Baker's Yeast Sampling and Frozen Dough Stability[1]

P. GÉLINAS,[2] M. LAGIMONIÈRE,[2] and C. DUBORD[3]

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ABSTRACT

The cryotolerance of six commercial baker's yeast strains grown under identical incremental feeding (fed-batch) conditions was evaluated in frozen doughs. Slight differences in survival were observed between yeast strains subjected to rapid freezing rates (about 10°C/min). All strains showed similar cryotolerance at slow freezing rates (about 1°C/min) followed by storage for 12 weeks. As part of a collaborative study between two laboratories, 21 commercial baker's yeast samples from seven trademarks (from which most strains were screened) were compared on the basis of their cryotolerance in frozen doughs. A rapid freezing test (measuring gas production) and three-month storage tests (measuring gas production, dough proofing time, and bread specific volume) showed that yeast cell cryotolerance varied greatly among the commercial yeast samples used to prepare frozen doughs. Because of the great variability in cryotolerance among yeast samples, none of the seven yeast trademarks tested from Canada, France, and the United States was superior to the others. These results show that the major effect on frozen dough stability is the commercial baker's yeast sampling rather than strain or trademark, and they stress the importance of quality control for cryotolerance of the baker's yeast samples used in frozen dough production.

Baker's yeast is an important ingredient in frozen dough production. Its cryotolerance varies according to strain (Hino et al 1987), growth conditions (Gélinas et al 1989), or commercial source (Kline and Sugihara 1968, Neyreneuf and van der Plaat 1991). Hsu et al (1979) stated that yeast from different batches and sources responded differently to freeze-thaw conditions during frozen dough production. However no extensive and comprehensive information has been reported on the relative importance of the sampling of baker's yeast to its survival of freeze-thaw cycles during frozen dough production and storage. The relative importance of yeast strain compared with commercial yeast sampling on the market is not known.

Variation in cryotolerance among commercial yeast samples is difficult to explain on the basis of their chemical composition, for example, the nitrogen (Hsu et al 1979), trehalose (Gélinas et al 1989), or lipid content (Gélinas et al 1991) of baker's yeast cells. In industrial bakeries or yeast plants, no significant quality control tool is available for checking the cryotolerance of baker's yeast samples before their incorporation into dough to be frozen. Bakers usually rely on the freshness of the baker's yeast samples to get optimal frozen dough stability, but they must wait a few weeks before getting information on yeast survival and dough proofing activity.

In the study reported in this article, we first compared the cryotolerance of six yeast strains isolated from representative commercial baker's yeast samples and grown in the laboratory under conditions similar to those used in manufacturing industrial baker's yeast. In a second series of experiments, we compared the cryotolerance of commercial baker's yeast samples (seven trademarks) dispatched from Canada, France, and the United States. A collaborative study was also attempted with another laboratory, using different protocols to evaluate yeast performance in frozen doughs. Our main objective was to determine the respective effects of yeast strain, sampling, and trademark; we also looked for possible discrepancies between results obtained from the two groups using different methodologies for frozen dough testing. A rapid test for yeast cryotolerance was also evaluated.

MATERIALS AND METHODS

Baker's Yeast Strains and Growth Conditions

Six commercial strains of baker's yeast were isolated (on malt agar, 30°C, 24 hr) from commercial samples dispatched from Canada, France, and the United States (C1, C2, F1, F2, U1, U3). All strains were further grown for 48-72 hr at 30°C on malt agar slopes supplemented with 0.7% bacto agar (Difco) and stored at 4°C.

Batch cultures were used as inoculum for fed-batch fermentations and were prepared as follows. Surface growth from malt

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[2]Food Research and Development Centre, Agriculture Canada, St. Hyacinthe, Quebec, Canada J2S 8E3.
[3]Campus Haute-technologie Agro-alimentaire de Saint-Hyacinthe.

agar was used to inoculate 4 × 40 ml of malt broth containing: malt syrup, 1,006 g; ammonium sulfate, 8.1 g; ammonium phosphate, 4.6 g; magnesium sulfate, 1.8 g; yeast extract, 1.2 g; water, 7.8 L, with pH adjusted to 5.5 with sulfuric acid. The 125-ml Erlenmeyer flasks containing the media were placed at 30°C and 120 rpm for 24 hr. Four 2,000-ml Erlenmeyer flasks, each containing 1,000 ml of malt broth, were then inoculated with the content of the previous culture and incubated at 30°C and 120 rpm for 18 hr. Cell content was then determined on malt agar. Cell biomass was centrifuged (4,000 × *g* for 13 min) and then washed twice with sterile distilled water. All batch fermentations were performed in duplicate.

Yeast dry weight was determined in triplicate by the following method. Yeast biomass (5 g) in an aluminum weight boat was diluted with 5 ml of 70% ethanol solution, dried at 110°C for 4 hr, and cooled; the resulting dried yeast sample was weighed.

