Water Distribution in Frozen Lean Wheat Doughs

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ABSTRACT

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Frozen storage increased the amount of liquid phase and decreased the storage modulus of water-flour mixtures. The liquid phase was studied by ultracentrifugation. The most significant change occurred during the first week of storage. The negative effects of ice crystals could be controlled by reducing the water content, which was seen as smaller amounts of liquid phase and higher dough rigidity after frozen storage (*G*′ values). Reduced water content also prevented an increase in the self-diffusion coefficient during frozen storage (^{1}H NMR studies). Prefermented frozen doughs were examined under different conditions: with and without S-kimo (additive from Puratos, Belgium), prefermentation time of 25 or 40 min, and reduced water content. The results obtained with autoradiographic method correlated best with the baking results and showed that S-kimo and shorter prefermentation time improve the water distribution of frozen prefermented doughs. Doughs contained small ice crystals after frozen storage and there were no large water patches in thawed doughs. Reduced water content and exclusion of S-kimo decreased the liquid phase of fermented doughs and increased dough rigidity. The baking properties of frozen prefermented doughs were better predicted by large deformation rheology (expansion potential of samples during oscillation). In general, flour quality had an obvious effect on the parameters. There was no correlation between the rheological properties and the values of liquid phase, but in most cases a high correlation between the total water content and rheological properties was observed.

Wheat flour dough has been described by Eliasson and Larsson (1993) as bicontinuous and phase-separated. It consists of two aqueous phases, the water-swelled protein phase (gluten) and the liquid phase (dispersed starch granules and solubles). In their latest work, Larsson and Eliasson (1996a) suggest a new model of phase separation for wheat dough based on ultracentrifugation studies. Five different phases are proposed: liquid, gel, gluten, starch, and unseparated dough; the relative amounts of each depend on the water content of the dough. The importance of the liquid phase in wheat flour doughs was originally noted by MacRitchie (1976). Although Larsson and Eliasson (1996a) found the degree of phase separation varied with flour quality, the water content of the different phases remained almost constant at ≈80% for gel, ≈55% for gluten, and ≈30% for starch. It has been assumed that the rheology of dough is dependent on the properties of the polymers (gluten). However, Larsson and Eliasson (1996a) observed changes in the rheology of the dough even when the water content of polymers remained constant. They also noted that the rheology can be divided into two regions. In the first region the rheology is strongly dependent on water content (no phase separation with ultracentrifuge, low water content), while in the second region it is less dependent on water content but still changes linearly with water content (phase separation with ultracentrifuge, high water content).

The major changes in frozen doughs, both in gluten structure and in yeast viability, are due to ice crystals. The damage is both direct (physical) and consequent (water distribution). Stauffer (1993) has stressed the role of ice crystals in damaging the gluten structure, but also notes the lack of convincing experimental support. Berglund and coworkers (1991) have nevertheless shown that in frozen doughs less water is associated with both gluten and starch fractions, and the water is instead concentrated into large patches of ice crystals. Also, our studies have shown the effect of ice crystals on the stability of frozen prefermented doughs. The pore structure of prefermented dough is extremely sensitive to ice crystals. To prevent physical changes in the pore structure, prefermentation time should be shorter, and water content reduced (Räsänen et al 1995, 1997a).

In the investigation reported here, we focused on the consequential changes (changes in water distribution) caused by ice crystals in frozen doughs. The water distribution was analyzed by ultracentrifugation, ^{1}H NMR, and autoradiography. The experiments were performed with four different flours, first in water-flour mixtures, then with various ingredients added, and finally in fermented yeasted doughs. The effects of water distribution on dough rheology were also monitored as small and large deformations (oscillation measurements and expansion potential of samples during oscillation, respectively).

MATERIALS AND METHODS

Flour Samples

The characteristics of the four flours used in these experiments are shown in Table I. The flours were milled from pure wheat cultivars (Bühler MLU-202 mill, Switzerland). Flour yield was 60%. Determinations were made of moisture, protein content (N × 5.7), falling number, wet gluten content, and farinograph absorption at 500 BU. Extensibility and resistance to extension were determined in an extensigraph (AACC 1995). The amount of water solubles was determined according to the procedure of Izydorczyk et al (1991). All analyses were performed in duplicate.

Sample Preparation

Wheat flour doughs were mixed in a 50-g bowl with a minorpin mixer (Henry Simon Ltd., UK) or a mixograph (National Mfg.) to obtain optimal dough development as determined with a farinograph. In water-flour dough, the flour (15 or 30 g), which had been milled from a pure cultivar, was mixed with the optimal amount of water and with a reduced amount (–2 percentage units). The optimal water content was measured as farinograph absorption at 500 BU (AACC 1995) as shown in Table I.

