparisons with the control (Camussi *et al.* 1986). The experimental design included three trials for each experimental condition.

## RESULTS

In this study, pizza doughs were characterized at the end of the leavening process at 23 °C, i.e. when the dough had reached 2.5 times the initial volume.

### Leavening performance

Leavening times (Table 1) ranged between 5.3 and 6.8 h, appearing shorter in the presence of *Lact. plantarum* when this micro-organism was in association with the yeast alone (starter 2) or with heterofermentative bacteria such as *Lact. sanfrancisco* (starter 8) or *Leuc. mesenteroides* (starter 9). *Enterococcus faecium*, present in dough 3 and 6, had a slight detrimental effect on yeast activity, while *Leuc. mesenteroides* as sole bacterial species in the starter did not influence the leavening process (dough 5). All other combinations (doughs 4, 7, 10, 11, 12 and 13) required longer or equal times of incubation for leavening, in comparison with the process performed by the yeast alone. Finally, when all micro-organisms were present (dough 14), a slight decrease in leavening time was observed. No volume change in the control was detected after 6.5 h of incubation at 23 °C.

### Pizza dough characteristics

Acidification properties of the different doughs (Table 1) were defined. The control dough samples, after 6.5 h of incubation, showed an average pH of 6.24, 0.60, an average value of total titratable acidity (TTA) and traces of lactic and acetic acids, consistent with their leavening behaviour. The same total acidity and low contents of lactic and acetic acids, but with a pH of 5.60, were detected in the doughs fermented by the yeast alone. In spite of higher acidity, the pH was also 5.60 in doughs fermented by yeast in association with *Ent. faecium*. Increasing the biodiversity of the starter culture, i.e. in the presence of more types of lactic acid bacteria in association with the yeast, generally led to a lower pH and higher TTA values. The lowest pH (4.71) was reached by

**Table 1** Fermentation properties of pizza doughs leavened by different starter cultures

	Starters	Time of leavening h*	pH†	TTA‡ ml 0.1 mol l <sup>-1</sup> NaOH 10 g <sup>-1</sup>	Lactic acid† g kg <sup>-1</sup>	Acetic acid† g kg <sup>-1</sup>	FQ‡
1	<i>Saccharomyces cerevisiae</i>	6.5	5.60	0.60	0.27	0.10	1.8
2	<i>Saccharomyces cerevisiae</i> + <i>Lactobacillus plantarum</i>	5.8	5.05	1.08	1.09	0.19	3.8
3	<i>Saccharomyces cerevisiae</i> + <i>Enterococcus faecium</i>	6.8	5.60	0.93	1.40	0.16	5.8
4	<i>Saccharomyces cerevisiae</i> + <i>Lactobacillus sanfrancisco</i>	6.3	5.39	0.95	0.63	0.28	1.5
5	<i>Saccharomyces cerevisiae</i> + <i>Leuconostoc mesenteroides</i>	6.5	5.12	0.96	0.75	0.57	0.9
6	<i>Saccharomyces cerevisiae</i> + <i>Lactobacillus plantarum</i> + <i>Enterococcus faecium</i>	6.5	5.07	1.00	1.73	0.15	7.7
7	<i>Saccharomyces cerevisiae</i> + <i>Lactobacillus sanfrancisco</i> + <i>Leuconostoc mesenteroides</i>	6.3	5.06	0.91	0.78	0.36	1.4
8	<i>Saccharomyces cerevisiae</i> + <i>Lactobacillus plantarum</i> + <i>Lactobacillus sanfrancisco</i>	5.5	4.88	1.20	1.27	0.24	3.5
9	<i>Saccharomyces cerevisiae</i> + <i>Lactobacillus plantarum</i> + <i>Leuconostoc mesenteroides</i>	5.3	4.71	1.13	0.96	0.20	3.2
10	<i>Saccharomyces cerevisiae</i> + <i>Lactobacillus plantarum</i> + <i>Lactobacillus sanfrancisco</i> + <i>Leuconostoc mesenteroides</i>	6.5	4.81	1.50	1.72	0.46	2.5
11	<i>Saccharomyces cerevisiae</i> + <i>Enterococcus faecium</i> + <i>Lactobacillus sanfrancisco</i> + <i>Leuconostoc mesenteroides</i>	6.8	4.96	1.76	1.23	0.39	2.1
12	<i>Saccharomyces cerevisiae</i> + <i>Lactobacillus plantarum</i> + <i>Enterococcus faecium</i> + <i>Lactobacillus sanfrancisco</i>	6.5	4.91	1.08	1.43	0.18	5.3
13	<i>Saccharomyces cerevisiae</i> + <i>Lactobacillus plantarum</i> + <i>Enterococcus faecium</i> + <i>Leuconostoc mesenteroides</i>	6.8	4.78	1.26	1.66	0.25	4.4
14	<i>Saccharomyces cerevisiae</i> + <i>Lactobacillus plantarum</i> + <i>Enterococcus faecium</i> + <i>Lactobacillus sanfrancisco</i> + <i>Leuconostoc mesenteroides</i>	6.3	4.82	1.46	1.56	0.36	2.9
15	Control	∞	6.24	0.60	0.20	0.01	12.9