For fed-batch fermentations, 12 g (dry weight equivalent) recovered from batch cultures was used as the inoculum. A 2.5-L fermentor (Bioengineering AG, Wald, Switzerland) was used throughout the study. A microcomputer (TRS-80 model 100; Tandy Corp., Fort Worth, TX) was used to control the stepwise incremental feeding of molasses and ammonia solutions by means of peristaltic pumps (model 520; Biochem Technology, Malvem, PA), according to current baker's yeast industrial production methodology. Ammonia addition was completed in 12 hr; molasses feeding was completed 1 hr later; and the fermentation itself stopped after another 1 hr. Fermentations were done at 30°C, 1,100 rpm, with 5 L of air per minute, corresponding to about 2.6 volumes of air per volume of growth medium per minute. The biomass yield was the amount of dried yeast (in grams) produced from 100 g of molasses (27%, yeast dry weight basis). All fed-batch fermentations were performed in duplicate. The composition of the growth medium and the complete methodology are described in Gélinas et al (1989).

Freezing Tests for Yeast Strains

Because of the limited amount of yeast biomass obtained under laboratory conditions, a different procedure for freezing was used in this section as compared with that for tests performed with commercial yeast samples. Cryotolerance of harvested baker's yeast was evaluated in duplicate in a dough, by both a rapid test and a storage test that measured gas-producing activity. Dough was prepared with a Swanson mixer (National Mfg. Co., Lincoln, NE) according to the no-time dough process, as follows (200 g, flour basis): flour, 100; water, 59; sugar, 4; yeast, 0.9 (dry weight basis); shortening, 3; salt, 2; ascorbic acid, 100 ppm; potassium bromate, 60 ppm. All dry ingredients were mixed for 1 min at low speed; the rest of the ingredients were then added and slowly mixed for 1 min. Intense mixing was done for 5 min; the resulting dough temperature was 25°C. The dough was divided by hand into 13 portions (one of 100 g, 12 of 15 g). All 15-g pieces of dough were rounded by hand. Half of them (standard) were directly placed into Risograph jars (RDesign, Pullman, WA) and placed in the water bath at 38°C; then the jars were connected to the Risograph. Gas measurements were immediately begun and were continued every 2 min for 90 min. The six other 15-g dough pieces were individually placed into plastic bags and sheeted mechanically. Bags containing doughs were then attached to a metallic rack and submerged in an ethanol bath at −45°C for 20 min, which corresponded to a freezing rate of 9.2°C/min. Each frozen sheet of dough was broken in two, removed from the bag, and placed in a Risograph jar. Closed jars (not connected) were left for 15 min in the water bath at 38°C before the 90-min collection of data was started. Gas production from rapid frozen-thawed doughs and nonfrozen doughs (standard) was compared and expressed as percent yeast survival.

The 100-g dough piece was sheeted-molded and placed in a storage freezer at −23°C; this corresponded to a freezing rate of about 1°C/min. After being held for 12 weeks, it was thawed at 0–2°C for 16 hr in a cardboard container. The 100-g dough piece was then divided into six 15-g portions to be placed into Risograph jars. Gas production from the yeast was then determined as described above except that measurements were made immediately. Gas production from stored frozen-thawed doughs and nonfrozen doughs (standard) was compared and expressed as percent yeast survival.

Commercial Yeast Samples

In all, three samples each from seven commercial yeast sources based in Canada (C1, C2), France (F1, F2), and the United States (U1–U3) were received at different times and tested separately. All samples were compressed yeasts, which were shipped in refrigerated containers either by air or ground transportation and tested as soon as possible upon reception (within two days). All yeast samples were tested within one week following the day of their manufacturing. The temperature of the yeast samples was kept at 2–4°C during transportation and evaluation.

Breadmaking Procedure (Laboratory 1)

The following procedure was used for testing commercial yeast samples in Lab. 1; the methodology employed in the second laboratory is presented in a later section.

Dough was prepared by the no-time dough process, as follows: (2.5 kg, flour basis): flour, 100; water, 59; sugar, 4; compressed yeast (30% dry weight), 3; shortening, 3; salt, 2; ascorbic acid, 100 ppm; potassium bromate, 60 ppm. All dry ingredients were mixed for 1 min at low speed in a Hobart mixer A 200-20. The rest of the ingredients were then added and slowly mixed for 1 min. Intense mixing (speed 2) was done for 12 min. Dough was divided by hand into 12 330-g portions and rounded mechanically. After resting for 10 min at room temperature, doughs were sheeted through sheeting rolls set at 9 mm, molded with a sheeter-molder (L & M Co. Ltd., Downsview, Ontario, Canada), and then separated into three groups. Group 1 contained fresh standards consisting of three pieces of dough immediately put into pans, proofed, and baked. Group 2 contained eight doughs that were immediately frozen and stored, and Group 3 contained one dough subsequently used for the rapid freezing test. Dough temperature was 20°C at this point. Each compressed yeast sample from each trademark was tested three times (three

TABLE I
Number of Replicates in Experimental Design for Testing of Commercial Baker's Yeast Samples, According to Analysis

Condition	Gas Production			Proof Time		Bread Volume	
	Lab. 1	Lab. 2	Rapid Test	Lab. 1	Lab. 2	Lab. 1	Lab. 2
Yeast sample/trademark	3	3	3	3	3	3	3
Batch of dough/yeast sample	3	1	3	3	1	3	1
Dough piece/batch of dough	1	1	1	2	2	2	6
Thawing replicate	2	2	1	2	2	2	2
Data/test on Risograph or Rheofermentometer	1[a]	1	1[a]	...	...	...	...
Data/yeast sample[b] (frozen dough)	6	2	3	12	2	12	2
Data/yeast sample[b] (standard)	3	1	3	9	1	9	1
Data/yeast trademark[b] (frozen dough)	18	6	9	36	6	36	6

[a] Mean calculated from six Risograph readings.
[b] Data used to calculate percentage means in Tables III–VI.

batches of dough per yeast sample). For this series of experiments involving commercial yeast samples, the number of repetitions according to procedure and test performed is presented in Table I.

