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Effect of proteolytic starter cultures as leavening agents of pizza dough

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Abstract

Lactic acid bacteria (LAB) and yeasts were selected on the basis of in vitro proteolytic activity against wheat gluten protein and then assayed as leavening agents for pizza dough. Trials were carried out to compare a proteolytic starter (Prt⁺), consisting of *Lactobacillus sakei* T56, *Weissella paramesenteroides* A51 and *Candida krusei* G271, and a non-proteolytic starter (Prt⁻), consisting of *Lb. sakei* T58, *W. paramesenteroides* A58 and *Saccharomyces cerevisiae* T22. The proteolytic activity of the starter cultures was monitored immediately after mixing of the dough and throughout the fermentation process. The proteolytic activity was assessed by analysing the salt-soluble protein (SSP) and the dioxane-soluble protein (DSP) fractions of the pizza dough by discontinuous SDS-PAGE. Only the Prt⁺ starter exhibited considerable qualitative and quantitative changes in the electrophoretic patterns of the protein fractions extracted. After the fermentation, the Prt⁺ and Prt⁻ doughs were tested to evaluate the influence of the proteolytic activity on the mechanical properties of the dough before and after baking. Indications emerged suggesting an influence of the proteolytic activity on the viscoelasticity of pizza dough. The pizza dough with Prt⁺ strains showed an increase in viscous properties during the fermentation as compared with the Prt⁻ dough. Moreover, an increase in the firmness of the crumb was observed in Prt⁺ baked pizza dough.

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1. Introduction

Microbial activities during leavening of bakery products, essentially involving volume rising, acidification and hydrolysis of proteins, have been shown to have extensive technological and sensory implications (Lönner and Preve-Åkesson, 1989; Torner et al.,

1992; Stoltz et al., 1993; Martínez-Anaya et al., 1994; Antuña and Martínez-Anaya, 1993; Röcken and Voysey, 1995; Gobbetti et al., 1995a,b,c; Röcken, 1996; Hammes et al., 1996). Sensorial attributes of bread may be enhanced by the release of nitrogen compounds that represent important flavour precursors influencing the overall aroma of the final product (Spicher and Nierle, 1988; Damiani et al., 1996; Schieberle, 1996; Stam et al., 1998). Amino acids released during dough fermentation can also promote growth and metabolic activities of microorganisms (Collar Esteve et al., 1992). Moreover, the proteolysis

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can influence rheological parameters (Mascarós and Collar, 1994; Martínez-Anaya, 1996), viscosity and gas retention (Scanlon et al., 1990). Interactions between the different constituents of the dough arise from the mechanical process occurring during the formation of the dough with physicochemical transformations largely involving flour proteins, leading to open polypeptide chains in which the starch granules are imbedded (Spicher, 1983). During dough fermentation, modifications of the nitrogen fractions especially are caused by microbial enzymatic activities, even though one third of total proteolytic activity is due to flour enzymes (Spicher and Nierle, 1988). Amino acids accumulation in fermented dough has been attributed to specific proteolytic activities of the starter cultures on high-molecular-weight nitrogen compounds (Spicher and Nierle, 1988; Collar Esteve et al., 1991, 1992; Gobetti et al., 1994). These activities generally have been monitored by analysing changes in the amounts of free amino acids although some authors (Mascarós and Collar, 1994) highlighted microbial proteolytic activity leading to changes in the electrophoretic profile of salt-soluble protein (SSP) and dioxane-soluble protein (DSP) fractions extracted from the dough.

Pizza doughs from restaurants of the Naples area have previously been characterised as having a low ratio of lactic acid bacteria to yeast (Coppola et al., 1996). It has also been shown that acidification and leavening depend on the microbiological association in the dough (Coppola et al., 1998). Moreover, preliminary studies have shown that the mechanical characteristics of pizza dough could be influenced by the activity of selected starter cultures (Formato and Sannino, 1999; Formato and Pepe, 2000).

The aim of this work was to select strains exhibiting proteolytic activity *in vitro* and to test the influence of their proteolytic potential on the mechanical properties of pizza dough.

