

Improvement of Frozen Dough Stability Using a Cryoresistant Yeast Strain and Refreshment

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Frozen doughs for baked goods are increasingly produced because they offer added value in terms of both convenience (ready for use) and storage. The quality of these products depends on the choice of raw materials and technological process, in which leavening is a major step involving lactic acid bacteria and yeasts. In particular, the time required to proof frozen doughs depends on their formulation, yeast quality and quantity, compounds released by yeast, fermentation before freezing, and freeze-thaw rates (Wolt and D'Appolonia 1984a; Ribotta et al 2001). Moreover, interaction between a wide range of species of lactic acid bacteria and yeasts during the fermentation process (Coppola et al 1996, 1998; Gobetti 1998) influences the peculiar leavened structure (Pepe et al 2003c), the flavor, and the shelf life of the final product (Spicher 1983; Gobetti et al 1994; Rocken and Voysey 1995; Pepe et al 2003b). Frozen bread dough, once defrosted, is not the end product because it must undergo the proofing process to allow proper rising before baking (Neyreneuf and der Plaat 1991). Dough-rising capacity of baker's yeast drops considerably when the doughs are frozen after the initial prefermentation period. Studies have suggested various formulations and processing conditions to improve freeze-thaw resistance and viability of yeast cells (Wolt and D'Appolonia 1984a,b; Rasanen et al 1995). The decrease in stress resistance is due to activation of the cyclic AMP (cAMP)-protein kinase A (PKA) pathway, which results in activation of trehalase and mobilization of trehalose concomitant with a rapid loss in stress tolerance (Park 1997; Thevelein 2000). Yeast strains with better freeze resistance have been identified among natural isolates (Oda 1986) or from mutants (Teunissen 2002). Structural changes in frozen and thawed doughs are determined by a weakening of the gluten network, resulting in poor gas retention and longer proof time of frozen dough (Autio and Sinda 1992). Loss of stability has a marked effect on the rheological and baking properties, resulting in a reduction of the overall quality of baked goods.

On the basis of these considerations, we speculate that the stability of the frozen dough could be improved by cryoresistant microbial cultures associated in the starter. Thus, after a preliminary selection of a cryoresistant starter culture among natural strains, we studied its effect on the rheological and structural characteristics of bread from frozen dough.

MATERIALS AND METHODS

Microbial Composition of Starters, Growth Conditions, and Inocula Preparation

Forty-two different starters were obtained combining 20 different *Lactobacillus plantarum* strains isolated from sourdough (Pepe et al 2003a) with two strains of *Saccharomyces cerevisiae* isolated

from pizza dough (Coppola et al 1996) and sourdough (Pepe et al 2003c) as reported in Table I. Doughs started only with yeasts were also included. Lactobacilli were grown in MRS broth and incubated overnight at 30°C, whereas the yeasts were cultured in malt extract broth for 48 hr at the same temperature with rotator shaking. Cells were collected by centrifugation (6,500 rpm for 10 min), washed, and resuspended in 1/4 strength Ringer's solution to obtain $\approx 5 \times 10^9$ microorganisms/mL (direct microscopic counts).

Dough Formation and Freeze-Thaw Conditions

The doughs for bread were obtained as previously described by Coppola et al (1998). The starter suspension was also added at this stage at viable counts of $\approx 5 \times 10^7$ cfu/g of both yeast and lactic acid bacteria in the final doughs. Doughs were shaped into loaves of ≈ 250 g and incubated in graduated glass containers at 30°C until a twofold rise in the initial volume was reached. The samples were immediately frozen in polyethylene bags and stored for two weeks at -18°C . After this period, the samples were thawed at 23°C for ≈ 2.5 hr and promptly analyzed.

Selection of a Cryoresistant Starter

Viable lactic acid bacteria (LAB) and yeast cells were counted on modified Chalmers agar plates (Pepe et al 2001) before and after dough freezing. Cryoresistance was expressed as the percentage of survival cells after freezing and thawing of doughs. Starter 37 (Table I), in which the *S. cerevisiae* F1 exhibited the highest value of cryoresistance, was chosen for further investigation.

