Studies on Frozen Doughs. III. Some Factors Involved in Dough Weakening During Frozen Storage and Thaw-Freeze Cycles[1]

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ABSTRACT

The mechanism of dough weakening during thaw-freeze cycles and storage in frozen condition was investigated by extensigraphy, protein solubility fractionation, and electrophoresis. Molded doughs were frozen and stored for one, seven, or 70 days or subjected to three thaw-freeze (3T-F) cycles during seven days of frozen storage. Extensigraph measurements showed that maximum resistance (R_{max}) decreased significantly after one day, after 3T-F cycles, and after 70 days, whereas dough extensibility increased significantly only after 70 days of frozen storage. Extensigraph results for nonfrozen doughs formulated with different yeast levels indicated that the decrease in R_{max} during frozen storage does not appear to be related to the concomitant drop in gassing power. In contrast, a very strong relationship was found between extensibility and gassing power ($r \geq -0.95$). Therefore, the substantial decrease in gassing power of the frozen dough stored for 70 days appears to be the probable cause of the significant increase of extensibility observed for this dough. Reducing sugar content of the doughs after 3T-F cycles was considerably lower than that of doughs subjected to other storage treatments, indicating that some fermentation occurred during the thaw part of the thaw-freeze cycle. Small but significant ($P<0.05$) increases in the proportion of water- and acetic-acid-soluble protein fractions were observed for doughs subjected to 3T-F cycles. For the dough subjected to 70 days of frozen storage, the proportion of 70% ethanol-soluble protein was significantly higher than for other doughs. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis performed under nonreducing conditions clearly showed changes in the high molecular weight region (glutenin oligomers) of the electrophoretic pattern of the doughs subjected to the 3T-F cycles. Factors responsible for dough weakening after prolonged frozen storage and for dough subjected to repeated thawing and refreezing appear to be different.

The overall quality of bread baked from frozen dough deteriorates gradually during frozen storage (Inoue and Bushuk 1991, 1992, and references cited therein). Two factors have been identified as possible reasons for the observed loss of baking quality: 1) decrease in gassing power due to a decline in yeast activity and 2) gradual loss of dough strength. Two explanations have been proposed for the loss of dough strength: 1) release of disulfide reducing substances from dead yeast cells (Kline and Sugihara 1968) and 2) disruption of the gluten network by ice crystals (Varriano-Marston et al 1980); neither has been confirmed by experimental evidence.

This article presents results of studies on the effect of frozen dough storage and thaw-freeze cycles on dough rheological properties, protein solubility, and composition that may be related to the observed loss of dough strength.

MATERIALS AND METHODS

Flour

The flour used was milled from a sample of No. 1 Canada Western Red Spring wheat of the 1989 crop year. It was of straight grade and contained 14.4% protein (N × 5.7) and 0.52% ash (both on 14% mb).

Yeast

Compressed baker's yeast was obtained from Fleischmann's Yeast Ltd. (Toronto, ON) and was used within one week of receipt.

Dough Formulation

The lean dough formula used in our previous study (Inoue and Bushuk 1991) was used. It comprised 100% flour, 5% yeast, 2.5% sugar, 1.5% shortening, 1% salt, 100 ppm ascorbic acid (flour basis), and an optimum amount of water.

Preparation of Frozen Dough

Frozen dough pieces (160 g) in the form of 16-cm long cylinders were prepared according to the procedure described previously (Inoue and Bushuk 1991). After freezing, the pieces were placed in polyethylene bags, vacuum-sealed, and stored in a chest freezer at −20°C. A total of nine dough pieces was prepared for each storage period.

In addition to the single-freezing storage and thawing treatments, nine doughs were subjected to three thaw-freeze (3T-F) cycles. After one day of frozen storage, the dough pieces were thawed in a retarder at −2°C for approximately 15 hr and then refrozen for 90 min (at −20°C) and stored in the chest freezer for one additional day. This thawing and refreezing treatment was repeated two more times. After the third cycle, the frozen dough was stored for approximately one day, then thawed, proofed, and baked.

