Factors Involved in the Stability of Frozen Dough. I. The Influence of Yeast Reducing Compounds on Frozen-Dough Stability[1]

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ABSTRACT

Freeze damage to yeast increased the amount of low-molecular-weight sulfhydryl (SH) compounds leached from yeast cells. The material leached from frozen yeast was incorporated with flour and studied in rheological and baking tests. Leachate from yeast frozen for six weeks produced effects on farinograph and extensigraph tests similar to the addition of 50 ppm pure glutathione. The incorporation of a six-week leachate into a "no-time" dough system did not cause a change in loaf volume. Similar results were found with the incorporation of 50 ppm glutathione. Extensigraph tests of dough cylinders frozen for up to 10 weeks indicated a decrease in extensibility of the dough with frozen storage, whereas addition of yeast leachates increased dough extensibility. Free SH content of yeasted and nonyeasted frozen doughs were evaluated over a storage period of 16 weeks. The free SH content of doughs with or without yeast in the system did not change significantly during the storage period. Rheological changes in both yeasted and nonyeasted doughs during frozen storage were not associated with changes in free SH content of the doughs.

Stability of frozen dough is the ability of a thawed dough to proof in an acceptable period of time and to bake into a loaf with normal volume and bread characteristics. Frozen-dough stability has been related to dough formulation, yeast quality, sulfhydryl (SH) compounds released by yeast, fermentation before freezing, and freeze-thaw rates (Davis 1981, Godkin and Cathcart 1949, Hsu et al 1979, Kline and Sugihara 1968, Lehmann and Dresse 1981, Lorenz 1974, Merrit 1960). The significance of many of these features is not totally understood.

Kline and Sugihara (1968) postulated that the dying of yeast cells during the frozen storage of dough is related to poor gas retention during proofing. These workers stated that the dough structure may be weakened by reducing substances released from dead yeast cells. The tripeptide glutathione (GSH) has been isolated from dry active yeast and has been associated with marked effects on the rheological and baking properties of dough (Ponte et al 1960). These effects include a shorter mixing time, increased extensibility, decreased loaf volume, and a greater oxidizing-improver requirement. Lorenz (1974) and Kline and Sugihara (1968) reported better overall bread quality with increased levels of potassium bromate in frozen-dough formulation. Both bromate and ascorbic acid are capable of counteracting the adverse effects of GSH (Freilich and Frey 1944, Johnston and Mauseth 1972).

The present study was undertaken to determine whether freeze damage to yeast results in the release of reducing compounds and to determine to what extent these compounds affect the gas-retention properties of frozen doughs.

MATERIALS AND METHODS

Yeast Samples

Compressed yeast samples of similar gassing powers were received by air express from the Universal Food Corporation, Milwaukee, WI. All yeast samples were stored under refrigeration and used within one week of their arrival.

Freezing and Thawing Conditions

To assess the effect of freezing on compressed yeast, 100-g samples were wrapped in aluminum foil and frozen on a laboratory-model plate contact freezer (Crepaco, Chicago, IL). The samples were analyzed fresh and after 2, 4, and 6 weeks of frozen storage. The frozen samples were allowed to thaw for 45 min at room temperature before being leached and before being tested for gassing power, baking activity, and percentage of dead cells.

Gassing Power and Baking Activity

Gassing power was determined by using a pressuremeter according to AACC (1962) method 22-13.

Baking activity was evaluated by the following "no-time" formulation: 100 g of flour (14.0% moisture basis), 4.0 g of sugar, 1.5 g of yeast, 100 ppm ascorbic acid, 30 ppm potassium bromate (flour basis), and an optimum amount of water. Each dough was mixed to optimum development in a pin-type mixer (National Mfg. Co., Lincoln, NE). The mixed doughs were fermented at 86° F and 80% rh for 20 min. After the fermentation period, the doughs were sheeted at a spacing of 3/16 in. and molded with the aid of a laboratory dough molder (National Mfg. Co., Lincoln, NE) with an 18-lb pressure-board setting. The molded doughs were panned and proofed at 86° F and 100% rh for 45 min and baked at 400° F for 20 min in a reel-type oven. Loaf volume was measured by rapeseed displacement 20 min after baking.