Bread Baked from Frozen Dough

The starter consisting of *L. plantarum* Q2 and *S. cerevisiae* T22 strains were tested in a frozen dough stability experiment. Frozen doughs, prepared as described above, were thawed after the storage time. One loaf (250 g) was refreshed by adding 150 g of flour and 25 mL of water and leavened at 30°C in aluminum pans until twice the initial volume was reached. The frozen loaves, with and without refreshment, were characterized by their microbial counts and leavening time and cooked in an oven at 250°C for 20 min. After cooling, the loaves were cut into two slices 3 cm thick and immediately submitted to structural and mechanical analysis. Images of the crumb were acquired by Snapscan 25 scanner (Agfa-Gevaert N.V., Morstel, Belgium) and processed by NHI Image 1.62 software (Wayne Rasband National Institutes of Health). The intensity of grey per area unit of crumb (10 cm) was determined and the values were expressed as gas cells/cm.

A mechanical test was performed using a Shimadzu dynamometer (mod EZ TEST). Cylindrical specimens were cut from the center of baked doughs and compressed between parallel plates at a deformation rate of 10 mm/min and load up to 2N. Three determinations per slice in duplicate analyses were made and the average values were reported. Unfrozen doughs were also assayed as control.

Statistics

Statistical treatment of data (analysis of variance) was performed using software for Macintosh v. 5.2.1 (www.systat.com).

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RESULTS

Cryoresistance of Lactic Acid Bacteria (LAB) and Yeast Strains in Frozen Dough

As reported in Table I, the cryoresistance observed, expressed as the percentage of survival after freezing (Meric et al 1995), depended on the bacterial and yeast strains associated in the starter ($0.01 \leq P \leq 0.05$). Almost all *L. plantarum* and yeast strains showed high sensitivity to low temperatures, with a cryoresistance variable from 0.1 to 10%. The freeze resistance improved when *L. plantarum* H1 and H18 strains were associated with *S. cerevisiae* T22, showing a cryoresistance of 39.9 and 79.5%, respectively (doughs 7 and 13). By contrast, *S. cerevisiae* T22 in association with the strains of *L. plantarum* L1, H1, H12, and H14 (doughs 2, 7, 10, and 11) showed a cryoresistance of 25–40%. This value increased to 50.2% when the yeast *S. cerevisiae* F1 was associated with *L. plantarum* Q2 (Table II, dough 37). The

TABLE I
Fermentation Properties and Cryoresistance in Doughs Leavened by *Lactobacillus plantarum* Strains Associated with *Saccharomyces cerevisiae* T22 and F1^a

Doughs	Starters ^b	Cryoresistance (%) ^c	
		<i>S. cerevisiae</i> ^d	<i>L. plantarum</i> ^d
1	T22	2.0	...
2	T22+L1	31.7	8.0
3	T22+L2	1.3	4.0
4	T22+L3	12.6	6.4
5	T22+L6	15.9	1.3
6	T22+L8	0.1	0.1
7	T22+H1	25.2	39.9
8	T22+H7	0.9	0.7
9	T22+H8	10.0	8.0
10	T22+H12	39.9	0.2
11	T22+H14	25.2	2.0
12	T22+H17	0.5	1.0
13	T22+H18	0.3	79.5
14	T22+H21	0.1	0.2
15	T22+Q1	0.1	4.0
16	T22+Q2	2.0	10.0
17	T22+Q3	0.2	0.2
18	T22+K13	10.0	0.2
19	T22+K14	0.1	1.6
20	T22+K21	0.1	0.4
21	T22+K22	0.1	0.3
22	F1	0.1	...
23	F1+L1	25.2	0.3
24	F1+L2	3.2	3.2
25	F1+L3	0.8	0.1
26	F1+L6	0.8	1.3
27	F1+L8	1.0	27.5
28	F1+H1	2.0	0.8
29	F1+H7	0.1	10.8
30	F1+H8	0.8	3.2
31	F1+H12	6.4	2.6
32	F1+H14	6.4	4.0
33	F1+H17	0.5	1.0
34	F1+H18	0.4	2.0
35	F1+H21	10.0	1.0
36	F1+Q1	3.2	1.0
37	F1+Q2	50.2	0.1
38	F1+Q3	1.0	0.2
39	F1+K13	1.0	0.1
40	F1+K14	2.6	1.6
41	F1+K21	0.6	0.1
42	F1+K22	6.4	10.0

^a Data are averages of triplicate measurements of duplicate analyses.