Data are means of triplicate analyses.

\*  $P < 0.05$ ; †  $P < 0.001$ ; ‡  $P < 0.002$ .

starter 9 containing both *Lact. plantarum* and *Leuc. mesenteroides*.

With regard to lactic and acetic acid production, *Lact. plantarum* and *Ent. faecium* produced the highest quantities of lactic acid and allowed the highest fermentation quotient (FQ = molar ratio of lactic/acetic acid) to be determined. By contrast, heterofermentatives produced the highest amounts of acetic acid and doughs with the lowest FQ < 1 in the case of the doughs fermented by *S. cerevisiae* and *Leuc. mesenteroides* (starter 5). The concurrence of the two homo-

fermentative species with the yeast (starter 6) enhanced lactic acid production, with an FQ of 7.7. No such effects with regard to production of acetic acid could be detected for starter 7, characterized by the contemporary presence of the two heterofermentative species. The presence of *Lact. sanfrancisco* with *Lact. plantarum* (starter 8) stimulated lactic acid production more than *Leuc. mesenteroides* (starter 9), without greatly modifying the FQ. In the case of other more complex associations, the FQ value ranked around 3 when the starter included two homofermentatives and two hetero-

**Table 2** Microbial contents (log cfu g<sup>-1</sup> ± S.D.) of pizza doughs leavened by different starter cultures

Starters*	<i>Saccharomyces cerevisiae</i>	<i>Lactobacillus plantarum</i>	<i>Lactobacillus sanfrancisco</i>	<i>Leuconostoc mesenteroides</i>	<i>Enterococcus faecium</i>	Total bacteria	Bacteria/yeasts
1	7.55 ± 0.1	—	—	—	—	—	—
2	7.76 ± 0.2	8.49 ± 0.6	—	—	—	8.49	3.97
3	7.55 ± 0.2	—	—	—	8.18 ± 0.1	8.18	3.99
4	7.88 ± 0.2	—	7.96 ± 0.4	—	—	7.96	1.26
5	7.66 ± 0.5	—	—	8.10 ± 0.3	—	8.10	3.17
6	7.02 ± 0.1	8.16 ± 0.3	—	—	7.60 ± 0.6	8.26	16.60
7	7.66 ± 0.4	—	8.25 ± 0.6	8.15 ± 0.6	—	8.45	7.14
8	7.81 ± 0.4	7.79 ± 0.4	7.98 ± 0.3	—	—	8.11	2.04
9	7.93 ± 0.6	7.97 ± 0.2	—	8.70 ± 0.3	—	8.76	7.30
10	7.08 ± 0.5	7.67 ± 0.4	7.98 ± 0.5	7.60 ± 0.7	—	8.20	12.62
11	7.72 ± 0.3	—	7.81 ± 0.5	7.89 ± 0.2	7.46 ± 0.2	8.16	2.91
12	7.40 ± 0.2	7.30 ± 0.3	7.77 ± 0.3	—	7.81 ± 0.4	8.12	5.30
13	7.95 ± 0.8	7.60 ± 0.3	—	7.60 ± 0.1	7.68 ± 0.3	8.07	1.50
14	7.48 ± 0.1	7.62 ± 0.2	7.49 ± 0.6	7.84 ± 0.3	7.23 ± 0.2	8.18	4.75
15	—	—	—	—	—	—	—

\* Starters composition is the same as Table 1.

fermentatives (starter 14); it was > 3 in the presence of two homo- and one heterofermentative species (starters 12 and 13) and < 3 with one homo- and two heterofermentative micro-organisms (starters 10 and 11).