2. Materials and methods

2.1. Microorganisms and growth conditions

A total of 108 lactic acid bacteria (LAB) and 21 yeasts, previously isolated from pizza doughs (Coppola et al., 1996), were used in this study. The

screening involved cultures belonging to the species: *Lactobacillus sakei* (9 isolates), *Lactobacillus plantarum* (9), *Lactobacillus paracasei* (4), *Lactobacillus pentosus* (2), *Lactobacillus confusus* (7), *Lactobacillus alimentarius* (2), *Lactobacillus rhamnosus* (1), *Lactobacillus minor* (1), *Lactobacillus sanfranciscensis* (1), *Lactobacillus viridescens* (1), *Weissella paramesenteroides* (9), *Leuconostoc pseudomesenteroides* (14), *Leuconostoc dextranicum* (4), *Leuconostoc gelidum* (14), *Leuconostoc mesenteroides* (4), *Leuconostoc amelibiosus* (2), *Leuconostoc argentinum* (2), *Leuconostoc carnosus* (1), *Oenococcus oeni* (2), *Enterococcus faecium* (1), *Lactococcus lactis* subsp. *lactis* (8), *Lc. lactis* subsp. *cremoris* (2), *Lactococcus raffinolactis* (2), *Lactococcus* spp. (11), *Candida krusei* (2), *Candida versatilis* (8), *Debaryomyces hansenii* (1), *Dekkera intermedia* (1), *Saccharomyces cerevisiae* (4) and 5 unidentified yeast isolates.

Lactobacilli and leuconostocs were inoculated in MRS broth; lactococci and enterococci in M17 broth and yeasts in malt extract broth (all media provided by Oxoid, Garbagnate Milanese, Italy). All microorganisms were incubated at 30 °C for 16 h.

2.2. Screening of proteolytic activity *in vitro*

Lactic acid bacteria and yeasts were grown twice overnight at 30 °C into Gluten Maltose Broth (GMB), a medium containing gluten as sole nitrogen resource. GMB contains the following (in g/l): maltose 10, glucose 10, gluten 2, K₂HPO₄ 2, sodium acetate 5, MgSO₄·7H₂O 0.2, MnSO₄·1H₂O 0.05, Tween 80 1 ml, pH 6.2–6.6. Gluten was extracted from wheat flour by phosphate-NaCl precipitation as described by Cappelli and Vannucchi (1990), freeze-dried, powdered and added to the medium. The GMB was sterilised at 121 °C for 15 min. Overnight GMB cultures were centrifuged at 10 000 × *g* for 10 min and supernatants were used to determine hydrolysed gluten proteins using the *o*-phthalaldehyde spectrophotometric assay (OPA) as described by Church et al. (1983). The *o*-phthalaldehyde reagent was supplied by Carlo Erba (Milan, Italy). The increase in absorbance at 340 nm (*A*₃₄₀) was measured using uninoculated GMB as control and the proteolytic activity of each culture was expressed as OPA value.

2.3. Pizza dough formation and leavening conditions

The proteolytic activity was studied during pizza dough fermentation by using two combinations of starter cultures selected from the examinations described above: (i) a proteolytic starter (Prt^+), composed by *Lb. sakei* T56, *W. paramesenteroides* A51 and *C. krusei* G271, and (ii) a non-proteolytic starter (Prt^-), composed by *Lb. sakei* T58, *W. paramesenteroides* A58 and *S. cerevisiae* T22. Pizza doughs were prepared by following a process previously described by Coppola et al. (1998). The leavening was considered complete after about 4.5 h of incubation at 23 °C when the dough volume reached about 2 times the initial volume. Uninoculated dough was used as a control.

2.4. Dough analysis

Microbial counts were determined on modified Chalmers agar plates (Vanos and Cox, 1986) as previously described (Coppola et al., 1998; Pepe et al., 2001). Total titratable acidity (TTA) and pH were determined by standard methods (AAAC, 1975).