The standard recipe for the yeasted doughs was 100% flour, 4% yeast (compressed baker's yeast), 2% sugar, 1.5% salt, 4% shortening, and 4% additive (S-kimo from Puratos, Belgium). The major components of S-kimo are wheat flour (32%), gluten (30%), and glucose (19%), as well as ascorbic acid and diacetyl

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TABLE I
Flour Characteristics (water-free basis)

Quality Tests	Flour 1	Flour 2	Flour 3	Flour 4
Protein content (N × 5.7), %	12.9	11.4	12.9	17.9
Ash content, %	0.5	0.5	0.5	0.6
Falling number value	470	315	395	290
Wet gluten, % (a)	30.7	25.8	29.4	44.4
Water soluble index, % (b)	4.15	3.79	3.53	4.22
b/a	0.14	0.15	0.12	0.10
Farinograph				
Absorption, %	56.5	52.3	54.0	61.8
Dough development time, min	10.3	2.2	2.3	17.4
Stability, min	17.7	9.8	4.5	16.5
Extensigraph (45 min)				
Maximum resistance, BU	810	770	815	915
Extensibility, mm	157	146	142	191

tartaric acid ester of mono-diglycerides (DATEM). Two different water contents were studied: optimal, to achieve optimal consistency, and –2 percentage units. After mixing, the yeasted doughs were left to rest for 20 min at room temperature. The fresh control doughs were proofed 40 min before analysis, and the test doughs were proofed 40 or 25 min before freezing at 34°C and 80% rh.

After mixing (nonyeasted) or fermentation (yeasted), the dough samples for the ultracentrifugation, autoradiography, and ^{1}H NMR studies were frozen immediately in a chest freezer. The freezing rate was considered to be sufficiently fast in view of the small size of the samples. Before freezing, the samples for rheological measurements were prepared with a noodle-maker machine (Atlas, Italy) to obtain sheets 3.0 mm thick. Loss of water from the samples during freezing and frozen storage was prevented by wrapping them in polymer film. The samples were analyzed after one, seven, and 14 days of frozen storage. All tests were performed at least twice.

Ultracentrifugation

Ultracentrifugation followed the procedure of Larsson and Eliasson (1996a). However, a larger sample was used (≈20 g) to increase the amount of liquid phase. Closable test tubes (25 mm dia. and 89 mm height) were centrifuged for 1 hr at 100,000 × *g* in a Beckman L8-55M ultracentrifuge. The amount of liquid phase was determined after one, seven, and 14 days of frozen storage at optimal and reduced water content. The changes in liquid phase were monitored based on the weight of liquid phase compared to the sample. The experiments were repeated twice for all conditions.

Rheological Measurements

A StressTech rheometer (ReoLogica Instruments AB, Sweden) was used to measure the rheological parameters of frozen doughs. The dough was thawed for 0.5 hr at room temperature before samples were taken. Circular samples, 20 mm dia. and 2.0 mm thick (nonyeasted dough) or 3.0 mm thick (yeasted dough), were prepared from dough sheets and measured with a parallel-plate system. The samples were compressed with the upper plate, the normal force being <2.0 N during compression. Before the oscillation measurements, which took 80 sec, the normal force was ≤1.0 N. Drying of the samples during measurements with the StressTech was prevented by coating them with silicon oil. The measurements were made at 25°C and 1 Hz with a constant stress of 10.0 Pa (corresponding to strain $\approx 1.5 \times 10^{-3}$). Nonyeasted doughs were measured with constant gap, but for yeasted doughs an autotension, limit normal force 0.1 N, was used. Thus, for yeasted doughs, the change in gap during the measurement was measured in addition to the storage (G') modulus (large deformation rheology). Tests were repeated at least four times.

^{1}H NMR

The self-diffusion of water-flour mixtures was analyzed at three water contents (optimal, +3, and –3 percentage units) after frozen storage times of up to 19 days. NMR spin-echo techniques have long been established as a valuable method for measuring the apparent self-diffusion coefficient D of mobile spins in a static magnetic field gradient. Variants of the spin echo techniques have been developed using a pulse-field gradient. The Minispec NMS 120 NMR Analyzer (Brucker, Germany) with an operating frequency of 20 MHz (^{1}H) was used with pulsed-field gradient spin echo (PFGSE) as defined by Tanner and Stejskal (1968). The conditions were: 100 µsec duration of magnetic-field gradient, delay of 4 msec between the 90° and 180° pulses, and recycle delay of 2 sec. The gradient amplitude was set at 5 (Bruker arbitrary units). The operation temperature was +40°C. Measurements were begun with the frozen sample and the stabilized values were recorded. The experiments were repeated twice under all conditions and the self-diffusion coefficient D was compared with D_0, the self-diffusion coefficient of water.