To provide material for the analyses described below (reducing sugars, protein solubility, and sodium dodecyl polyacrylamide [SDS-PAGE]), four dough pieces for each treatment were subjected to freeze-drying. Two dough pieces were freeze-dried directly and ground in a coffee grinder. The remaining two dough pieces were used for extensigraph and gassing power measurements.

Extensigraph Measurements

The extensigraph procedure used was described previously (Inoue and Bushuk 1991), except that the final proofing time in the present study was 75 min. Three dough pieces were tested for each treatment to check replicability; average tests are reported.

Gassing Power

Gassing power of doughs, fermented for 90 min at 30°C, was measured using the procedure previously described (Inoue and Bushuk 1992). Duplicate samples of two different dough pieces were tested for each treatment; average results are reported.

Extensigraph Studies of Doughs with Variable Gassing Power

To determine the relationship between gassing power and rheological properties of fermenting nonfrozen doughs, doughs differing in yeast content (0, 1.25, 2.50, 3.75, and 5.00%, flour basis) were mixed in the GRL-200 mixer to just beyond peak development, as indicated by the mixing curve. For each dough, 100 g of flour was used. Dough temperature during mixing was maintained at 28 ± 0.5°C. Doughs (160 g) were scaled and molded

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into 16.0-cm long dough cylinders immediately after mixing, thus eliminating the 20 min of fermentation used in the case of the frozen dough treatments and any related premolding effects. In addition to being used for gassing power determination, replicate dough cylinders were clamped in the extensigraph holder, proofed for 30, 45, or 60 min in the fermentation cabinet, and stretched, using the straight stretching bar (Kilborn and Preston 1982). In addition, the dough cylinders with 0, 1.25, and 2.50% yeast were proofed for 75 min and stretched. Three dough pieces were tested for each treatment.

Reducing Sugar Content

Reducing sugar content of ground freeze-dried frozen doughs was determined by AACC method 80-60 (1982) and expressed as milligrams of maltose per 10 g of the ground freeze-dried frozen dough. Triplicate samples were analyzed for each treatment; average results are reported.

Protein Solubility

Ground freeze-dried frozen dough samples (1 g) were extracted with 20 ml of distilled water, 20 ml of 70% ethanol solution, or 20 ml of 0.05*M* acetic acid solution. Extraction was for 2 hr at room temperature in 50-ml centrifuge tubes with constant shaking. Suspensions were centrifuged at 28,000 × *g* and 20°C for 30 min. The proportion of protein extracted in the supernatant was determined as N (by micro-Kjeldahl method) and expressed as the percentage of N in the freeze-dried dough sample. Extraction of protein from unthawed frozen dough samples with distilled water at 4°C was also done in one experiment to check the possibility of proteolysis occurring during the extraction at room temperature; no activity was found.

SDS-PAGE

For SDS-PAGE analysis, flour and ground freeze-dried doughs were extracted with 2% SDS buffer (Ng and Bushuk 1987) with or without reduction. SDS-PAGE was done using an LKB 2001 apparatus according to a modified Laemmli procedure (Ng and Bushuk 1987).

Statistical Analysis

Analysis of variance using the general linear model procedure with *t*-tests (SAS Institute, Cary, NC) was used to evaluate the data.

RESULTS AND DISCUSSION

Extensigraph and Gassing Power Results

Maximum resistance of doughs decreased significantly for all treatments (Table I). On the other hand, extensibility of the doughs remained relatively constant for all treatments except for the 70-day dough, where it increased significantly. The maximum resistance of the dough subjected to 3T-F cycles was significantly lower (110 BU) than that of the dough subjected to a single freezing and seven days of frozen storage before thawing. These results indicate that doughs become weaker during frozen storage and during repeated thaw-freeze cycles, but the mechanisms of weakening under the two treatments appear to be different.

Gassing power of the doughs decreased significantly after 3T-F cycles and decreased further during 70 days of frozen storage (Table I) to approximately 82 and 55%, respectively, of the gassing power of the nonfrozen doughs. This result is consistent with the conclusion of Kline and Sugihara (1968) that the proportion of active yeast cells, and hence gassing power, declines during extended storage of frozen dough.