2.5. Assessment of microbial proteolysis during pizza dough fermentation

The doughs were sampled and immediately freeze-dried after the initial mixing and 1.5, 3.0, 4.5 and 24 h of fermentation at 23 °C. Salt-soluble protein (SSP) and dioxane-soluble protein (DSP) fractions were extracted using the method described by Mascarós and Collar (1994), freeze-dried and stored at 4 °C. The extracts were preliminarily characterised for primary amine nitrogen by the OPA method (Church et al., 1983) and for total protein content by using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Milan, Italy). To obtain polypeptide profiles, SSP and DSP fractions were applied to discontinuous SDS-PAGE (5% in the stacking gel, 14% in the running gel) according to Laemmli (1970) at constant voltage (200 V) for 45 min. Concentrations of 100 mg ml⁻¹ of SSP fraction or 20 mg ml⁻¹ of DSP fraction were obtained in sample buffer consisting of 62.5 mM Tris-HCl (pH 6.8), 19% glycerol, 5% β -mercaptoethanol, 2% SDS and 0.05% bromophenol blue. The samples were then heated at 90 °C for 4 min and run in a Mini-Protean II vertical dual cell apparatus (Bio-Rad Laboratories,

Richmond, CA, USA). The gels were dried at 60 °C for 1 h (Model 580 Gel Dryer, Bio-Rad). Electrophoretic profiles were acquired by an Arcus 2 scanner (Agfa-Gevaert, Morstel, Belgium) and processed by Phoretix 1D software (Phoretix International, Newcastle upon Tyne, UK). Apparent molecular weight (MW) and quantification of polypeptides and protein subunits were accomplished by reading intensity tracings of stained gels, using an internal calibration standard represented by the molecular weight marker in which the size and the mass of each band was known (Perfect Protein™ Markers, Broader Size, 10–225 kDa, Novagen, Madison, WI, USA).

2.6. Assessment of the mechanical properties of doughs before and after baking

Mechanical characterisation of the two kinds of leavened pizza dough was performed by using a “Modified Alveograph” (Formato and Sannino, 1999; Formato and Pepe, 2000). The variation in the pressure of air (ΔP) acting on a disk of a standard piece of pizza dough was determined as well as the consequent variation in volume (ΔV) of the bubble of the pizza dough until it was broken. For each test, the ΔP_{max} , the ΔV_{b} (at break-up), the energy value E_{b1} necessary up to ΔP_{max} , the energy E_{b2} required from ΔP_{max} to ΔV_{b} and the total energy E_{bT} required to break the bubble were evaluated. In addition, characteristic ratios $E_{\text{b1}}/E_{\text{bT}}$ and $E_{\text{b2}}/E_{\text{bT}}$ were calculated. Unleavened dough was analysed as a control.

Baked doughs crumbs were then submitted to mechanical test by uniaxial compression using an Instron UTM 4301 dynamometer (Instron, High Wycombe, Great Britain) equipped by a 1000-N load cell. Cylindrical specimen (30 mm in depth, 25 mm in diameter) were cut from the centre of baked doughs and compressed between parallel plate at deformation rate of 10 mm min⁻¹. Data were elaborated by Instron Series IX, ver. 4.1 Software (Automated Materials Testing System, Instron, 1992). The Young's modulus (kgf/mm²) was calculated from the force–deformation curves.

2.7. Statistics

Statistical treatment of data (S.D. and *t*-test) was performed using Systat software for Macintosh. The

Table 1

Proteolytic activity (OPA values \pm S.D.) of microorganisms isolated from pizza dough detected in Gluten-Maltose-Broth (GMB)

Microorganisms	OPA values*
<i>Lactobacillus sakei</i> T56	0.49 \pm 0.01
<i>Lactobacillus plantarum</i> E5	0.20 \pm 0.02
<i>Weissella paramesenteroides</i> A51	0.34 \pm 0.02
<i>Leuconostoc pseudomesenteroides</i> LM195	0.20 \pm 0.02
<i>Enterococcus faecium</i> A86	0.46 \pm 0.03
<i>Candida krusei</i> G271	0.21 \pm 0.01

Only Prt⁺ strains (OPA values \geq 0.2) are reported.

* Values are means \pm S.D. of triplicate analyses.

experimental design included the analysis of at least three trials for each independent experiment.