Freezing Tests for Commercial Baker's Yeast Samples

Frozen doughs were evaluated according to two types of freezing tests: three-month storage tests and a rapid freezing test.

Storage tests. For each batch of dough, three pieces of dough (Group 1) were put into pans 65 mm high (100 × 210 mm at the top of the pan) and proofed at 40°C and 100% rh. Proof times were noted when the top of the dough was 15 mm over the pan rim. Baking was done for 20 min at 213°C in an electric revolving oven (L. P. Inc., Victoriaville, Quebec, Canada). After cooling for 1 hr, the three breads were weighed and their volumes measured by rapeseed displacement.

During that period, eight doughs (Group 2) were frozen at −50°C for 45 min in a cryogenic (CO_2) freezer (Ultrafrost, Küleg, Germany). They were placed into double plastic bags, and stored for 12 weeks at −30°C. Upon completion of the storage time, thawing was done on two different days (two series of four doughs each). Each time, four doughs were put into a cardboard container; doughs were placed along each side of the box, between two plastic bags, and were kept at 0–2°C for 16 hr, or until the temperature at dough center was 0–2°C, as measured with a thermometer. Three of the four thawed doughs were placed into pans, proofed, and baked under the same conditions as the standards. Proof times (transformed to the reciprocal, $1/t$) and bread volumes were compared with those of the standards (from the same yeast sample) and expressed as percent residual performance.

For the determination of yeast gas production, the remaining piece of dough (330 g) was divided into six 25-g portions, each rounded by hand and deposited into Risograph jars. These jars were placed in the water bath at 38°C, and gas production measurements were started immediately after the jars were connected to the Risograph. After 90 min of fermentation, pooled data from the two repetitions of six Risograph readings (from thawing on two different days) were compared with the mean of six Risograph readings for nonfrozen doughs (standard) obtained from the rapid test described in the following section. Results were expressed as percent residual performance (gas production).

Rapid freezing test. A rapid freezing test was performed concurrently with the storage test. After sheeting and molding, one nonfrozen dough (Group 3) was further divided into 12 25-g pieces. Six of the pieces (the standard for all gas-production tests for commercial samples) were immediately rounded by hand, placed into Risograph jars, and put into the water bath at 38°C; gas production readings were then registered every 2 min for 90 min. The six remaining dough pieces were rolled by hand into a cylinder form, placed into plastic bags, slightly pressed by hand, and then sheeted mechanically. Bags containing doughs were then attached to a metallic rack and submerged in an ethanol bath at −45°C for 20 min. Each frozen sheet of dough was broken in two, removed from the bag, and placed into a Risograph jar. Closed jars (not connected) were left for 15 min in the water bath at 38°C before the 90-min collection of data was started. Gas production from rapid frozen-thawed doughs and nonfrozen doughs were compared and expressed as percent residual performance.

Collaborative Study (Laboratory 2)

Besides the tests described above, a collaborative study was set up to confirm results and, indirectly, to evaluate the effect of frozen dough testing methodology on the precision of the results. Four of the seven commercial yeast trademarks from the latter study were tested in a laboratory based in France.

A french white pan bread, without fat and sugar, was prepared as follows: flour, 100; water, 59; compressed yeast (30% dry weight), 3; salt, 2.25; bread improver (vital wheat gluten, soya lecithin, ascorbic acid, and α-amylases in unknown proportions), 1 (Neyreneuf and van der Plaat 1991). Each batch was produced from 10 kg of flour in an inclined-arm mixer (3 min at low speed, 40 × *g*, then 17 min at high speed, 80 × *g*). Yeast and salt were added at 5 and 2 min, respectively, before the end of mixing. The final dough temperature was about 20°C.

Upon dividing, three shapes of doughs were prepared: five balls of 315 g (for gas production measurements), 10 balls of 30 g (to be used as proofing indicators for the rectangular doughs), and 60 rectangular slabs of 165 g (170 × 70 × 10 mm) (to be used to measure bread volume).

For each yeast sample, standards (nonfrozen doughs) consisted of one 315-g ball, two 30-g balls, and 12 165-g doughs. Twenty minutes after mixing, the gas pressure measurements were performed for 3 hr at 28.5°C by the Chopin rheofermentometer (Groupe Tripette & Renaud, Villeneuve-la-Garenne, France). Each bread was prepared from two slabs of dough piled inside the pan before fermentation (25°C and 85% rh); baking was done at 225°C for 25 min after both 30-g proofing indicators had attained 60 mm of height (one mean value). After breads had cooled for 30 min, volumes were measured, three breads at a time, with a volumeter; results were pooled (one mean) and reported as volumes without consideration of bread weight.