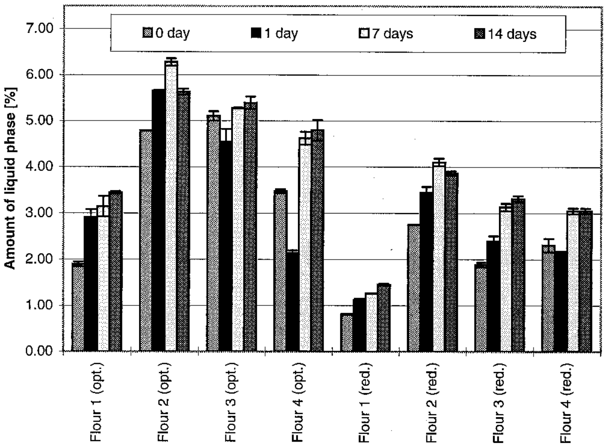

Fig. 1. Effect of frozen storage time and water content on the amount of liquid phase in water-flour mixtures (opt. = optimal water content and red. = reduced water content).

Autoradiography

Tritiated (^{3}H) water was substituted for part of the water in dough. Tritium is a β-emitter (E_{max}= 18.6 keV) with a half-life ($T_{0.5}$) of 12.3 years. The range in biological material is 2 µm (Rogers 1979) and the range in air is 5 mm (Evans 1955). The ^{3}H-labeled water was bought as 185 MBq/mL (5.00 mCi/mL) solution, and was diluted to 20 mL of water. In baking, the radioactivity of doughs was adjusted to ≈30 MBq total activity. The appropriate activity was selected after experiments with total activity from 20 to 50 MBq.

After frozen storage, the doughs were cut into 5-mm sections with a guillotine. Cutting was done in a cold-storage room (+4°C) to avoid thawing of samples. The sections were held in a –70°C freezer for a minute or two and transferred onto a film (15 × 24 cm) (Hyperfilm ^{3}H, Amersham). The film was prepared for direct autoradiography for tritium β-activity; the sensitivity and resolution of the film were good and excellent, respectively. Two weeks exposure was conducted at –70°C to minimize tritium diffusion, background blackening, distribution of water, and growth of ice crystals. The dough sections were removed from the film after the exposure and briefly placed in a freezer before photography with a scanner (Ricoh FS 2). The film was developed with Kodak D-19 for 5 min and attached with Kodak AL-5 for 5 min. Between development and attachment the film was washed in flowing water for 5 min, and after attachment for 10–15 min. The distribution of water in prefermented frozen doughs (black spots in film) was studied with an image analyzer (Global Lab Image ver 3.0). At least three sections were taken from each dough for exposure, and two or three different doughs were prepared for each sample.

Other Methods

The statistical significance of the results was analyzed with analysis of variance (ANOVA) and multiple range tests by using the Statgraphics Plus 2.1 application. All tests were conducted at 5% significance level. Thus, the results are statistically significant when the *P*-value is smaller than the significance level ($P < 0.05$).

RESULTS AND DISCUSSION

Flour Samples

The present study was based on the earlier baking tests with prefermented frozen doughs (Räsänen et al 1995, 1997a). In the baking tests S-kimo, shorter prefermentation time, and reduced water content were favorable for fresh-like baking properties with frozen storage times up to 14 days. The flour samples (Table I) were same as those described previously (Räsänen et al 1997a), where the flour characteristics were also discussed more closely. However, the number of samples was decreased from six to four; the numbering was changed to flours 1, 2, 3, and 4, which correspond to flours 1, 3, 4, and 5, in the previous study (Räsänen et al 1997a). In Table I, protein and ash contents are expressed on a water-free basis, wet gluten and water soluble index are expressed on an as is basis, and other parameters are expressed at 14% moisture level.

Water-Flour Mixtures

Amount of liquid phase. Effects of frozen storage time and water content on the amount of liquid phase in water-flour mixtures were discussed previously (Räsänen et al, unpublished). The amount of liquid phase increased during frozen storage because of the growth of ice crystals (Fig. 1). The ice crystals cause redistribution of water in frozen doughs, resulting in drying of polymers and in large water patches. The growth of ice crystals could be controlled by reducing the water content, which was observed as a smaller amount of liquid phase in dough (Fig. 1). Larsson and Eliasson (1996a) also reported a marked effect of water content on the phase separation and liquid phase of dough. The most dramatic increase in the amount of liquid phase was seen during the first week of frozen storage, after which the liquid phase remained almost constant ($P < 0.01$). A similar result was obtained in baking tests with prefermented frozen doughs (Räsänen et al 1997a). Although the amount of liquid phase in dough with reduced water content also increased during frozen storage, the level, in most cases, remained lower than in fresh doughs with optimal water content.