3. Results and discussion

3.1. Proteolytic activity of starter cultures

The GMB medium was prepared in order to select microbial strains able to use crude gluten as the sole

nitrogen source. The LAB *Lb. sakei* T56, *Lb. plantarum* E5, *W. paramesenteroides* A51, *Ln. pseudomesenteroides* LM195 and *E. faecium* A86 showed growth and proteolytic activity in GMB, giving OPA values higher than 0.2 (Table 1). *Lb. sakei* T56 showed the highest activity with an OPA value of 0.49.

For the yeast strains, *C. krusei*, *C. versatilis*, *D. intermedia* and *S. cerevisiae* were able to grow in the GMB, but only *C. krusei* G271 showed proteolytic activity with an OPA value of 0.21 (Table 1). The occurrence of proteolytic strains was low; however, there is limited information, if any, to compare the findings.

3.2. Characterisation of pizza doughs

No significant quantitative and qualitative changes in yeast and LAB populations were detected during the leavening process. In fact, all microbial cultures achieve viable counts of about 5×10^7 CFU g⁻¹ after 4.5 h of incubation at 30 °C (data not shown) as also

Table 2

Salt-soluble protein subunits (SSP) expressed as μ g/10 mg of extract (dry weight) of pizza doughs fermented with Prt⁺ and Prt⁻ starter

Subunits (kDa)	Control		Inoculated dough					
	0 h ^a	24 h ^a	Prt ⁺			Prt ⁻		
			0 h ^b	3 h ^c	4.5 h ^d	0 h ^b	3 h ^c	4.5 h ^d
75	4.88 \pm 0.57	4.28 \pm 0.02	0	0	0	0	0	0
70	13.20 \pm 0.28	13.42 \pm 0.17	0	2.15 \pm 0.00	1.53 \pm 0.00	0	0	0
60	57.66 \pm 0.39	60.85 \pm 0.34	5.18 \pm 0.44	7.71 \pm 0.17	6.91 \pm 0.63	6.84 \pm 0.30	5.95 \pm 0.25	5.70 \pm 0.49
56	39.09 \pm 0.43	38.02 \pm 0.22	18.20 \pm 0.46	22.7 \pm 0.31	27.35 \pm 0.29	19.06 \pm 0.18	20.50 \pm 0.83	19.76 \pm 0.17
52	38.25 \pm 0.56	38.58 \pm 0.31	8.31 \pm 0.38	7.54 \pm 0.70	11.21 \pm 0.68	11.48 \pm 0.36	5.84 \pm 0.47	6.24 \pm 0.69
45.6	31.07 \pm 0.67	31.64 \pm 0.28	2.56 \pm 0.86	3.90 \pm 0.78	3.27 \pm 0.88	5.54 \pm 0.75	1.90 \pm 0.37	2.64 \pm 0.24
40	30.97 \pm 0.23	31.27 \pm 0.16	0	0	0	0	0	0
37	54.02 \pm 0.24	51.70 \pm 0.26	3.55 \pm 0.74	6.74 \pm 0.17	7.18 \pm 0.45	4.93 \pm 0.99	3.96 \pm 0.94	4.76 \pm 0.44
35	12.48 \pm 0.13	13.01 \pm 0.23	10.22 \pm 0.07	12.28 \pm 0.37	13.48 \pm 0.23	13.51 \pm 0.33	8.84 \pm 0.23	9.16 \pm 0.68
32	9.22 \pm 0.07	8.48 \pm 0.32	2.74 \pm 0.40	2.66 \pm 0.19	2.47 \pm 0.41	4.06 \pm 0.19	1.95 \pm 0.13	2.00 \pm 0.29
25.2	43.18 \pm 0.25	44.56 \pm 0.13	24.32 \pm 0.20	24.70 \pm 0.41	23.44 \pm 0.76	28.65 \pm 0.99	22.34 \pm 0.77	21.54 \pm 0.85
23.5	21.61 \pm 0.18	20.49 \pm 0.14	8.49 \pm 0.55	9.19 \pm 0.89	8.52 \pm 0.56	10.30 \pm 0.53	7.49 \pm 0.22	7.71 \pm 0.15
21	22.99 \pm 0.17	22.74 \pm 0.20	5.81 \pm 0.16	8.22 \pm 0.90	8.31 \pm 0.47	9.21 \pm 0.06	5.73 \pm 0.75	6.60 \pm 0.27
18.8	14.55 \pm 0.15	14.05 \pm 0.08	4.30 \pm 0.09	3.24 \pm 0.65	3.48 \pm 0.13	4.86 \pm 0.69	3.45 \pm 0.90	2.74 \pm 0.05
16	34.13 \pm 0.21	33.04 \pm 0.33	13.24 \pm 0.81	15.18 \pm 0.25	15.68 \pm 0.25	18.76 \pm 0.96	11.59 \pm 0.67	13.94 \pm 0.87
13.5	53.96 \pm 0.18	52.98 \pm 0.22	16.49 \pm 0.34	20.85 \pm 0.64	27.95 \pm 0.95	20.89 \pm 0.69	17.07 \pm 0.12	18.04 \pm 0.01
12.2	56.50 \pm 0.33	46.55 \pm 0.11	14.30 \pm 0.91	16.36 \pm 0.45	19.35 \pm 0.90	17.90 \pm 0.83	14.43 \pm 1.42	15.17 \pm 0.12
11.5	34.49 \pm 0.27	35.24 \pm 0.15	12.65 \pm 0.36	17.79 \pm 0.19	17.84 \pm 0.11	17.22 \pm 0.79	15.54 \pm 0.20	15.02 \pm 0.39
10	26.31 \pm 0.33	30.26 \pm 0.22	40.42 \pm 0.73	31.28 \pm 0.65	31.10 \pm 0.96	37.22 \pm 0.76	29.63 \pm 0.47	29.10 \pm 0.95
<10	39.26 \pm 0.27	40.47 \pm 0.21	31.10 \pm 0.70	29.41 \pm 0.42	31.80 \pm 0.74	30.31 \pm 0.00	25.75 \pm 0.00	30.86 \pm 0.00

