Frozen Doughs: Rheological Changes and Yeast Viability

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ABSTRACT

Stress-relaxation and small deformation oscillatory techniques were used to study the rheological changes in doughs subjected to freezing and thawing. Relative to fresh doughs, the relaxation modulus and relaxation time both decreased. Plots of storage modulus (G') and tan δ versus temperature showed a decrease in G', an increase in tan δ, and an increase in the onset temperature of starch gelatinization in frozen and thawed doughs. Yeast viability was studied by measuring the gas volume of the dough. The viability decreased slightly during two weeks of storage at −18°C. Dead yeast cells did not affect the rheological properties of the doughs.

Cereal Chem. 69(4):409-413

Frozen doughs are increasingly being produced in the baking industry even though they generally provide bread with lower loaf volume than does fresh (unfrozen) dough. Several investigators have reported that the major changes in doughs that have been frozen are related to yeast; dead yeast cells release reducing agents such as glutathione, which weaken the gluten network, resulting in poor gas retention and longer proof time (Kline and Sughiara 1968, Hsu et al 1979). On the other hand, Varriano-Marston et al (1980) and Wolt and D'Appolonia (1984) have suggested that the structural changes in thawed doughs are not associated with the release of reducing substances from yeast cells but with a weakening of the dough network.

During dough fermentation, yeast produces carbon dioxide and flavor compounds. The gas-forming ability of yeast depends on the strain, the number of yeast cells, cell activity, and the amount of fermentable sugars. The amount of fermentable sugars in wheat flour is less than 1%, which is not enough for yeast without the additional sugar produced from the starch by α- and β-amylase (Reed and Peppler 1973, Oura et al 1982). Some of the detrimental changes in frozen dough are related to the freezing and thawing

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conditions. Fast freezing reduces the number of yeast cells (Lorenz 1974) and their gas-producing capacity (Sinda 1989). To maximize the yeast viability, doughs should be frozen slowly and thawed rapidly (Lorenz 1974).

Most of the work done on the rheology of frozen doughs has involved instruments such as the extensigraph, which exerts large deformations. However, these instruments do not measure any well-defined physical parameter. Dynamic rheological (small-deformation) techniques have been used to determine the effect of flour protein content, absorption, and mixing time, etc., on the fundamental physical properties of nonleavened dough (Hibberd 1970, Navickis et al 1982, Abdelrahman and Spies 1986).

An important property of wheat dough is its ability to change from viscoelastic dough to an elastic bread. Freezing and thawing dough may cause changes that become manifested only during the baking process. The small-deformation technique has been used to monitor the structure formation of complex food systems during heating (Dea et al 1983, Bohlin et al 1987, Autio et al 1989) and recently for the study of dough systems (Dreese et al 1988).

The formation of gluten disulfide is important in the rheology of gluten. According to Carlson (1981), the film-forming ability of the continuous matrix of dough is related to the characteristic relaxation time of the dough. The addition of excess thiol reagent, such as glutathione, has been suggested to interfere with gluten disulfide formation (Eliasson 1990). Mita and Bohlin (1983) reported that cysteine decreases the relaxation time of gluten.

To understand the altered textural properties of frozen doughs, a knowledge of both the rheological changes and yeast viability is required. In this work, we studied the rheological changes in unyeasted frozen doughs by basic rheological methods, the gas-forming ability of yeast in frozen doughs, and the effect of yeast metabolites on the rheological properties of fresh and frozen doughs.

MATERIALS AND METHODS

Materials and Dough Preparation

Flours (A and B) were commercial Finnish wheat flour mixtures. The protein content (conversion factor 5.7), as determined by Kjeldahl nitrogen, was 12.9 for flour A and 13.4 for flour B. The α-amylase activity (unit per gram), as determined by the Biocon ceralpha method (McCleary and Sheehan 1987), was 0.174 for flour A and 0.191 for flour B.

Fresh-pressed baker's yeast was obtained from the Rajamäki Yeast Factory (Rajamäki, Finland) and was kept for at least two days at 4°C. Reduced glutathione (GSH) was purchased from Sigma Chemical Co. (St. Louis, MO). Yeast homogenate was prepared by agitating 10 g of fresh yeast with 10 ml of cold (2°C) distilled water and 25 ml of glass pearls in a B Brown homogenizer (B. Braun Melsungen AG, Germany). The cold yeast-glass pearl mixture was homogenized for 1 min three times, after which the pearls were separated from yeast homogenate by filtration through a sinter.