To investigate further the interrelationship of yeast activity (gassing power) and extensigraph parameters, we used nonfrozen doughs containing 0, 1.25, 2.5, 3.75, and 5.0% yeast to first determine the relationship between the amount of yeast and gassing power. The gassing power of these yeasted doughs was directly proportional to the amount of yeast (Table II). The 5% yeast dough in this experiment is equivalent to the control dough of Table I, except that the latter was subjected to an additional 20 min of fermentation. The difference in gassing power between the two doughs (459 vs. 538 mm of Hg) is attributed to the depletion of a small amount of fermentable sugar during the extra 20 min of fermentation.

On the basis of the observed linear relationship between the percentage of yeast and gassing power (Table II), the one- and seven-day frozen doughs had gassing powers equivalent to about 4.9% yeast. The 3T-F cycle and 70-day doughs had gassing powers equivalent to about 4.1 and 2.8% yeast, respectively. These data give an estimate of the loss of yeast activity under the four treatments used in the study.

Changes in extensigraph parameters appear to be only partially related to changes in gassing power. The decrease in maximum resistance and the increase in extensibility during the initial 30

TABLE I
Extensigraph Properties and Gassing Power of Nonfrozen and Thawed Frozen Doughs[a]

Frozen Storage Time	Properties			
	Maximum Resistance[b] (BU)	Extensibility[b] (mm)	Gassing Power[c] (mm Hg)	(%)
0 days (control)	627 ± 6 a	121 ± 3 a	459 ± 6 a	100
1 day	530 ± 10 b	122 ± 3 a	447 ± 7 a	97
7 days	523 ± 12 b	123 ± 4 a	451 ± 16 a	98
7 days[d]	407 ± 6 c	121 ± 4 a	378 ± 9 b	82
70 days	360 ± 20 d	136 ± 4b	254 ± 10 c	55

[a] Values are means ± standard deviations. Means with different letters within a column are significantly different ($P < 0.05$).
[b] Means ± SD of three replicates.
[c] Mean ± SD of two replicates.
[d] Subjected to three thaw-freeze cycles.

TABLE II
Gassing Power of Nonfrozen Doughs Containing Different Amounts of Yeast

Yeast (%)	Gassing Power[a] (mm Hg)	(%)
5.00	538 ± 23	100
3.75	402 ± 18	75
2.50	280 ± 16	52
1.25	138 ± 2	26

[a] 30 g of dough after 90 min at 30 ± 0.5°C.

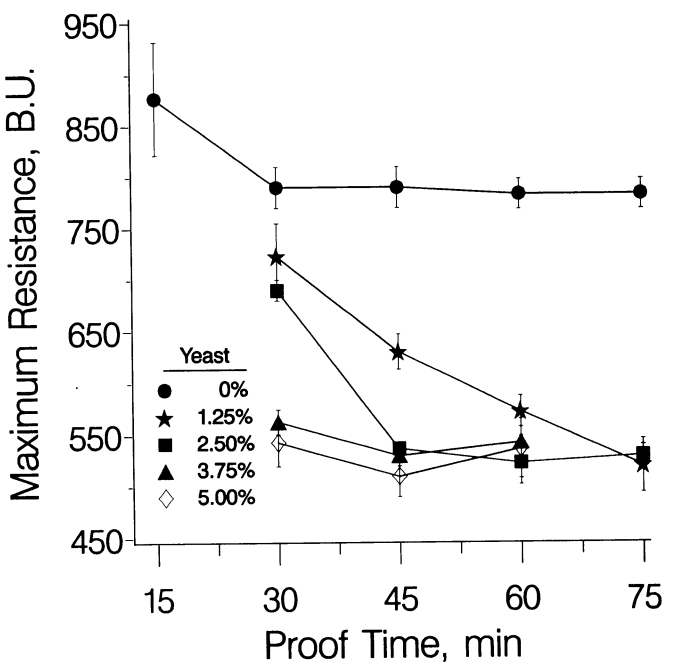

Fig. 1. Relationship between extensigraph maximum resistance and proof time of fermenting doughs at different yeast levels.