The conditions suggested by Mills (1941) and Thiessen (1942) were used to determine the percentage of dead cells in yeast samples. Yeast (0.5 g) was suspended in 100 ml of tap water. A 5-ml aliquot of this suspension was mixed with 5 ml of methylene blue (1:10,000 dilution) and agitated for 3 min on a roto torque mixer. A drop of this suspension was examined under a microscope on a Neubauer hemocytometer. An average of 1,000 cells were counted, and all stained cells were counted as dead.

Leaching Procedure and Analysis of Leachate

Yeast samples were leached according to the modified procedure of Ponte et al (1960). Yeast (10 g) was suspended in 100 ml of distilled water and allowed to rest 20 min at room temperature. The suspension was then centrifuged 15 min at 16,300 × *g*, and the supernatant decanted and vacuum-filtered. A GF/A glass filter was used to remove yeast cells.

Total solids of the leachate were determined by pipetting 10 ml of leachate into a tared aluminum moisture dish, which was then heated for 3 hr in a forced-draft air oven at 100° C. Results were expressed as milligrams of total solids per gram of yeast (dry weight basis).

Nitrogen content of the leachate was determined by the micro-Kjeldahl procedure, AACC (1962) method 46-13. Protease activity of the leachate was determined by the method described by Preston et al (1978).

Free SH group content was determined with the use of 5',5'-dithiobis-2-nitrobenzoic acid (DNTB), according to the conditions specified by Ellman (1959). A tris-glycine buffer (pH 8.0) used by

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Beveridge (1974) was substituted for Ellmans phosphate buffer. A standard curve using a $3.0 \times 10^{-4} M$ stock solution of GSH was prepared. Aliquots of the stock solution were brought to a total volume of 5 ml with the tris-glycine buffer. Color was developed by the addition of 0.02 ml of DNTB solution (39.6 mg/100 ml in tris buffer). After 20 min, the absorbance was determined at 412 nm. A diluted sample solution without DNTB was used to adjust the absorbance to zero. Free SH content was expressed as milligrams of GSH per gram of dry yeast.

Use of Leachate in Rheological and Baking Tests

Volumes of leachate equivalent to the amount of material leached from 5% yeast (flour basis) were evaluated in farinograph and extensigraph tests and in a baking test.

For the farinograph test, AACC (1962) method 54-21 and the 50-g bowl were utilized. Leachate (25 ml) and distilled water were used to center the curve on the 500-Brabender-unit line. Values for absorption, dough development time, and stability were obtained.

A modification of the extensigraph procedure also was used to evaluate the leachates. Flour (100 g on 14.0% moisture basis) was mixed with 1.0% sodium chloride U.S.P., 50 ml of leachate, and sufficient distilled water to bring the absorption to a level predetermined by the farinograph absorption. Mixing was performed in a 200-g National Dough Mixer with mixing times determined relative to the farinograph mixing time. Doughs were scaled after mixing to 150 g, rounded, molded, and placed in the extensigraph saddles and allowed to rest 45 min in a cabinet controlled at 86° F and 80% rh before being stretched on the extensigraph.

The baking procedure previously stated for evaluation of baking activity was used to determine the effects of the leachates on loaf volume.

Effects of GSH on Rheological and Baking Properties

The farinograph employing AACC (1962) method 54-21 (50-g bowl and constant flour weight method) and the extensigraph procedure previously outlined were used to investigate the effects of various levels of GSH on physical dough properties. The effect of GSH was also tested in the presence of ascorbic acid and potassium bromate.