Twenty minutes after mixing, the rest of the doughs (48 165-g slabs, four 315-g balls, and eight 30-g balls) were placed in a blast freezer (Pierre Pont, Villefranche-sur-Saône, France) at −40°C until the dough centers were −12°C. All doughs were placed in plastic bags and stored at −20°C. Thawing was done after 1, 2, 90, and 91 days (each time, one 315-g ball, two 30-g balls, and 12 165-g slabs were used). The 315-g balls were thawed rapidly for 1 hr at 28°C, and the other doughs were defrosted slowly (15 hr, 0°C). Results from doughs thawed after one and two days were used as an indication of the quality of the frozen dough process itself; only a minor drop in residual performance was observed (not shown).

Results for gas production, proof time ($1/t$), and bread volume were expressed as percent residual performance, comparing results from frozen-thawed doughs with those from nonfrozen doughs (standard).

Number of Replicates and Statistical Analysis

Data were analyzed according to Tukey's Studentized range test at the level of probability of 0.05. Table I gives information on the experimental design and the number of data replications used to calculate the means used in later tables for yeast samples and trademarks.

RESULTS AND DISCUSSION

Effect of Yeast Strain

Similar biomass yields were obtained after production by incremental feeding of the six baker's yeast strains tested (results not shown). The first stages of yeast cell growth also gave similar yields (8–13 × 10^7 cells per milliliter), so it was not necessary to standardize the inoculum to be used for the fed-batch fermentations. All strains were relatively easy to grow in the fed-batch mode, using a specific growth rate of 0.117 hr^{-1}. This indicates that the commercial baker's yeast strains tested did not differ much in their nutritional requirements.

Baker's yeast biomass produced was incorporated into a bread dough formulation; the yeast strains showed similar gas-producing activity of about 5.5 ml of gas per gram of dough after 90 min at 38°C (results not shown). This indicates that the choice of the baker's yeast strain itself, among the six strains tested here, had no effect on gas-producing activity in conventional (nonfrozen) breadmaking fermentations.

The survival after freeze-thaw of the six baker's yeast strains tested is presented in Table II. Results are shown in decreasing order of cryotolerance according to data obtained after rapid freezing.

Cryotolerance of the baker's yeast strains was generally similar. After rapid freezing and without a storage period, strains U1 and C2 showed a slightly lower cryotolerance compared to that of strain U3 (24–25% vs. 32%). Slow freezing rates (about 1°C/min), without storage, did not affect the gas-producing activity of the yeast cells no matter what the strain (results not shown).

Other tests also showed that freezing doughs, without storage, at −20, −25, −30, or −40°C did not affect yeast survival (which was about 100%) when the freezing rate was low (about 1°C/min). After a three-month storage period, there was a general drop in survival, but no single strain was markedly more cryotolerant than the others (Table II). Under rapid freezing conditions such as those met in the rapid freezing test (9.2°C/min), survival dropped to 24–32% compared to that of standard (nonfrozen) doughs; under slow freezing conditions (about 1°C) followed by storage at −23°C, survival was higher (48–61%). This indicates that rapid freezing in dough was much more deleterious to yeasts, regardless of the strain, as compared to slow freezing.

TABLE II
Percent Yeast Residual Gas Production in Frozen-Thawed Doughs, According to Yeast Strain and Freezing Condition

Strain	Freezing Condition[a]	
	Rapid	Slow
U3	32.00 a	56.00 a
F2	28.61 ab	54.53 a
C1	27.91 ab	61.72 a
F1	25.79 ab	50.33 a
U1	24.79 b	48.46 a
C2	24.08 b	57.00 a
General mean	27.20	54.67

[a]Means with the same letter are not significantly different within each column ($P < 0.05$; Tukey). Each yeast strain is identified according to the country of origin of its trademark (Canada = C1, C2; France = F1, F2; United States = U1, U3). Percentage means were calculated from four data (one datum = one ratio calculated by comparing six Risograph readings from frozen-thawed doughs with six readings from nonfrozen doughs).

TABLE III
Gas Production in Nonfrozen Doughs and Percent Yeast Residual Gas Production in Frozen-Thawed Doughs, Frozen Rapidly, According to Yeast Sample and Trademark[a]

Yeast Trademark and Sample Number	Gas Production, Nonfrozen Doughs (ml)	Residual Gas Production, Frozen Doughs	
		Sample (%)	Trademark (%)
F1-1	120.6 e–g	47.65 d–f	52.82 a
F1-2	135.4 cd	55.30 a–c	
F1-3	128.7 de	55.50 ab	
F2-1	114.9 fg	53.34 a–c	52.66 a
F2-2	130.0 c–e	47.76 d–f	
F2-3	130.5 c–e	56.89 a	
C2-1	147.5 ab	51.28 b–d	50.53 a
C2-2	151.5 a	47.39 d–f	
C2-3	128.2 de	52.93 a–c	
U3-1	135.1 cd	41.98 g–i	48.17 a
U3-2	112.1 g	53.20 a–c	
U3-3	139.2 bc	49.33 c–e	
C1-1	128.2 de	46.30 d–g	47.51 a
C1-2	137.2 cd	51.24 b–d	
C1-3	111.4 g	45.00 e–g	
U1-1	138.9 b–d	38.67 i	45.17 a
U1-2	156.1 a	43.48 f–h	
U1-3	137.8 b–d	53.35 a–c	
U2-1	135.3 cd	32.12 j	37.86 a
U2-2	148.1 ab	43.42 f–h	
U2-3	123.8 ef	38.03 i	
General mean	127.2	47.82	