The yeasted dough was made by the procedure used by the Finnish yeast industry for determining yeast activity (Parisi 1970, Suomalainen et al 1972). All of the ingredients were kept overnight at 4°C. The flour (A or B, 560 g) was mixed with 340 ml of water containing 8 g of NaCl and 10 g of pressed yeast for 5 min with a Kenwood Kneader Major KM 230 (Kenwood Limited, Hampshire, England). The dough temperature was 21 ± 0.5°C. Unleavened dough was prepared similarly, without the addition of yeast.

Freeze-Thaw Conditions

Doughs for freezing studies were frozen in a home-type freezer at −18 ± 0.5°C and stored for two weeks at this temperature. Samples were thawed for 2.5 hr at 30°C or 17 hr at 4°C.

Measurement of Gas Volume

Immediately after mixing, the yeasted dough was divided into three pieces (300 g), and two of the dough pieces were sealed in plastic bags and frozen. The nonfrozen dough piece was used as a control. The gassing power of the yeast was determined with an SJA fermentograph (no. 451, Nässjö, Sweden), the three closed cabinets of which allowed the separate measurement of the total volume of gas produced in three dough samples at the same time. Fermentation temperature was 35°C, and the relative humidity was maintained. After 1 hr of proofing, the dough was molded into a ball and proofed for another hour in the fermentograph. Replicates were made with two independently prepared doughs. Percent error of the mean of the method was lower than 5%.

In another experiment, cold yeast homogenate was added at concentrations of 0.5 and 1.0 g per 300 g of dough when dough mixing was begun to determine the effect of dead yeast cells on the fermenting power. Yeast homogenate was added in place of the normal yeast or in addition to it.

To determine the effect of glutathione on the rheological properties of the dough, glutathione was added to the dough at 50 and 100 mg/kg after dough mixing had started.

Rheological Measurements

Rheological measurements were made with a Bohlin rheometer VOR (Bohlin Reologi Ab, Lund, Sweden) with a parallel plate geometry, operating in the oscillatory or relaxation mode.

Dynamic rheological measurements. Immediately after mixing, fresh nonyeasted dough was placed in a plastic bag and stored at 4°C for 2 hr. Because the water content of the dough was not optimized, the rheological measurements were repeated with a new sample of the dough after different periods of time. After 90 min, the storage modulus reached a stable value and the heat-induced rheological properties were measured. Three hundred grams of the dough then was frozen.

An overview of the small-deformation technique can be found in Dea et al (1983). A high-temperature cell (30–350°C) was used, where thermally conditioned gas was forced into the cell. The parallel plate measuring system was located in the cell. The dough sample was slowly compressed by the upper plate (about 2 min), until the gap between the plates (25 mm diameter) was 1.5 mm, and the expelled dough was carefully trimmed off with a razor blade. The sample was allowed to rest for 2.0 min. An O-ring was used in the lower plate and silicon oil was applied around the plate edges to prevent the sample from drying. The temperatures of the dough and the gas were measured by two thermocouples. The heating rate of the dough was about 3°C/min. The

TABLE I
Standard Errors of Means for Rheological Parameters

Method	Parameter[a]	Temperature (°C)	Standard Errors of Means (percent error of the mean)
Oscillation	G' (nonyeasted dough)	50	2.2
	δ (nonyeasted dough)	50	0.6
Relaxation	G_{rel} (nonyeasted dough)	25	1.7
	$T_{1/2}$ (nonyeasted dough)	25	12.0

[a] G' = storage modulus; G_{rel} = relaxation modulus; $T_{1/2}$ = half relaxation time.

TABLE II
Effects of Freezing and Thawing Temperature and Time on the Gassing Power of Dough

Flour	Storage and Thawing Conditions	Gas Volume (ml) 1 hr	Gas Volume (ml) 2 hr
A	Fresh	150	625
	14 days, −18°C; 17 hr, 4°C	145	520
	14 days, −18°C; 2.5 hr, 30°C	140	540
B	Fresh	375	685
	14 days, −18°C; 17 hr, 4°C	210	525
	14 days, −18°C; 2.5 hr, 30°C	270	580

effect of testing frequency (1.0–5.0 Hz) was studied with fresh doughs. The moduli and tan δ increased slightly with the increase of frequency. In freeze-thaw experiments, the measurements were performed at a fixed frequency of 5 Hz and at a strain of 0.04. Tan δ was calculated from the ratio G''/G'.