The effect of GSH on loaf volume was evaluated using the no-time dough procedure previously described, as well as a 2-hr straight-dough procedure. The following straight-dough formulation was used: 100 g of flour, 5.0 g of sugar, 2.0 g of salt, 3.0 g of vegetable shortening and, an optimum amount of water. Doughs were mixed to optimum development in a pin-type mixer (National Mfg. Co., Lincoln, NE). The doughs were fermented at 86° F and 80% rh for 2 hr. After 90 min the doughs were scaled to 135 g, punched, and returned to the cabinet. At the end of 2 hr the doughs were processed as previously described for the no-time dough procedure.

TABLE I
Effects of Freezing on Compressed Yeast Activity and Leachate Properties

	Weeks of Frozen Storage			
	0	2	4	6
Yeast analysis				
Gassing power (mm HG)	470	465	465	460
Baking activity (cc)	840	820	795	795
Dead yeast cells (%)	4.9	11.4	14.3	21.7
Yeast leachate analysis				
Glutathione (mg/g of dry yeast)	0	2.08	2.12	2.87
Total solids (mg/g of dry yeast)	5.3	50.1	47.4	72.7
Nitrogen (mg/g of dry yeast)	0	2.8	4.2	5.29
Protease activity (relative units)	0.12	0.06	0.06	0.05
Incorporation of yeast leachates in rheological and baking tests				
Farinograph test with 25 ml of leachate[a]				
Dough development time (min)	9.5	5.5	5.0	6.0
Mixing tolerance (min)	16.0	7.5	9.5	7.5
Extensigraph test with 50 ml of leachate[a]				
Extensibility (E), cm	21.0	23.0	22.5	23.0
Resistance (R), cm	4.9	4.4	4.1	4.2
R/E	0.233	0.191	0.187	0.182
Baking test with 50 ml of leachate[a] (in volume from control in cc)	0	+20	+25	+45

[a] Equivalent to material leached from 5% yeast on flour basis.

SH Content of Yeasted and Nonyeasted Frozen Doughs

The no-time dough formulation and procedure previously given was utilized to produce doughs containing 0 and 5% compressed yeast. After molding, the dough cylinders were cut into 35-g pieces, placed in plastic bags, and frozen at −10° F. After one day of frozen storage, samples of each dough type were freeze-dried and ground on a Wiley mill (60-mesh screen) and evaluated for urea-dispersible SH content. Subsequent samples were analyzed every four weeks for a total of 16 weeks. Urea-dispersible SH content was determined by suspending 0.4 g of lyophilized dough powder in 1 ml of tris-glycine buffer and then adding 9.0 ml of $8M$ urea (in tris-glycine buffer, pH 8.0). The suspension was centrifuged (12,800 $\times$ g, 20 min) and filtered through Whatman No. 4 paper. The supernatant was diluted with three volumes of $8M$ urea in tris-buffer, and the absorbance measured at 412 nm. A standard curve using mercaptoethanol was used, and results were expressed as micromoles of SH per gram of dough powder.

Extensigraph Studies of Frozen Dough Cylinders

Doughs were prepared for the extensigraph using the no-time formulation previously mentioned. Doughs were scaled after mixing to 150 g, rounded, molded, and placed on waxed paper in a cabinet controlled at 30° C and 80% rh. After a 30-min rest, the doughs were frozen at −10° F and then stored in plastic bags at the same temperature. Frozen dough cylinders were placed in extensigraph holders and thawed to a temperature of 25 ± 1.5° C in a cabinet maintained at 30° C and 80% rh. Yeasted and nonyeasted dough cylinders were evaluated fresh, one day after freezing, and every two weeks thereafter, for a total of 10 weeks.

RESULTS AND DISCUSSION

Table I shows the yeast evaluation data for fresh yeast and yeast samples frozen for two, four, and six weeks. Both gassing power and baking activity of the yeast decreased during frozen storage. Higher levels of yeast (5–6%) are normally used in frozen dough formulations to compensate for losses in yeast gassing power during extended storage (Lorenz 1974, Javes 1971, Drake 1970, Merritt 1960). The fresh yeast contained an appreciable amount of dead cells (4.9%), but the leachate of the fresh yeast contained no detectable amounts of GSH. As frozen storage increased, the number of dead cells and the amount of GSH leached from the yeast increased. Total solids and nitrogen leached from the yeast increased with frozen storage.