Paired *t*-test between of two groups of data: ^a*P*>0.05; ^b*P*>0.05; ^c*P*<0.01; ^d*P*<0.01.

Values are averages and standard deviations of three different experiments.

detected by Coppola et al. (1998) in a previous work. Moreover, after 4.5 h of fermentation, doughs prepared with Prt^+ and Prt^- starters showed pH values of about 5.0 and total titratable acidity (TTA) of 1.0. After 24 h of incubation, the pH dropped to about 4 and TTA increased to 2. The uninoculated dough used as control appeared unfermented after 24 h of incubation at 23 °C with a pH around 6 and TTA value of 0.1.

3.3. Proteolytic activity during pizza dough fermentation

The effect of the proteolytic and non-proteolytic starter cultures on the nitrogen fraction of pizza dough was evaluated by comparing their SSP and DSP electrophoretic patterns during the fermentation process. Quantification of the protein subunits of SSP fractions extracted from pizza doughs obtained by proteolytic and non-proteolytic starters, respectively, is reported in Table 2; electrophoretic patterns of SSP subunits are shown in Figs. 1 and 2. The addition of a microbial starter into the pizza dough formula led to a decrease of all the protein subunits ($P < 0.01$), as observed immediately after mixing by comparing the extracts from the inoculated and uninoculated doughs (Table 2; Figs. 1 and 2, lanes a and b). The decrease was particularly associated to the 75, 70 and

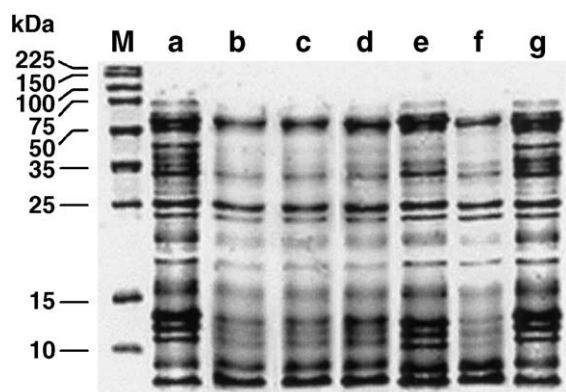


Fig. 1. SDS-PAGE of salt-soluble protein (SSP) fractions of pizza dough started with proteolytic (Prt^+) strains. Lanes a and g: uninoculated controls immediately after mixing and after 24 h of fermentation, respectively; lanes b, c, d, e and f: samples immediately after mixing and after 1.5, 3, 4.5 and 24 h of fermentation.