Stress-relaxation measurements. Immediately after mixing, the nonyeasted dough was placed in plastic bag and stored at 4°C. The stress-relaxation measurements were repeated with a new piece of dough after different periods of time. After 1 hr of storage, rheological properties were measured, and 300 g of the dough was frozen.

In stress-relaxation experiments, a sudden strain is applied to the sample, and the stress decay at constant strain is measured as a function of time. The method is described in detail by Bohlin and Carlson (1980). A parallel plate (30 mm diameter) measuring system was used. An O-ring and silicon oil were used and the gap between the plates was set at 1.5 mm to prevent the sample from drying. The dough was allowed to rest for 10 min. The stress relaxation was measured between 0.02 and 600 sec. The strain applied was 0.147. The parameters calculated were the relaxation modulus (G_{rel}) and the half relaxation time ($T_{1/2}$). Typical standard errors of means for the rheological measurements are shown in Table I.

RESULTS AND DISCUSSION

Effects of Freezing and Thawing on Gas Volume

The effects of freezing and the thawing temperature on gas volume are summarized in Table II. Freezing and thawing slightly decreased the gassing power of the yeast. The gas production was lower, particularly during the second hour, for frozen and thawed doughs. Gas volume was slightly greater for the rapidly thawed doughs. Table III shows that the addition of yeast homogenate to doughs with baker's yeast had no effect on the gassing power. Yeast homogenate alone produced hardly any gas.

TABLE III
Effect of Yeast Homogenate on the Gas Volume

Dough (flour)	Gas Volume (ml) 1 hr	2 hr
B + 3.3% BY[a]	300	1,050
B + 3.3% BY + 0.33% YH[b]	310	1,120
B + 3.3% BY + 0.16% YH	310	1,120
B + 0.33% YH	70	70

[a]BY = baker's yeast.
[b]YH = yeast homogenate.

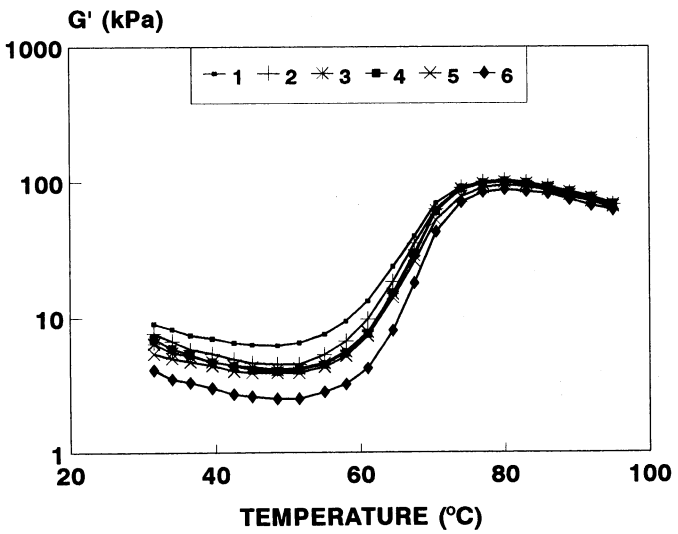

Fig. 1. The effect of freezing and thawing temperature and time on the relationships between storage modulus (G') and temperature for nonyeasted dough B. 1 = A nonfrozen control, 2 = thawing at 30°C for 2.5 hr, 3 = thawing at 4°C for 17 hr, 4 = thawing at 4°C for 19.5 hr, 5 = thawing at 4°C for 23 hr, and 6 = thawing at 4°C for 41 hr.

Heat-Induced Changes in Fresh Dough

Continuous evaluation of storage modulus (G') and tan δ during heating of the doughs (Figs. 1 and 2) showed G' values to increase and tan δ to decrease between 62 and 78°C. Extrapolation of this part of the curve to the x-axis allowed determination of the onset temperature of the rapid storage modulus increase (Fig. 3). A study of the heat-induced rheological changes of doughs made from blends of commercial starch and commercial gluten suggested to Dreese et al (1988) that the changes in moduli and tan δ between 55 and 75°C were attributable to starch gelatinization.