[a]Means with the same letter are not significantly different within each column ($P < 0.05$; Tukey). Each yeast trademark is identified according to its country of origin (Canada = C1, C2; France = F1, F2; United States = U1–U3). Yeast samples within a trademark are numbered (e.g., F1-1, F1-2, F1-3). For yeast samples and trademarks, percentage means were calculated from three and nine data, respectively.

Effect of Yeast Sample and Trademark

In a separate series of experiments, commercial baker's yeast samples were tested for cryotolerance in frozen doughs; all were screened from representative trademarks from Canada, France, and the United States (three samples per trademark). Except for trademark U2, each individual yeast trademark tested was used as a source for strain selection (results presented above).

Gas Production Tests

Gas-producing activity. Despite the fact that commercial yeast samples were dispatched from different sources and shipped by air or ground transportation, all tested samples were in very good condition upon reception. Table III presents the gas-producing activity of the 21 baker's yeast samples when tested in nonfrozen doughs. A general mean of 127.2 ml of gas was produced in 25 g of dough after 90 min at 38°C; this corresponds to 5.1 ml of gas per gram of dough at 38°C, which is slightly lower than 5.5 ml, the mean gas volume obtained for fresh yeasts produced in our laboratory using different yeast strains (results presented above). According to our experience and under the conditions of the test performed, including the calibration value for the Risograph, gas production values of 4.8–5.0 ml/g of dough (90 min, 38°C), about 120–125 ml for 25 g of dough, are acceptable, but values of 5.5–6.2 obtained for fresh yeast samples are optimal. This means that three yeast samples (F2-1, U3-2, and C1-3) had lower activity than the others and might have suffered from transportation. However, as presented below, these considerations did not appear to affect cryotolerance of the three yeast samples, whatever the freezing conditions.

Rapid freezing test. Besides storage tests, a rapid freezing test was performed. Results are presented in decreasing order in Table III. The rapid test is based on the fact that rapid freezing is more deleterious to yeast than slow freezing, at least for freezing rates close to those met in frozen dough production. Results con-

TABLE IV
Percent Yeast Residual Gas Production of Frozen-Thawed Doughs Stored for Three Months, According to Yeast Sample and Trademark[a]

Yeast Trademark and Sample Number	Evaluation Method			
	Laboratory 1		Laboratory 2	
	Sample (%)	Trademark (%)	Sample (%)	Trademark (%)
F1-1	91.92 a	80.27 a	81.60 ab	74.37 a
F1-2	80.82 a–c		69.19 ef	
F1-3	68.07 c–g		72.33 de	
F2-1	87.29 ab	77.57 a	82.10 a	73.41 a
F2-2	77.21 b–e		60.24 hi	
F2-3	68.21 c–g		77.88 bc	
U2-1	79.22 c–g	73.69 a	...	...
U2-2	72.96 a–d			
U2-3	68.90 c–g			
U3-1	75.62 b–f	71.54 a	...	...
U3-2	72.24 c–g			
U3-3	66.76 c–g			
U1-1	72.38 c–g	70.29 a	...	...
U1-2	72.68 c–g			
U1-3	65.81 d–g			
C2-1	74.80 b–f	66.50 a	67.34 fg	62.34 a
C2-2	64.58 e–g		59.16 i	
C2-3	60.12 g		60.54 hi	
C1-1	74.31 b–f	67.96 a	76.30 cd	67.86 a
C1-2	63.03 fg		63.46 gh	
C1-3	66.54 d–g		63.84 gh	
General mean	72.64		69.50	

[a]Means with the same letter are not significantly different within each column ($P < 0.05$; Tukey). Identification of yeast trademarks and samples is presented in Table III. For yeast samples and trademarks, percentage means were calculated from six and eighteen data, respectively (Lab. 1) and two and six data, respectively (Lab. 2).

cerning yeast cryotolerance are available within 2.5 hr, which makes this test a potential quality control tool.

According to the results of the rapid freezing test (Table III), there were important differences in cryotolerance among the 21 yeast samples tested, even within the same trademark. Survival of yeast samples varied from 32 to 55%, but no significant difference was seen among cryotolerance of the seven yeast trademarks tested. These results indicate that yeast samples available to the baker vary markedly in ability to survive high freezing rates, despite the fact that the strains used to produce them commercially have similar cryotolerance. These major differences in cryosurvival of baker's yeast samples reflect fluctuations in the quality of commercial baker's yeasts.

Three-month storage tests. Table IV presents the residual gas producing activity of yeast samples incorporated into frozen-thawed doughs stored for three months (according to results from Labs. 1 and 2). Initial freezing was performed at 1.3°C/min, which is considerably slower than in the rapid freezing test (9.2°C/min). All data are presented in decreasing order, based on results from Lab. 1.