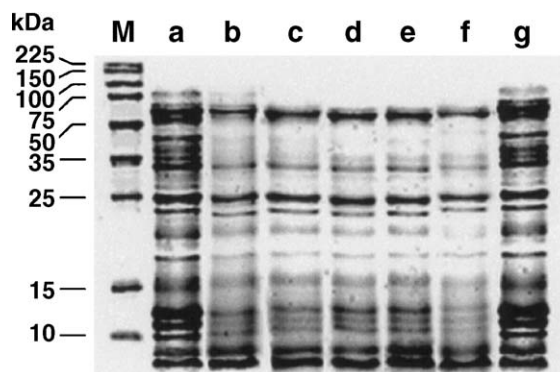


Fig. 2. SDS-PAGE of salt-soluble protein (SSP) fractions of pizza dough started with non-proteolytic (Prt^-) strains. Lanes a and g: uninoculated controls immediately after mixing and after 24 h of fermentation, respectively; lanes b, c, d, e and f: samples immediately after mixing and after 1.5, 3, 4.5 and 24 h of fermentation.

40 kDa subunits in both the types of inoculated doughs (Table 2; Figs. 1 and 2, lanes a and b). These changes, also observed by other researchers during doughmaking (Mascarós and Collar, 1994), may be due to the mechanical input during mixing, responsible for changes to cellular active sites for protein binding and, consequently, for changes to the chemical structure of the proteins. Only the bands with a MW below 10 kDa remained unchanged during the whole fermentation process.

Significant differences in SSP subunits between Prt^+ and Prt^- doughs were observed after 3 and 4.5 h of fermentation, by comparing the corresponding electrophoretic patterns (Figs. 1 and 2). The addition of Prt^+ starter resulted in an increased concentration of the protein subunits, especially of the fragments of 56 (+50%), 37 (+103%), 13.5 (69%), 12.2 (35%) and 11.5 (41%) kDa (Table 2; Fig. 1, lanes d and e), whereas modifications were not apparent to the same extent in SSP fragments from Prt^- dough during the fermentation ($P < 0.01$). As defined by MacRitchie et al. (1991), the subunits from 80 to 51 kDa, 40 to 28 kDa and the subunits ≤ 28 kDa correspond to high-molecular-weight albumins and globulins, to C groups of low-molecular-weight glutenins and to low-molecular-weight albumins and globulins, respectively. Such of these changes are reported to affect the bread-making and technological quality of the dough and the bread. In particular, LMW glutenins were defined as

quality-contributing glutenins able to improve loaf volume and dough strength (MacRitchie et al., 1991; Mascarós and Collar, 1994).

Nevertheless, after 24 h of fermentation, all SSP protein subunits extracted from Prt⁺ and Prt⁻ pizza doughs decreased to the same level ($P>0.05$), apart from the subunits of 60, 18.8 and <10 kDa (Figs. 1 and 2, lane f). The disappearance of the differences in the electrophoretic profiles of Prt⁺ and Prt⁻ doughs after 24 h of fermentation was probably due to a gradual SSP subunits degradation that could increase with the decrease of pH. In fact, a rise in acidity increases the rate of swelling and peptidization of the dough-proteins and controls the activity of the proteolytic enzymes (Stear, 1990).

The electrophoretic profiles of DSP fractions showed that Prt⁺ and Prt⁻ pizza doughs were the same in terms of presence of bands even if increases in DSP subunits were observed in both pizza doughs during the fermentation, albeit to a greater extent in Prt⁺ samples ($0.01 < P < 0.05$) (data not shown).

In agreement with Gobetti et al. (1994), dough without starter showed unchanged subunits during 24 h of incubation ($P>0.05$) for both SSP (Table 2; Figs. 1 and 2, lanes a and g) and DSP (data not shown) fractions, indicating that wheat flour endogenous enzymes did not contribute to protein degradation.