Except for tan δ, which remained constant, the rheological properties of the freshly mixed dough stored at 4°C changed—G' increased, gelatinization temperature decreased, and the gelatinization peak height increased (results not shown). These changes probably were attributable to the transport of water from gluten to starch. Szczesniak (1988) has suggested that the changes due to water distribution and relaxation after mixing can be minimized by letting the dough reach equilibrium before measurement.

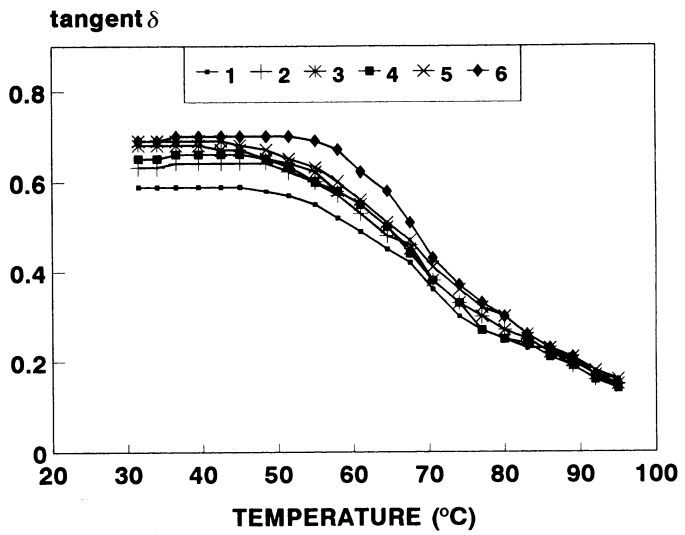

Fig. 2. The effect of freezing and thawing temperature on the relationships between tan δ and temperature for nonyeasted dough. 1 = A nonfrozen control, 2 = thawing at 30°C for 2.5 hr, 3 = thawing at 4°C for 17 hr, 4 = thawing at 4°C for 19.5 hr, 5 = thawing at 4°C for 23 hr, and 6 = thawing at 4°C for 41 hr.

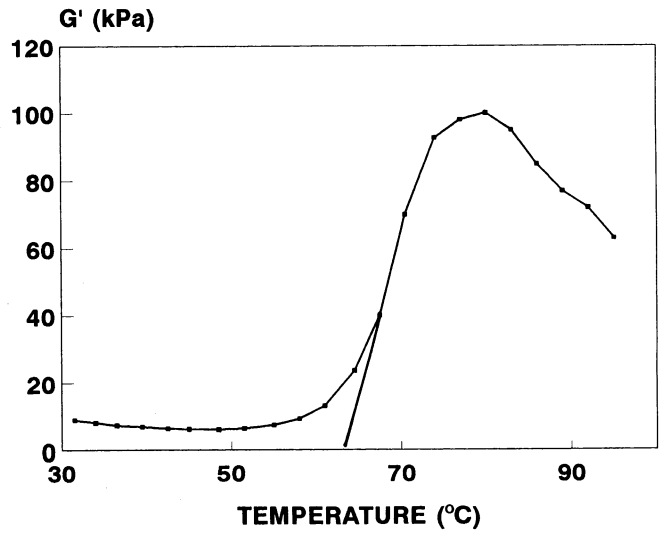

Fig. 3. Determination of the onset temperature of starch gelatinization.

Effects of Freezing and Thawing on the Heat-Induced Rheological Changes

Freezing and thawing caused three major changes in the heat-induced rheological properties of doughs—the storage modulus (G') of the dough decreased, the tan δ of the dough increased, and starch gelatinization was delayed. The decrease of G' accompanied by an increase of tan δ suggests a loss of polymer cross-linking. Two processes might be involved: weakening of the gluten network and separation of starch granules from the gluten network (Berglund et al 1991).