### Rheological properties

Rheological measurements (data not shown) were subjected to analysis of variance and Dunnett's test. No significant difference among the means was detected ( $P > 0.05$ ) when the analysis of variance was applied to data, while it was possible to distinguish significantly ( $\alpha = 0.05$ ) Young's Modulus (elastic modulus) of leavened doughs from the control. The leavened doughs had an average elastic modulus of 0.025 kg mm<sup>-2</sup>, approximately three times lower than the control value. All doughs showed Stress and Energy mean values of about 0.0018 and 0.0031, respectively.

### Cell counts

The microbial contents of the doughs are reported in Table 2. They were determined by exploiting the capability of modified Chalmers agar to distinguish between colonies belonging to the different micro-organisms used in the starters. Yeast colonies were typically very large and creamy. *Lactobacillus plantarum* grew in convex pale pink colonies of about 2 mm with a very small fuchsia-coloured centre and surrounded by a CaCO<sub>3</sub> dissolution halo. *Lactobacillus sanfrancisco* had small, flat and pale pink colonies with a large fuchsia centre, without a CaCO<sub>3</sub> dissolution halo. Colonies of *Leuc. mesenteroides* were

small, flat, pale pink, with a small fuchsia centre and without a dissolution halo, and those of *Ent. faecium* were small, convex, entirely dark fuchsia-coloured with a dissolution halo.

The most favourable association, allowing the best growth of both the yeast and bacteria, was that including *Lact. plantarum* and *Leuc. mesenteroides* (dough 9); this has already been shown by its leavening and acidification kinetics. Significant bacterial growth could also be detected in the doughs fermented by the yeast in association with *Lact. sanfrancisco* and *Leuc. mesenteroides* (dough 7), rather different from the former because it was characterized by a longer leavening time (6.3 h), a higher pH (5.06), a lower lactic acid content and higher acetic acid content. The dough with the lower bacterial content was that fermented by *S. cerevisiae* and *Lact. sanfrancisco* (dough 4), which also showed the lowest bacteria/yeast ratio (1.26). However, in spite of such microbial characteristics, the starter in question did not fail in leavening performance. The highest bacteria/yeast ratios (16.60 and 12.62) were detected in the doughs where yeasts appeared to be inhibited by the other partners, showing significant decreases of cfu g<sup>-1</sup> in comparison to the size of the inoculum (doughs 6 and 10, respectively). Generally, the bacteria reached cumulative counts of 10<sup>8</sup> cfu g<sup>-1</sup>, while only minor variations could be detected after the leavening process as far as yeast counts are concerned.

### Principal Component Analysis (PCA)

In order to determine the main factors influencing the characteristics of pizza doughs, a Principal Component Analysis

(PCA) based on the acidimetric parameters (pH, LA, AA, TTA and FQ), was performed. Fourteen pizza doughs obtained by different starters (excluding the control) constituted the training set of multivariate analysis. A 2-PCs model accounted for about 85% of the total variance. The factor loadings for the first and the second PC were plotted to investigate the significance of each PC (Fig. 1). The first PC with which FQ and AA variables were correlated explained 48% of the variance; the pH, TTA and LA correlated with the second PC (37%). The variables were well represented on the principal plain as shown in Fig. 1; orthogonality means lack of correlation between them (FQ and TTA, FQ and pH). In all other cases, the variables were highly correlated. As expected, the pH was inversely correlated with TTA, AA and LA as well as FQ with AA, whereas TTA correlated directly with LA and AA as well as FQ with LA.