min of proofing time can be explained on the basis of the rapid relaxation of the internal stresses of a work-hardened dough that occurs immediately after mixing and molding (Dempster et al 1952; Kilborn and Preston 1982). The additional changes in extensigraph properties beyond the 30-min proof time cannot be related to this relaxation and must result from other factors. Beyond 30 min, maximum resistance (Fig. 1) and extensibility (Fig. 2) both decreased with increasing proof time and increasing yeast level. The control dough (0% yeast) showed no change in resistance and only a small increase in extensibility with increasing proof time. The two sets of curves for the doughs with 3.75 and 5.0% yeast were essentially the same, suggesting that, at this higher level of yeast, the available fermentable sugar may be a limiting factor in the magnitude of gassing power.

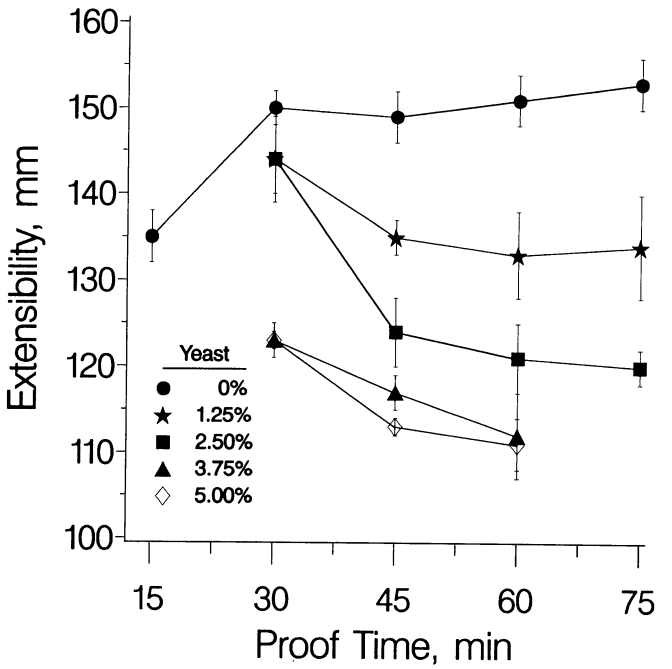

Fig. 2. Relationship between extensigraph extensibility and proof time of fermenting doughs at different yeast levels.

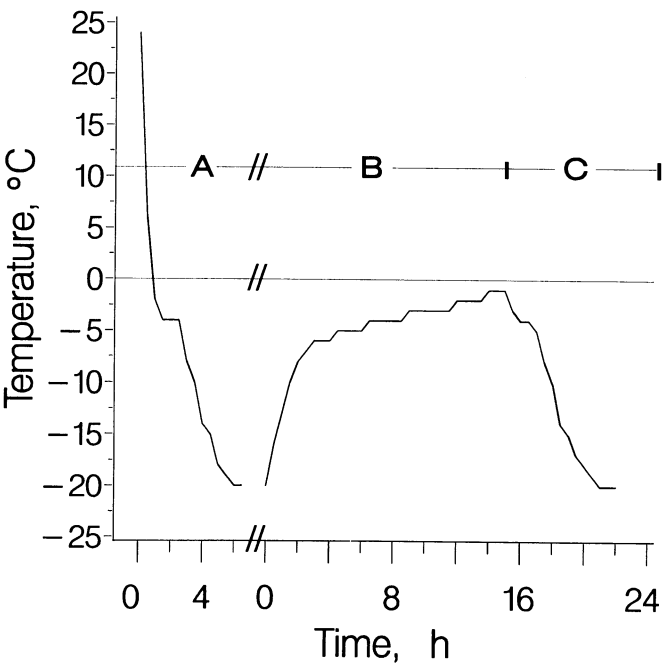

Fig. 3. Dough core temperature changes during freezing (**A**) and thaw (**B**)-freeze (**C**) cycles.