Freezing and thawing increased the onset temperature of starch gelatinization (Table IV). In native starches, the gelatinization temperature measured by differential scanning calorimetry depends on the extent and type of crystallinity in the granule, total moisture content, and moisture distribution (Levine and Slade 1990). The growth of ice crystals during frozen storage means that water in the dough is separated into large pools (Berglund et al 1991). The increase of the onset temperature of starch gelatinization in frozen doughs may thus be attributable to a delay in the diffusion of water into the starch granules or to the increased crystallinity of starch granules.

Doughs containing dead yeast cells. Dead yeast cells had little effect on the heat-induced changes in dough. Only at the highest concentration did the storage modulus of dough thawed at 30°C decrease slightly (data not shown).

Effect of Freezing on Stress Relaxation

Values of the relaxation modulus and relaxation time before and after freezing are given in Table V. Storage of fresh dough at 4°C for several hours resulted in a decrease in the relaxation modulus. Freezing and thawing caused a decrease in both G_{rel} and $T_{1/2}$, suggesting a weakening of the gluten network. There were no differences in the results of the relaxation test when the thawing time at 4°C varied between 17 and 21 hr (Table V). The relaxation time of doughs containing GSH decreased substantially as a result of freezing and thawing, suggesting that GSH causes a reducing reaction in gluten.

TABLE IV
Effect of Freezing and Thawing Temperature and Time on the Onset Temperature of Starch Gelatinization

Sample	Thawing Conditions	Onset Temperature (°C)
Nonfrozen	...	62.5 ± 0.5
Frozen	30°C for 2.5 hr	63.5 ± 0.5
	4°C for 17 hr	63.5 ± 0.5
	4°C for 19.5 hr	63.5 ± 0.5
	4°C for 23 hr	63.6 ± 0.5
	4°C for 41 hr	64.1 ± 0.5

TABLE V
Effects of Storage Conditions on the Relaxation Properties of Doughs

Dough (flour)	Storage Conditions	Thawing Conditions	G_{rel}[a] (kPa)	$T_{1/2}$[b] (sec)
A	1 hr, 4°C		8.2	0.27
	4 hr, 4°C		7.6	0.30
	5.5 hr, 4°C		5.8	0.26
	14 days, −18°C	17 hr, 4°C	5.4	0.22
	14 days, −18°C	21 hr, 4°C	5.4	0.21
	14 days, −18°C	2.5 hr, 30°C	6.0	0.24
B + GSH[c]	1 hr, 4°C		7.3	0.21
	14 days, −18°C	17 hr, 4°C	5.2	0.13
	14 days, −18°C	2.5 hr, 30°C	5.6	0.11
B + DY[d]	1 hr, 4°C		9.2	0.20
	14 days, −18°C	15 hr, 4°C	7.7	0.18
	14 days, −18°C	2.5 hr, 30°C	8.2	0.17

[a] Relaxation modulus.
[b] Half relaxation time.
[c] Reduced glutathione.
[d] Dead yeast cells.

Freezing and thawing decreased the gas production of the yeast. However, because freezing and thawing changed the rheological properties of the nonyeasted dough, a loss of bread loaf volume as a result of freezing and thawing must be attributable not only to decreased gas production but also to structural changes in the dough.

CONCLUSIONS

Viscoelastic measurements allow the following conclusions about the rheological changes induced by the freezing and thawing of doughs. First, a decrease in G' and an increase in tan δ in frozen and thawed dough suggested a loss of polymer cross-linking. Second, both the relaxation modulus and relaxation half-life decreased in frozen dough. The decrease of relaxation half-life indicates a weakening of the gluten network. And finally, the addition of dead yeast cells to dough did not affect the rheological properties, indicating that the structural changes in frozen and thawed doughs are not associated with the release of reducing substances from yeast cells.

LITERATURE CITED

ABDELRAHMAN, A., and SPIES, R. 1986. Dynamic rheological studies of dough systems. Pages 87-103 in: Fundamentals of Dough Rheology. H. Faridi and J. Faubion, eds. Am. Assoc. Cereal Chem.: St Paul, MN.

AUTIO, K., KIESVAARA, M., and POLVINEN, K. 1989. Heat-induced gelation of minced rainbow trout (*Salmo gairdneri*): Effect of pH, sodium chloride and setting. J. Food Sci. 54:805.

BERGLUND, P. T., SHELTON, D. R., and FREEMAN, T. P. 1991. Frozen bread dough ultrastructure as affected by duration of frozen storage and freeze-thaw cycles. Cereal Chem. 68:105-107.