Within a trademark or not, yeast survival after freeze-thaw varied greatly among baker's yeast samples: from 60 to 92%, as compared with the nonfrozen dough standard obtained for each sample. These results confirm those obtained in the rapid freezing test and probably explain the difficulty in identifying one specific commercial yeast trademark as the most stable for frozen dough production. At least for tests performed in Lab. 1, these trends cannot be attributed to reproducibility problems because slight variations of about 2–5% were seen among results from three repetitions on the Risograph (not shown). In both laboratories, yeast trademarks from France had some of the best samples for retaining gas production after freeze-thaw but also had very cryosensitive samples (e.g., F1-3).

In general, baker's yeast samples retained about 70% of their fermentative activity in doughs stored for 12 weeks at −20 or −30°C and prepared according to protocols used in both laboratories. This percentage is in accordance with data obtained by Dubois and Brockcolsky (1986) but may vary markedly depending on experimental conditions. For example, a residual gas production of 40% (Bruinsma and Giesenschlag 1984) or 90% (Bultmann 1988) has been reported for frozen doughs stored for 12 weeks. Procedures used to prepare frozen doughs, including formulation and dough temperature at the end of mixing, are considered major factors.

TABLE V
Percent Residual Proof Time ($1/t$) of Frozen-Thawed Doughs Stored for Three Months, According to Yeast Sample and Trademark[a]

Yeast Trademark and Sample Number	Evaluation Method			
	Laboratory 1		Laboratory 2	
	Sample (%)	Trademark (%)	Sample (%)	Trademark (%)
F2-1	68.66 a–d	68.77 a	50.26 a	46.97 a
F2-2	73.51 a		44.13 b–d	
F2-3	64.13 b–f		46.52 ab	
F1-1	70.21 a–c	67.89 a	50.26 a	46.97 a
F1-2	71.15 ab		44.13 b–d	
F1-3	62.32 c–f		46.52 ab	
U2-1	67.93 a–d	66.77 a	...	...
U2-2	64.30 b–e			
U2-3	68.09 a–d			
U3-1	68.51 a–d	66.54 a	...	...
U3-2	69.70 a–d			
U3-3	61.41 d–f			
U1-1	63.93 b–f	64.38 a	...	...
U1-2	67.37 a–e			
U1-3	61.83 c–f			
C1-1	64.52 b–e	62.99 a	45.48 bc	41.18 a
C1-2	61.61 d–f		41.80 cd	
C1-3	62.85 b–f		36.26 e	
C2-1	66.75 a–e	60.46 a	42.10 cd	41.84 a
C2-2	58.94 ef		42.23 cd	
C2-3	55.69 f		41.18 d	
General mean	65.40		44.24	

[a] Means with the same letter are not significantly different within each column ($P < 0.05$; Tukey). Identification of yeast trademarks and samples is presented in Table III. For yeast samples and trademarks, percentage means were calculated from 12 and 36 data, respectively (Lab. 1) and two and six data, respectively (Lab. 2).

Dough Proofing Tests

Table V presents results from dough proofing tests performed in both laboratories. Raw data were transformed to the reciprocal of proof time ($1/t$) and compared to standards on a percent basis. In general, these results confirmed those obtained in gas production tests (Tables III and IV): large fluctuations appeared among yeast samples (within a trademark or not), but no significant difference existed among cryotolerance levels of the seven yeast trademarks tested.

Dough proof time results give information on gas production by yeasts but are influenced by the gas retention properties of the dough, which are affected by dough formulation and processing. This method is usually less precise than are direct gas pressure measurements. In Lab. 2, some of the yeast samples could not be distinguished by the dough proofing test.

Bread Specific Volume Tests

According to bread volume results (Table VI), there were only minor differences in cryotolerance among yeast samples. Yeasts that had the shortest proof time or the highest activity for gas production did not make bread with the highest volume. Bread specific volumes seem of limited value to evaluate yeasts for frozen doughs because the test is not very precise and is rather a measure of the oven spring capacity of doughs than a real screening test

TABLE VI
Percent Residual Bread Specific Volume from Frozen-Thawed Doughs Stored for Three Months, According to Yeast Sample and Trademark[a]

Yeast Trademark and Sample Number	Evaluation Method			
	Laboratory 1		Laboratory 2	
	Sample (%)	Trademark (%)	Sample (%)	Trademark (%)
U3-1	99.19 ab	99.07 a	...	...
U3-2	98.77 ab			
U3-3	99.24 ab			
C2-1	94.14 ab	98.07 a	93.79 b–d	94.06 ab
C2-2	96.47 ab		98.81 ab	
C2-3	103.60 a		89.58 cd	
F1-1	100.85 ab	97.78 a	100.79 a	99.11 a
F1-2	97.93 ab		97.48 ab	
F1-3	94.57 ab		99.07 ab	
C1-1	97.32 ab	97.04 a	87.01 d	92.01 b
C1-2	96.32 ab		98.81 ab	
C1-3	97.49 ab		89.96 cd	
F2-1	97.24 ab	96.86 a	98.87 ab	97.43 ab
F2-2	96.55 ab		93.35 a–c	
F2-3	96.79 ab		99.07 ab	
U2-1	93.22 b	96.04 a	...	...
U2-2	97.88 ab			
U2-3	97.00 ab			
U1-1	94.03 ab	95.85 a	...	...
U1-2	97.30 ab			
U1-3	96.26 ab			
General mean	97.24		95.65	