^b *Saccharomyces cerevisiae* T22 and F1 associated to *Lactobacillus plantarum* L1, L2, L3, L6, L8, H1, H7, H8, H12, H14, H17, H18, H21, Q1, Q2, Q3, K13, K14, K21, K22 strains.

^c Cryoresistance is expressed as the percentage of survival cells after freezing and thawing of doughs.

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

latter starter was employed in breadmaking experiments that included refreshment of the frozen dough with flour and water. The large number of surviving cells of the yeast was necessary to allow leavening of the dough after freezing and thus to evaluate the stability of the bread. Refreshed thawed doughs, obtained with the starter consisting of *S. cerevisiae* F1 and *L. plantarum* Q2, proofed in ≈ 8 hr, while the frozen doughs without refreshment did not increase their volume even after a long period of incubation. Moreover, frozen doughs prepared with other starter cultures did not proof in an acceptable period of time, even when the doughs underwent refreshment (data not shown).

Structural and Mechanical Analysis of Bread from Frozen Doughs

Frozen loaves, with and without refreshment, and unfrozen doughs underwent structural and mechanical analysis. As shown in Table II, an increase in Young's modulus was observed in bread from frozen dough without refreshment with respect to two other bread types ($0.01 \leq P \leq 0.05$). Breadcrumb from frozen dough

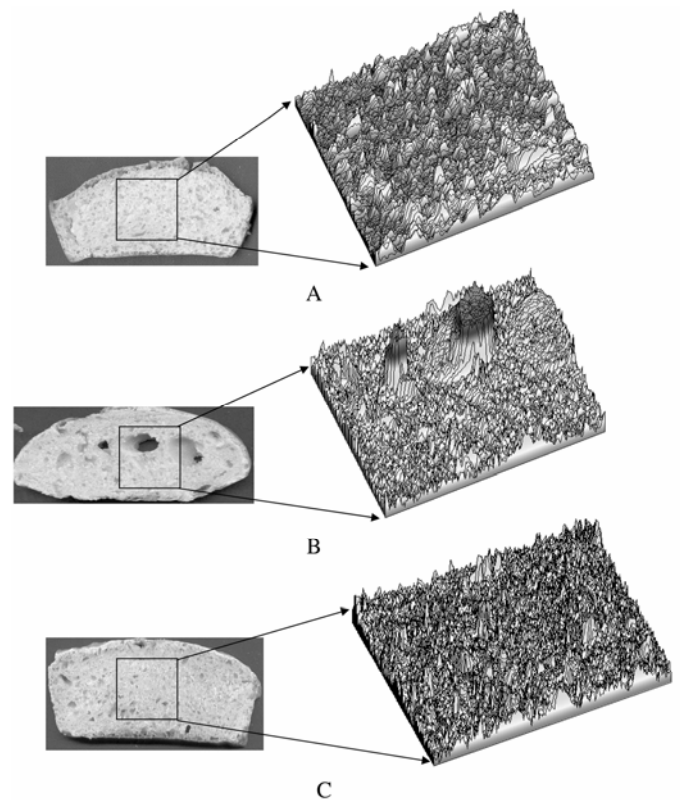


Fig. 1. Crumb appearance of breads made with dough prepared with *Saccharomyces cerevisiae* F1 and *Lactobacillus plantarum* Q2. Bread from fresh dough (A), bread from thawed dough (B), and bread from refreshed frozen dough (C). Graphics show surface plots of the selected area of the crumbs analyzed.