Extensigraph maximum resistance of yeasted doughs leveled off at about 525 BU, notwithstanding large differences in gassing power. Apparently, the observed decrease in resistance (Table I) below 525 BU (as for the 3T-F cycle and 70-day doughs) results from unknown factor(s) other than yeast activity. This result is consistent with the scanning electron microscopy results of Varriano-Marston et al (1980) and Berglund et al (1991), who observed an alteration of the gluten network in doughs subjected to repeated (three) thawing and refreezing cycles.

In contrast to changes in maximum resistance (Fig. 1), no similar plateau effect was seen for the extensibility data (Fig. 2). Doughs containing 2.5 and 1.25% yeast fermented for 75 min were significantly more extensible than doughs with 5.0 and 3.75% yeast after 60 min of fermentation. The correlation coefficients between gassing power and extensibility at 45 and 60 min were −0.98 and −0.95, respectively. We speculate that this relationship results from the fact that considerable dough "development" occurred during fermentation.

Reducing Sugar Content

Reducing (fermentable) sugar content was determined in selected doughs as a possible indicator of the extent of fermentation (Table III). Reducing sugar content of the dough subjected to 3T-F cycles was significantly lower than that of other doughs. This result suggests that a significant amount of fermentation occurred in these doughs during the repeated thawing and refreezing conditions used in this study, where dough core temperatures were between −5 and −2°C for ~12 hr per cycle (Fig. 3).

Protein Solubility in Doughs

Changes in protein solubility was examined as a possible explanation of dough weakening during frozen storage or during repeated thaw-freeze cycles. Results (Table III) for the doughs that were freeze-dried directly after frozen storage showed statistically significant changes in protein solubility in the dough that was subjected to 3T-F cycles. Solubility results for the 70-day dough were not significantly different from those for the one- and seven-day doughs.

The solubility results provide additional evidence, albeit not very strong, that the factors involved in the weakening of doughs during repeated thawing and refreezing cycles may be related to the observed increase in the magnitude of water- and acetic-acid-soluble protein.

The protein solubility results observed in the present study are not consistent with the hypothesis by Kline and Sugihara (1968), i.e., that dough weakening (and presumably protein solubility) during frozen storage is caused partly by the release of reducing substances from dead yeast cells. Doughs subjected to 70 days of frozen storage suffered almost a 50% reduction in gassing power (i.e., contained substantially more dead yeast cells) but did not show any significant change in protein solubility (Table III).

SDS-PAGE Results

Changes in protein composition under the various treatments used in this study were determined by SDS-PAGE under reducing

TABLE III
Reducing Sugar Content[a] and Protein Solubility (%) of Frozen Doughs[b]

Frozen Storage Time	Reducing Sugar	Distilled Water	70% Ethanol	0.05*M* Acetic Acid
1 day	482 ± 4 b	20.0 ± 0.5 b	49.4 ± 0.6 a	68.1 ± 0.5 a
7 days	485 ± 4 a	20.1 ± 0.4 b	49.3 ± 0.1 a	68.1 ± 0.2 a
7 days[c]	459 ± 2 c	21.8 ± 0.2 a	49.6 ± 0.5 a	68.9 ± 0.4 b
70 days	482 ± 4 b	20.1 ± 0.5 b	50.1 ± 0.5 a	68.2 ± 0.1 a

[a] Maltose (mg per 10 g of freeze-dried sample).
[b] Values are means ± standard deviations of three replicates after frozen storage without thawing. Means with different letters within a column are significantly different ($P < 0.05$).
[c] Subjected to three additional thaw-freeze cycles during storage.