[a] Means with the same letter are not significantly different within each column ($P < 0.05$; Tukey). Identification of yeast trademarks and samples is presented in Table III. For yeast samples and trademarks, percentage means were calculated from 12 and 36 data, respectively (Lab. 1) and two and six data, respectively (Lab. 2).

for gas production by yeast. If proof times are not excessively long, bread specific volumes are expected to be quite constant, considering that all others factors are kept constant (formulation, proofing and baking conditions, etc.). Bread volume would be indicative of the quality of the whole breadmaking process rather than a screening test for yeast cryotolerance.

Contrary to what was observed in Lab. 1, where most yeast samples could not be differentiated according to bread volumes, some yeast samples (C1-1, C1-3) tested in Lab. 2 gave lower bread volumes than the standard. This lead to significant differences between trademarks F1 and C1, but these results were not confirmed by the proofing or gas production tests.

Comparison of Screening Tests

Figure 1 presents the overall percent residual performance of the 21 baker's yeast samples, according to Lab. 1. The gas production test for doughs stored for three months was used as the reference curve because it was the most direct measure of yeast activity among the tests; yeast samples are presented in decreasing order according to their cryotolerance. In the figure, points were connected to facilitate comparison between screening tests and to calculate correlation coefficients.

In general, yeast survival after rapid freezing was not related to its survival after slow freezing (followed by storage for 12 weeks). Hence, results from the rapid freezing test could not always predict well the long-term cryotolerance of the 21 yeast samples incorporated in frozen doughs and stored for 12 weeks. However, variations in the results from the rapid freezing test are certainly indicative of yeast cryotolerance to high freezing rates and stress such as those encountered at the surface of doughs. Freezing rates of 9.2°C/min were obtained at the center of the 25-g doughs immersed in the ethanol bath (rapid test), as compared to 1.3°C/min at the center of 330-g doughs placed in the cryogenic freezer at −50°C; these freezing temperatures were measured between −5°C and a temperature 5°C higher than the final temperature. The rapid freezing test, as presented here, may be a thorough research tool or a quality control test to screen baker's yeasts that do not survive well at high freezing rates.

Good correlation was observed between proof time and three-month gas production tests (Pearson correlation coefficient = 0.749). This indicates that results from dough proof times could also accurately predict yeast cryotolerance. However, the most cryotolerant yeasts were not screened adequately by the dough proofing test.

As expected, bread volumes of frozen-thawed doughs stayed high (90–100%, compared to the standard) and were not related to results from gas measurements. According to the data presented, loaf volumes cannot be considered good indicators of yeast survival after freezing. Dubois and Blockcolsky (1986) came to similar conclusions and stated that loaf volumes from frozen-thawed doughs, as compared to gas production or proof time, remained fairly constant after storage of doughs for up to 20 weeks.

Figure 2 presents the effect of test methods used in Lab. 2 to screen yeast survival after freeze-thaw. As in Figure 1, the reference curve is for gas production after storage for three months (obtained in Lab. 1). Surprisingly, correlation was not very good between results from gas production tests performed in the two laboratories. This suggests that the methodology used for measuring gas generation is important, including the human factor. The rheofermentometer considers one reading at a time (Lab. 2), whereas the Risograph used in Lab. 1 can do up to 12 readings; six readings were used in Lab. 1 to calculate each data. Also, for each yeast sample tested, six repeats were done in Lab. 1 as compared to two in Lab. 2 (Table I).

In Lab. 2 (Fig. 2), there was good correlation between gas production and proof time (Pearson correlation coefficient = 0.669). Proof times for frozen-thawed doughs from Lab. 1 were also shorter than those from Lab. 2. In Lab. 1, the use of sugar in the bread formulation and higher proofing temperatures (40 instead of 25°C in Lab. 2) also contributed to a more rapid fermentation startup by the frozen-thawed yeasts. The use of a bread formulation without fat or sugar in Lab. 2 might also have enhanced the observed differences because these ingredients might have offered some protection to frozen-thawed yeasts in Lab. 1. Gas retention for doughs was superior in Lab. 1 compared to Lab. 2, probably because of better oxidation conditions (100 ppm of ascorbic acid + 60 ppm of potassium bromate compared to 120 ppm of ascorbic acid) and higher flour protein content (13.9 compared to 11.9%; 14% moisture basis). Maitre (1985) and Neyreneuf (1990) both have insisted that there are gas retention problems in French bread doughs.