The protease activity of the leachates decreased as frozen storage of the yeast increased. The relatively low level of protease activity in the leachate indicates that proteolytic enzymes leached from damaged yeast are not a factor in frozen-dough stability.

Farinograph properties were altered as the concentration of GSH in the leachate increased (Table I). Table II shows the effect of pure GSH on farinograph and extensigraph properties with incorporation of various levels of oxidation. GSH decreased both dough-development time and mixing tolerance in the farinograph test. As the level of GSH was increased in the extensigraph test, the proportional number (resistance/extensibility) decreased, indicating a weakening of the gluten structure by GSH. Table II also shows that $KBrO_3$ and ascorbic acid are only partially effective in counteracting the rheological effects of GSH. The effect on dough development time and mixing tolerance with the incorporation of the leachate obtained from yeast frozen for six weeks was similar to the addition of 50 ppm GSH (Tables I and II). Analysis of this leachate for GSH indicated that approximately 45 ppm GSH was incorporated into the farinograph test.

As the amount of GSH increased in the leachate, the

TABLE II
Effects of Glutathione on Farinograph and Extensigraph Properties

Glutathione (ppm)	Farinograph			Extensigraph				
				Resistance (R)		Extensibility (E)		R/E
	Absorption (%)	Dough Development Time (min)	Mixing Tolerance (min)	45 min (cm)	180 min (cm)	45 min (cm)	180 min (cm)	180 min
No oxidant								
0	66.5	10.5	15.0	4.1	4.0	21.0	24.6	0.163
50	66.5	5.5	8.5	3.3	2.8	23.8	23.4	0.110
100	67.2	4.0	4.3	2.4	1.9	23.7	19.5	0.091
150	67.9	3.0	3.0	1.7	1.5	27.2	17.7	0.085
30 ppm $KBrO_3$								
0	66.5	10.0	17.0	3.8	5.6	24.0	20.5	0.273
50	66.5	5.5	8.0	3.6	5.9	22.2	22.7	0.385
100	65.6	4.0	5.0	3.2	4.8	24.9	17.7	0.271
150	62.5	3.0	3.0	2.2	3.4	26.9	21.2	0.160
100 ppm ascorbic acid								
0	68.8	10.0	15.5	5.5	10.2	21.0	13.3	0.767
50	66.8	6.5	10.0	4.7	8.0	20.0	14.0	0.571
100	69.5	5.5	5.0	3.5	6.0	24.0	15.5	0.387
150	67.5	4.5	4.5	2.2	4.0	23.0	18.8	0.213
100 ppm ascorbic acid, 30 ppm $KBrO_3$								
0	66.5	10.5	17.0	6.0	15.2	21.7	11.3	1.345
50	66.5	7.5	13.0	6.1	14.1	20.0	7.5	1.880
100	67.2	5.0	7.5	4.7	14.8	22.8	8.2	1.805
150	67.9	4.5	8.0	3.4	10.3	21.0	9.4	0.913

TABLE III
Sulfhydryl Content of Yeasted and Nonyeasted Doughs at Different Periods of Frozen Storage[a]

Storage Time	Yeasted Dough	Nonyeasted Dough
1 day	1.02	0.55
4 weeks	1.05	0.52
8 weeks	1.11	0.55
12 weeks	0.91	0.52
16 weeks	0.92	0.51

[a] Results expressed as micromoles of sulfhydryl per gram of dough powder.

TABLE IV
Influence of Frozen Storage on the Extensigraph Properties of Dough

Storage Time	Proportional Number[a]	
	Yeasted	Nonyeasted
fresh	0.278	0.212
1 day	0.280	0.181
2 weeks	0.283	0.210
4 weeks	0.290	0.239
6 weeks	0.305	0.283
8 weeks	0.311	0.312
10 weeks	0.376	0.429

[a] Proportional number = resistance/extensibility after thawing to 25°C.

extensigraph proportional number (resistance/extensibility) decreased (Table I), indicating a slackening of the dough. The slackening effect of the six-week leachate on the dough was similar to that obtained by the addition of 50 ppm pure GSH (Table II).