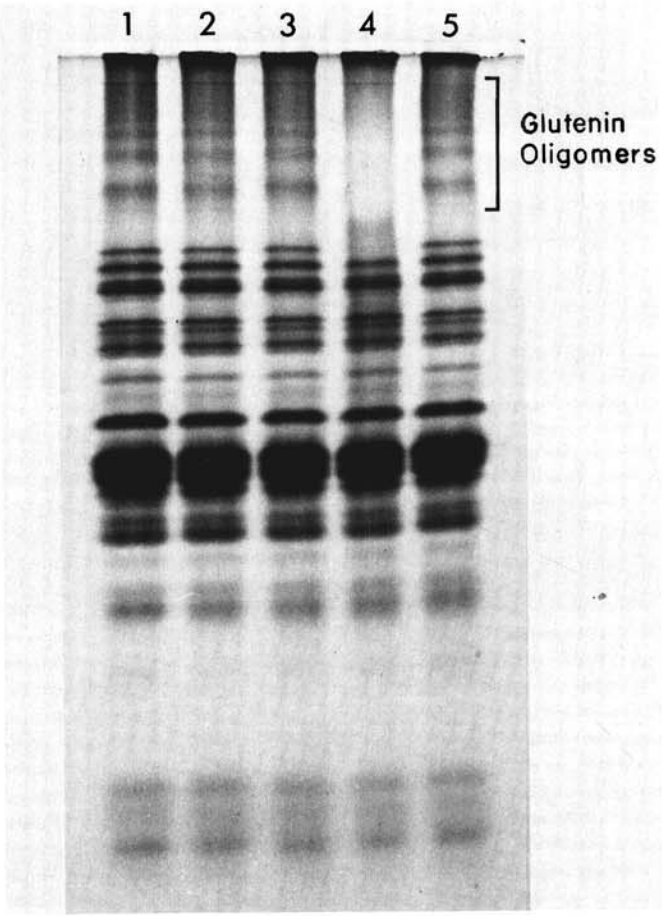

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of 0.05*M* acetic acid protein extracts of frozen doughs under nonreducing conditions: lane 1, control flour; lane 2, one-day frozen dough; lane 3, seven-day frozen dough; lane 4, seven-day frozen dough subjected to three thaw-freeze cycles; and lane 5, 70-day frozen dough.

and nonreducing conditions. For SDS-PAGE under reducing conditions, no differences were seen among the patterns of the ethanol, acetic acid, or SDS-buffer protein extracts (results not shown). On the other hand, SDS-PAGE results for the acetic-acid-soluble proteins under nonreducing conditions showed that the pattern of the 3T-F dough was noticeably different from the patterns of the other doughs (Fig. 4). In the 3T-F pattern, several diffuse high molecular weight bands were absent, and a new band with slightly higher mobility was present. Similar observations were noted for the ethanol and SDS-buffer extracts analyzed by SDS-PAGE (results not shown). The change in protein composition is interesting because the new band is located in the zone of closely migrating bands that result during mixing of doughs to which trace amounts of reducing agents have been added (Lawrence and Payne 1983; Graveland et al 1985; Gao et al 1992). Bands in this zone correspond to oligomers of glutenin subunits. Although the change is a minor one, it does suggest that the structure of glutenin protein is definitely altered by repeated thawing and freezing. Depolymerization of glutenin into oligomers of lower molecular weight would be consistent with the observed dough weakening and solubility results.

Since the reduction in gassing power of the 3T-F cycle doughs was relatively small, the observed change in protein solubility and SDS-PAGE pattern appears to be caused by a factor other than the presumed release of reducing substances from dead yeast cells. The pattern of the 70-day frozen dough, which had the lowest gassing power, i.e., was presumed to contain the highest amount of dead yeast cells (Table I), was the same pattern as those of the one- and seven-day doughs. The mechanism of the postulated depolymerization occurring in the doughs subjected to repeated thaw-freeze cycles remains to be discovered. The observation that a significant amount of fermentation occurred only in frozen doughs with this treatment may still implicate yeast as the causal factor.

CONCLUSIONS

This study showed that the mechanisms of dough weakening after prolonged frozen storage and after repeated thawing and refreezing appear to be different. Changes during frozen storage appear to be related to yeast activity, with concomitant effects on dough extensibility. On the other hand, dough weakening with related protein solubility and compositional changes during repeated thawing and refreezing appears to be caused by another mechanism such as ice crystallization, release of carbon dioxide during freezing, or some other unknown factor. The results of the present study suggest that a yeast having reduced activity at low temperatures, compared with the activity of currently used yeast, might be more suitable for bread production from frozen dough. Further research is needed to confirm the findings of the preliminary study reported in this article.

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