Differences in freezing conditions might also have contributed slightly to differences between results from the two laboratories. In Lab. 1, higher freezing rates were obtained with cryogenic freezing. The results presented here do not indicate that cryogenic freezing is the method of choice for frozen doughs. However, considering the overall results presented here, frozen doughs prepared in Lab. 1 retained their activity over three months better

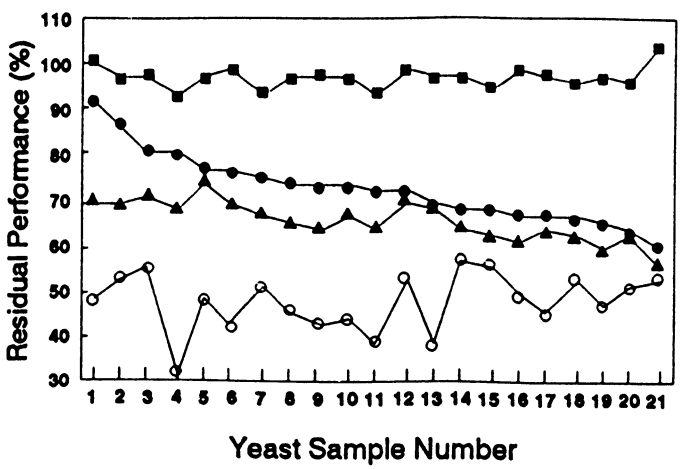

Fig. 1. Comparison among screening tests for yeast cryosurvival, as performed in Laboratory 1. Yeast samples are presented in decreasing order of freeze-thaw survival according to the reference curve (gas production for doughs stored for three months): 1 = F1-1, 2 = F2-1, 3 = F1-2, 4 = U2-1, 5 = F2-2, 6 = U3-1, 7 = C2-1, 8 = C1-1, 9 = U2-2, 10 = U1-2, 11 = U1-1, 12 = U3-2, 13 = U2-3, 14 = F2-3, 15 = F1-3, 16 = U3-3, 17 = C1-3, 18 = U1-3, 19 = C2-2, 20 = C1-2, 21 = C2-3. ● = gas production test (stored, Lab. 1), ○ = gas production test (rapid), ▲ = proof time, ■ = bread volume.

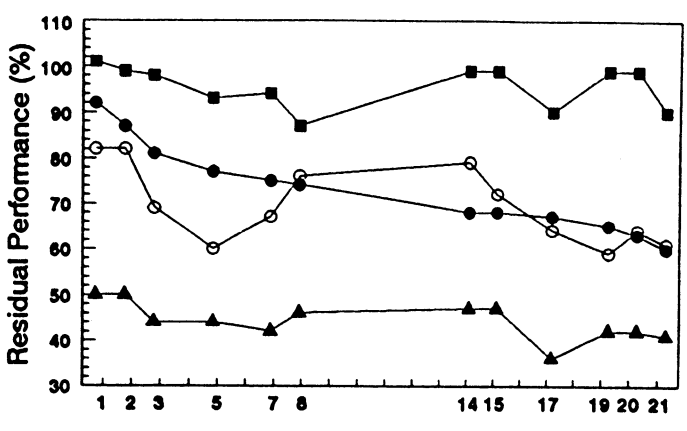

Fig. 2. Comparison among screening tests for yeast cryosurvival, as performed in Laboratory 2. Yeast samples are presented in decreasing order of freeze-thaw survival according to the reference curve (gas production [Lab. 1] for doughs stored for three months): 1 = F1-1, 2 = F2-1, 3 = F1-2, 5 = F2-2, 7 = C2-1, 8 = C1-1, 14 = F2-3, 15 = F1-3, 17 = C1-3, 19 = C2-2, 20 = C1-2, 21 = C2-3. ● = gas production test (stored, Lab. 1), ○ = gas production test (stored, Lab. 2), ▲ = proof time, ■ = bread volume.

than those from Lab. 2. This suggests that a better protocol was used in Lab. 1 for the preparation of frozen doughs. Even considering such differences in protocols for preparing frozen dough, results from both laboratories agreed on the main results: freeze-thaw tolerance of commercial baker's yeast samples varied markedly, despite the fact that the strains themselves had similar cryotolerance.

A most interesting question remains unanswered: What is the reason for the variations of cryotolerance among yeast samples, especially those taken from the same manufacturer? Growth conditions could partly explain some of the observed differences of freeze-thaw tolerance (Gélinas et al 1989), suggesting that variations in the baker's yeast manufacturing process are involved.

CONCLUSIONS

Results presented here show that the strain of a regular baker's yeast is not a major factor for frozen dough stability when the yeasts are grown under similar conditions. There were major variations in cryotolerance among yeast samples found on the market, either in France, Canada, or the United States. No significant difference of cryotolerance was seen among the seven yeast commercial trademarks tested from these three countries. Direct measurement of gas production by yeast was the most reliable method to screen freeze-thaw-tolerant yeast samples.

It is theoretically possible to use the rapid freezing test presented here to screen freeze-thaw-sensitive yeasts. The basis of this test is yeast survival to high freezing rates. Cryosurvival of the 21 yeast samples also varied according to freezing conditions such as freezing rate (1.3 or 9.2°C/min).

On the whole, this article stresses the importance of quality control for yeast in frozen dough production. Cryotolerance of baker's yeast samples available on the market is subject to fluctuations that must be checked before frozen dough production, either in yeast factories or at the bakery plant. However, the basis of such fluctuations among batches of yeast is still obscure.

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