TABLE II
Effect of Refreshment on Mechanical and Textural Properties of Breads Baked from Frozen Dough^a

Sample	Young's Modulus (Kgf/mm) ^b	Gas Cells/cm ^b
F1+Q2 ^c	0.039	2.7
F1+Q2 WR ^d	0.096	6.0
F1+Q2 R ^e	0.066	4.2

^a Data are averages of triplicate measurements of duplicate analyses.

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

^c Fresh leavened dough.

^d Frozen dough without refreshment.

^e Frozen dough baked after refreshment.

showed higher values with regard to gas cell area than the corresponding crumbs from nonfrozen and refreshed doughs (Table II), suggesting a structure with a higher proportion of gas ($0.01 \leq P \leq 0.05$). Moreover, the crumb structure of the bread from frozen dough baked without refreshment showed an irregular distribution of the gas (Fig. 1B) with a more open structure with respect to the homogeneous appearance of the other surface plots of the other two breadcrumb sections (Fig. 1A,C). Mechanical and structural values of bread from dough with refreshment were between those of bread made from fresh dough and frozen dough without refreshment (Table II).

DISCUSSION

The prime aim of this work was to evaluate the cryoresistance of yeast strains associated with lactic acid bacteria in the starter cultures used for the preparation of frozen dough. Cryoresistance has been defined by Meric et al (1995) as the microbial ability to withstand the whole freezing treatment (freezing, thawing, cold storage stresses). The loss of microbial viability is caused by ice crystals that physically puncture the surface of the microbial cells or by the accumulation of metabolic products resulting in the autolysis of the cells (Hsu et al 1979). The variability observed in the cryoresistance of the starter could depend on the *L. plantarum* strains used in this study that, as observed in previous research (Pepe et al 2003a), exhibited molecular and technological diversity affecting fermentation in the dough. *S. cerevisiae* F1 and *L. plantarum* Q2 constituted an interesting starter in which the yeast showed highest cryoresistance and was used for the evaluation of stability of the breads from frozen doughs. Stability of frozen dough is defined as the ability of thawed dough to proof in an acceptable period of time and to bake into a loaf with normal volume and bread characteristics (Wolt and D'Appolonia 1984a). Because the refreshment of thawed doughs improved the leavening of the frozen dough, we speculate that the refreshment of the frozen dough allowed the formation of a new gluten network, improving CO₂ retention. This effect was possible only in doughs leavened by the selected cryoresistant starter containing a large number of yeast cells that survived freezing. Indeed, frozen doughs prepared with other starter cultures did not proof in an acceptable period of time, even when the doughs underwent refreshment. It is commonly known that freezing followed by storage in frozen conditions and thawing affects the gassing power of yeast due to the weakening of the frozen dough structure. This is caused by the deterioration of the gluten network, probably resulting from ice crystals forming during freezing (El Hady et al 1996) that lead to physical breakage of the gluten, disruption of hydrophobic bonding, and redistribution of water in the gluten structure (Varriano-Marston et al 1980; Berglund et al 1991; Inone and Bushuk 1991; Autio and Sinda 1992; Rasanen et al 1995).

Refreshment improved the mechanical characteristics of breads from frozen dough that increased softness. Indeed, the decrease in Young's modulus is related to the firmness of the crumb, as observed in a previous study (Pepe et al 2003c). Moreover, refreshment resulted in a crumb with a lower proportion and a regular distribution of gas, typical of bread from fresh dough.

CONCLUSIONS

In conclusion, freeze-thawing and storage of dough at -18°C generated loss in bread quality reflected by a longer fermentation time, an increase in the proportion of gas cells, and less elasticity of the bread dough. Combining the use of selected cryoresistant starters and the refreshment of the thawed dough, we prepared bread from frozen dough with improved stability, and structural and mechanical characteristics that were closer to bread made from fresh dough than bread made from frozen dough without refreshment. Studies are in progress to demonstrate a causal link between cryoresistance and microbial activity in dough.

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