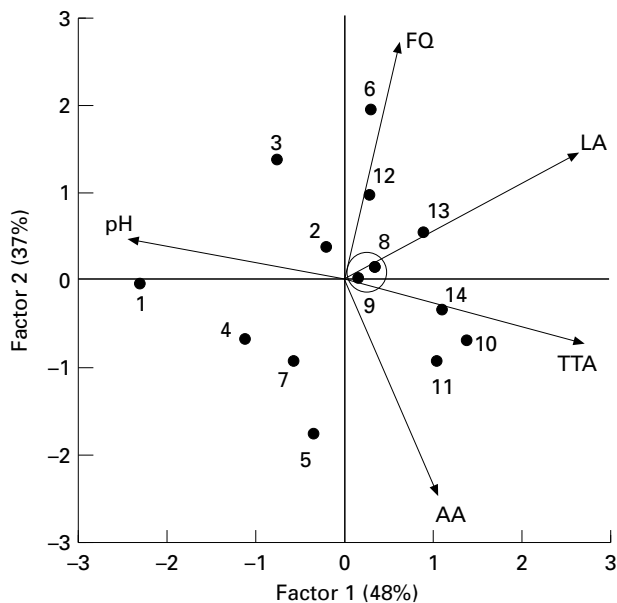
Scatters of pizza doughs were distributed in the plain formed by the two principal axes of PCA (Fig. 1). On the upper half of the plain, doughs were found which were obtained with starters containing homofermentative lactic acid bacteria alone (doughs 2, 3 and 6) or associated with a single heterofermentative strain (doughs 8, 9, 12 and 13). The doughs were well separated into six different groups, one of which was formed by doughs 8 and 9, both prepared with starters containing homo- and heterofermentative bac-

teria (1:1). Doughs 2 and 3, obtained from a single homofermentative species, were found in the upper left part. In the lower half of the plain, doughs were found obtained with starters in which heterofermentative bacteria prevailed (doughs 10, 11, 4, 5 and 7), or in which all the species occurred (dough 14). In particular, doughs 10, 11 and 14 (homo- and heterofermentative bacteria together) were localized on the lower right part of the plain. Doughs 4, 5 and 7, all fermented by only heterofermentative bacteria, were found on the left. Dough 7 (*Lact. sanfrancisco* and *Leuc. mesenteroides* together), near to dough 4 (*Lact. sanfrancisco* alone), showed unexpected lower acidification activity with respect to dough 5 (*Leuc. mesenteroides* alone). Dough 1 (*S. cerevisiae* alone) constituted a single, well separated group, located on the boundary between the lower and upper part of the plain.

## DISCUSSION

Although each restaurant or bakery in the Naples area, and probably elsewhere, has 'personal' methods for pizza preparation, the procedure described above allowed us to obtain pizzas which closely resembled those currently available on the market. The procedure was chosen with respect to the different artisanal preparation methods reported by producers or in the popular literature. Within the chosen procedure, pizza doughs were considered as ready to be garnished and baked when their volume reached 2.5 times the initial volume. Results showed that starter cultures characterized by different combinations of yeast and lactic acid bacteria can promote the leavening processes, achieving the required increase in volume, producing doughs with different rheological characteristics. In fact, all fermented doughs had similar values for Young's Modulus, Stress and Energy, showing clearly that the 2.5 times volume increase corresponds to the achievement of similar characteristics of consistency and elasticity. Only unfermented dough showed a higher value for Young's Modulus, indicating that the leavening process significantly affected dough elasticity.

The time required for leavening appeared to be affected by interactions between the species used in the starter. This was probably due to the metabolic capacity of associated strains, e.g. sugar uptake (Stoltz *et al.* 1993; Antuña and Martínez-Anaya 1993) and proteolytic activity, that allowed synergism or could represent a rate-limiting step in the dough process. In this regard, Spicher and Nierle (1988) and Gobetti *et al.* (1994b, 1996a, b) observed that the proteolysis of lactic acid bacteria increased the availability of free amino acids, enhancing the yeast growth, and among lactic acid bacteria, *Lact. plantarum* and *Lact. sanfrancisco* showed high proteolytic activity during sour dough fermentation. The leavening time can be of the utmost importance during pizza-making at both the industrial and artisanal level; requiring a



**Fig. 1** Score plot (●) and loading plot (arrows) of first and second principal component after Principal Component Analysis of acidimetric characteristics of pizza doughs leavened by different starter cultures (starters 1–14 of Table 1). Scatters included in the circle were grouped

shorter production time, it is worth noting that associations of yeast with *Lact. plantarum*, alone (starter 2) or in combination with heterofermentatives such as *Lact. sanfrancisco* (starter 8) or *Leuc. mesenteroides* (starter 9), can be regarded as the most effective.