Primary amine nitrogen measured in SSP and DSP extracts of pizza doughs obtained using Prt⁺ strains

increased after 4.5 h of fermentation, while it showed no significant increase in Prt⁻ doughs (results not shown). Total protein content in the extracts from both Prt⁺ and Prt⁻ pizza doughs, as determined by the Bio-Rad Protein Assay, remained unchanged after 4.5 h of fermentation. Both values were also unchanged after 24 h of incubation in the uninoculated control dough (data not shown).

3.4. Effect of proteolysis on the mechanical characteristics of dough before and after baking

Mechanical measurements showed differences among the doughs, as shown in Table 3. The leavening process resulted in a decrease of the variation of pressure (ΔP_{\max}) and volume (ΔV_b), of the energy value E_{b1} necessary up to ΔP_{\max} , and an increase of the energy E_{b2} required from ΔP_{\max} to ΔV_b . The trend appeared more evident when the control and the Prt⁺ manufactures were compared ($0.01 \leq P \leq 0.05$). The proteolysis increased the values of E_{b2} and E_{b2}/E_{bT} indexes ($P \leq 0.05$), related to the viscous component, as well as lower values of E_{b1} and E_{b1}/E_{bT} indexes ($P \leq 0.01$), related to dough elasticity. Compressibility data (Table 3) showed a significant difference ($P < 0.001$) between Prt⁺ and Prt⁻ baked doughs as well. An increase in Young's modulus, related to the firmness of the crumb, was observed in Prt⁺ baked pizza doughs compared to Prt⁻ doughs. These data

Table 3

Numerical results (means \pm S.D.) of mechanical behaviour of leavened pizza doughs before and after baking fermented with proteolytic (Prt⁺) and non-proteolytic starter (Prt⁻)

Sample	Mechanical parameters ^a						Baked dough Young's modulus (kgf/mm ²)
	Dough						
	ΔP_{\max} (Pa)	ΔV_b (cm ³)	E_{b1} (10 ⁻⁶ J)	E_{b2} (10 ⁻⁶ J)	E_{b1}/E_{bT}	E_{b2}/E_{bT}	
Prt ⁺	133.60 \pm 11.43 ^b	18.14 \pm 2.21 ^b	143.47 \pm 34.13 ^c	3980.80 \pm 756.16 ^d	0.035 ^c	0.970 ^d	0.006 \pm 0.001 ^c
Prt ⁻	188.29 \pm 46.22 ^b	19.44 \pm 1.56 ^b	217.01 \pm 24.93 ^c	2024.86 \pm 544.96 ^d	0.097 ^c	0.900 ^d	0.004 \pm 0.001 ^c
Control ^f	195.67 \pm 18.55 ^g	20.04 \pm 1.22 ^g	234.91 \pm 73.86 ^g	625.88 \pm 186.00 ^g	0.270 ^b	0.730 ^b	nd ^h

Data are means and standard deviations of triplicate analyses.

^a For the symbols, see the text.

^b $P>0.05$.

^c $P \leq 0.01$.

^d $P \leq 0.05$.

^e $P < 0.001$.

^f Unleavened dough tested as control.

^g $0.01 \leq P \leq 0.05$.

^h Not determined.

demonstrated that the degradation of the gluten, as a result of the proteolytic activity of the Prt⁺ starter culture, affected the viscoelastic properties of the dough with a loss of elasticity. As a consequence, the dough became softer with a reduced capacity to retain CO₂. Moreover, in agreement with Stear (1990), an increased crumb density of the baked dough was observed.

4. Conclusion

The selection of lactic acid bacteria and yeast strains utilising the GMB media appears to be useful for determining the incidence of proteolytic and non-proteolytic cultures as leavening agents for pizza dough. Different modifications of protein fractions were obtained by using Prt⁺ and Prt⁻ starters for the fermentation of pizza dough. Prt⁺ starter cultures showed significant changes in some protein subunits while no major protein modifications were observed in the dough added Prt⁻ cultures. Knowledge on the technological performance of proteolytic strains during the manufacture of pizza dough will enable the leavening process to be controlled and will further help to improve and standardise the industrial production of pizza dough. Further studies are focusing on the evaluation of the effect of Prt⁺ and Prt⁻ starter cultures on the aromatic and sensorial properties of pizza dough.

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