BOHLIN, L., and CARLSON, T. L-.G. 1980. Cone-plate instrument for stress relaxation measurements. J. Colloid Interface Sci. 73:61.

BOHLIN, L., AUTIO, K., and PUOLANNE, E. 1987. A new measuring system to study the rheological properties of meat during heating. Page 399 in: Proc. Int. Congr. Meat Sci. Technol., 33rd.

CARLSON, T. L-.G. 1981. Law and order in wheat flour dough. Colloidal aspects of the wheat flour dough, and its lipid and protein constituents in aqueous media. Ph.D. thesis. University of Lund, Lund, Sweden.

DEA, I. C. M., RICHARDSON, R. K., and ROSS-MURPHY, S. B. 1983. Characterization of rheological changes during processing of food materials. Pages 357-360 in: Gums and Stabilisers for the Food Industry. G. O. Philips and D. J. Wedlock, eds. Pergamon Press: Oxford.

DREESE, P. C., FAUBION, J. M., and HOSENEY, R. C. 1988. Dynamic rheological properties of flour, gluten and gluten-starch doughs. I. Temperature-dependent changes during heating. Cereal Chem. 65:348-353.

ELIASSON, A.-C. 1990. Rheological properties of cereal proteins. Pages 67-110 in: Dough Rheology and Baked Product Texture. H. Faridi and J. Faubion, eds. Van Nostrand Reinhold: New York.

HIBBERD, G. E. 1970. Dynamic viscoelastic behaviour of wheat flour doughs. III. The influence of the starch granules. Rheol. Acta 9:501.

HSU, K. H., HOSENEY, R. C., and SEIB, P. A. 1979. Frozen dough. I. Factors affecting stability of yeasted doughs. Cereal Chem. 56:419-424.

KLINE, L., and SUGIHARA, T. F. 1968. Factors affecting the stability of frozen bread doughs. I. Prepared by the straight dough method. Baker's Dig. 42(5):44.

LEVINE, H., and SLADE, L. 1990. Influences of the glassy and rubbery states on the thermal, mechanical, and structural properties of doughs and baked products. Pages 157-330 in: Dough Rheology and Baked Product Texture. H. Faridi and J. Faubion, eds. Van Nostrand Reinhold: New York.

LORENZ, K. 1974. Frozen dough present trend and future outlook. Baker's Dig. 48:14.

MCCLEARY, B. V., and SHEEHAN, H. 1987. Measurement of cereal α-amylase: A new assay procedure. J. Cereal Sci. 6:237.

MITA, T., and BOHLIN, L. 1983. Shear stress relaxation of chemically modified gluten. Cereal Chem. 60:93-97.

NAVICKIS, L. L., ANDERSON, R. A., BAGLEY, E. B., and JASBERG, B. K. 1982. Viscoelastic properties of wheat flour doughs: Variation of dynamic moduli with water and protein content. J. Texture Stud. 13:249.

OURA, E., SUOMALAINEN, H., and VISKARI, R. 1982. Breadmaking. Pages 87-146 in: Economical Microbiology. Vol 7. A. H. Rose, ed. Academic Press: Oxford.

PARISI, F. 1970. Yeast quality. Pages 81-93 in: Welt-Getreide- und Brotkongress, Dresden, DDR, Kongressbericht 5.
REED, G., and PEPPLER, H. 1973. Pages 103-164 in: Yeast Technology. Avi Publishing: Westport, CT.
SINDA, E. 1989. Taikinan ja hiivan pakastus. Leipuri 87:6.
SUOMALAINEN, H., DETTWILDER, J., and SINDA, E. 1972. α-Glucosidase and leavening of baker's yeast. Process Biochem. 7:5.
SZCZESNIAK, A. S. 1988. Applying rheology to dough and baked goods. Cereal Foods World 33:841.
VARRIANO-MARSTON, E., HSU, H. K., and MAHDI, J. 1980. Rheological and structural changes in frozen dough. Baker's Dig. 54:32.
WOLT, M. J., and D'APPOLONIA, B. L. 1984. Factors involved in the stability of frozen dough. I. The influence of yeast reducing compound on frozen dough stability. Cereal Chem. 61:209-212.

[Received March 29, 1990. Accepted January 15, 1992.]