Figure 1 shows data obtained by incorporating pure GSH into a no-time and a 2-hr straight-dough system. GSH was found to lower loaf volume in both baking systems, but it was more pronounced in the straight-dough formulation in which no oxidizing agents were added. Leachate from fresh yeast contained no detectable amounts of GSH or nitrogen and had no effect on loaf volume when incorporated into the baking tests (Table I). The yeast leachates were shown to have no adverse effect on loaf volume in the no-time dough system.

Data obtained with the addition of pure GSH into the no-time dough system (Fig. 1) indicate that loaf volume decreased when

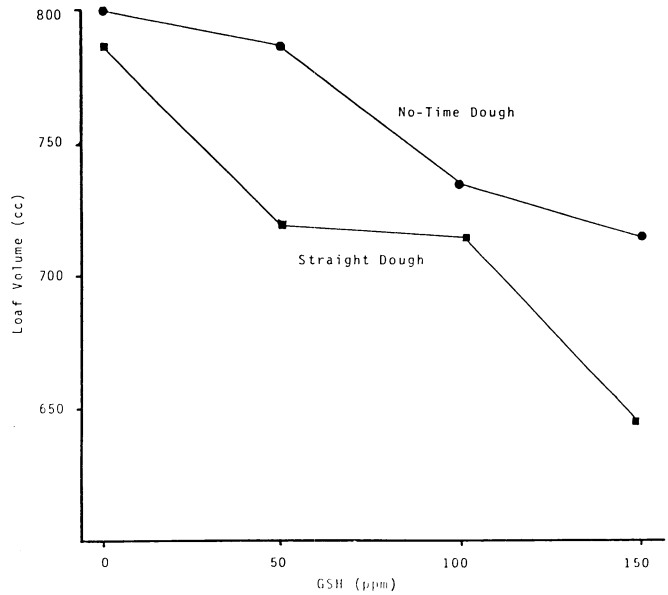

Fig. 1. Effect of glutathione on loaf volume in "no-time" and straight-dough procedures.

levels greater than 50 ppm GSH were incorporated into the dough system. Analysis of the yeast leachates indicated that less than 50 ppm of GSH would be incorporated into a dough system after six weeks of frozen storage.

Freezing yeast in a dough system is different from direct freezing of yeast. Yeast in a dough system is under osmotic pressure and is in a state of active fermentation, which may increase its susceptibility to damage when frozen. On the other hand, the physical nature of the dough may protect yeast cells during the freezing process. Using a plate-count method, Kline and Sugihara (1968) reported substantial losses in the number of viable cells in frozen dough. The losses reported by these workers are similar to what is reported in this study for frozen compressed yeast.

Table III shows that no significant change in free SH content occurred in yeasted and nonyeasted doughs over 16 weeks of frozen storage. The yeasted doughs showed higher levels of SH groups than the nonyeasted doughs because of the free SH content of the yeast. The GSH leached from freeze-damaged yeast accounts for

only part of the difference between free SH content of yeasted and nonyeasted doughs. A substantial amount of free SH groups is assumed to remain in the yeast cells even after extensive freeze damage and leaching.

Extensigraph studies of yeasted and nonyeasted dough cylinders over 10 weeks of frozen storage are shown in Table IV. Both yeasted and nonyeasted doughs increased in proportional number (resistance/extensibility) with storage. Similar findings were reported by Varriano-Marston et al (1980).

The rheological changes of dough during frozen storage are contrary to what would be expected if compounds leached from yeast were weakening the dough structure. Although freeze damage does cause reducing compounds to be released from yeast, the data do not support the theory that these reducing compounds are a factor in the gas-retention properties of frozen dough.

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