The acidification properties of the different doughs also varied with the starter cultures used; the proportion of lactic and acetic acid produced by lactic acid bacteria was in good agreement with the theory for their hexose fermentation (Kandler 1983). The dough obtained with *Lact. sanfrancisco* and the yeast had lower acidimetric values and bacterial contents than all the other starter combinations. This could be explained by the fact that *Lact. sanfrancisco* strains are generally characterized by a relatively long latency phase and by a decrease in the metabolism of soluble carbohydrates when in association with *S. cerevisiae*, as demonstrated by Gobbetti *et al.* (1994a, 1995, 1996a).

Bacteria/yeast ratios arising from microbial enumerations confirmed that pizza doughs are characterized by lower values than sour doughs or bread doughs (Hamad *et al.* 1992; Gobbetti *et al.* 1994a; Iorizzo *et al.* 1995; Rocken and Voysey 1995; Ottogalli *et al.* 1996) as previously reported (Coppola *et al.* 1996). As a  $\geq 90\%$  recovery was reached on modified Chalmers medium for all five types of micro-organisms, our experience indicates that generally speaking, the size of the yeast population does not change at an important rate during the leavening process, while bacteria show some growth corresponding to a maximum of around one (dough 4) to four (dough 9) duplications during leavening.

Principal Component Analysis allowed us to distinguish, on the basis of the acidification properties, 13 types of pizza dough obtained using 14 different starters. According to the metabolic behaviour of the species involved in the starters, the pizza doughs were found in well separated areas of the plain formed by 2-PCs. Doughs obtained by starters containing only homofermentative or only heterofermentative species occupied opposite positions with respect to their fermentation products; doughs produced with a mix of hetero-homofermentative species had an intermediate location. The position of dough 14 could mean that heterofermentatives can affect acidimetric characteristics more than homofermentative bacteria. The unexpected lower position of dough 5 (one heterofermentative strain) with respect to dough 7 (two heterofermentative strains) could be due to the ability of *Lact. sanfrancisco*, as well as other sour dough lactobacilli, to prevent competitors by glucose repression (Stoltz *et al.* 1993). The association between *Lact. plantarum* and *Lact. sanfrancisco* or *Leuc. mesenteroides* led to similar acidimetric characteristics, enabling the doughs to be appropriately grouped. Excluding doughs showing strong acidimetric characteristics, generally located in the extreme position of the PCA plane and resembling bread doughs (Collar Esteve *et al.* 1994), all others could produce pizza with satisfactory

sensorial attributes. Most of them are characterized by the presence, in the starter composition, of heterofermentative strains that, through the production of acetic acid and other compounds, could positively influence the flavour and the taste of the baked products. In particular, *Lact. sanfrancisco* is known to produce a wide, homogeneous and characteristic profile of volatile compounds so that it may be considered as a unique and irreplaceable lactic acid bacterial species in bakery productions (Gobbetti *et al.* 1996a). Moreover, the significant differences between doughs with respect to acidimetric characteristics and leavening ability could allow the selection of appropriate starter cultures for use in specific applications in the bakery industry. Starters producing doughs with a strong acidification and a higher amount of acetic acid could be of interest for extending the shelf-life of packaged pizza, because they appear to possess the greatest ability of preventing growth of moulds and rope bacteria (Lonner and Preve-Åkesson 1989; Röcken 1996). Therefore, considering the various requirements of the industry, we hypothesize that more than one microbial combination could be of practical use.

In conclusion, each starter culture used in this study had a different effect on the characteristics of pizza doughs. The interaction between lactic acid bacteria and *S. cerevisiae* influenced the time of leavening, while the association between lactic acid bacteria species led to interactive effects on acidification properties. We obtained 13 types of pizza doughs employing 14 different starters. These results suggest the general desirability of controlling contamination in dough preparation in order to be able to manage the leavening process through the microbiological composition of the starter culture. Further knowledge regarding the role of leavening microflora and its effect on sensorial properties could lead to an expansion in the range of pizza types available and especially, to improvements in the quality of industrially-prepared products.

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