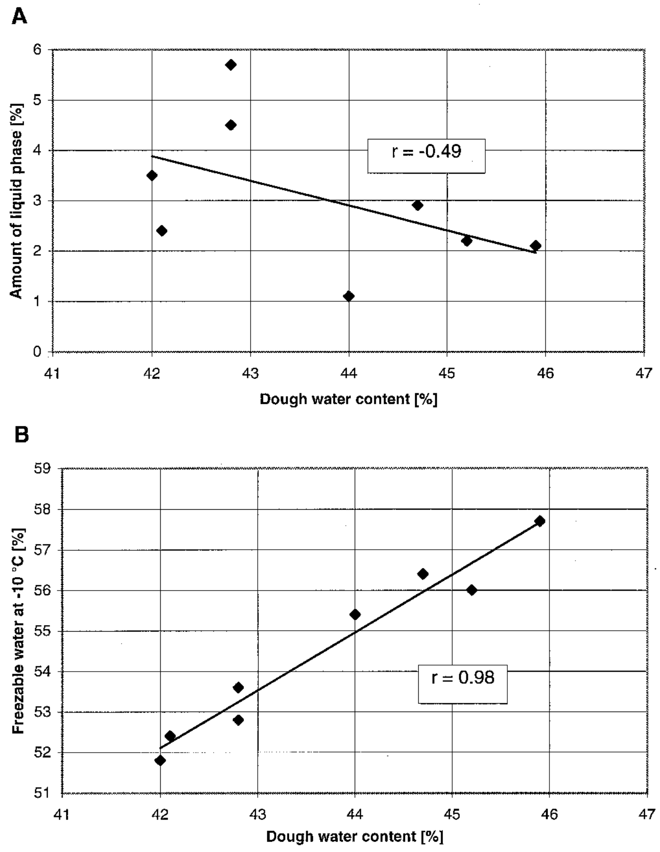

Fig. 2. Correlation between total water content of dough and (**A**) the amount of liquid phase and (**B**) freezable water at –10°C. Results for water-flour mixtures after one day of frozen storage.

Flour quality always had a statistically significant effect on the amount of liquid phase in water-flour mixtures (Fig. 1) ($P < 0.01$). Under all conditions investigated, flour 2 had the highest and flour 1 the lowest level of liquid phase. However, Fig. 2a shows that after one day of frozen storage, a higher total water content in dough resulted in lower amount of liquid phase. The correlation between the amount of liquid phase and the total water content of dough was highest after one day of storage (r = –0.49). These results show that the amount of liquid phase was not only dependent on the amount of water in the dough, but also on the amount and properties of water-binding components in the flour (gluten and pentosans). It was also established earlier by Eliasson and Larsson (1996a,b) that there is no simple relationship between the amount or properties of separated phases in different cultivars. Flours 1 and 4 had the highest protein content and water-soluble index, and a smaller amount of liquid phase (Table I). The situation was completely different when the freezable water was determined by ^{1}H NMR in water-flour mixtures at –10°C (Räsänen et al, unpublished). The correlation between freezable water and total water content after one day storage is seen in Fig. 2b (r = 0.98). One explanation for such a great difference between the results of these two methods of describing the water state in dough could be that the water that cannot be separated by ultracentrifuge is also freezable.

Rheological properties. Rheological properties of water-flour mixtures were analyzed under the same conditions as the amount of liquid phase (Fig. 3). Greater rigidity of dough, seen as higher storage modulus (i.e., G' values [$P < 0.01$]) was almost always associated with reduced water content. The only exception was flour 4. In general, one day of storage had only a minor effect on G' values, whereas longer frozen storage decreased G', making doughs softer. A decrease in the G' of water-flour mixtures during frozen storage was also recorded by Autio and Sinda (1992). The

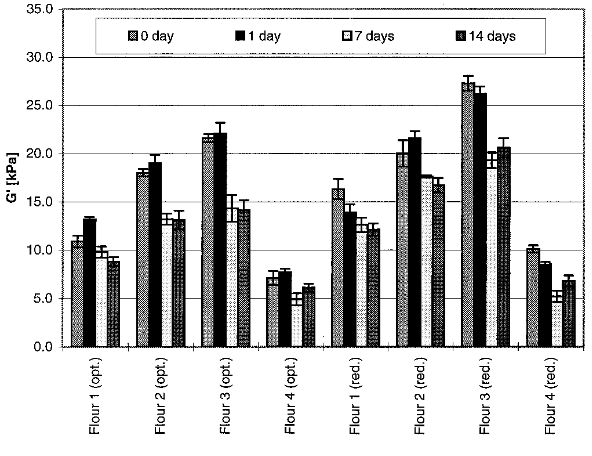

Fig. 3. Effect of frozen storage time and water content on the storage modulus (G') in water-flour mixtures (opt. = optimal water content and red. = reduced water content).

greatest decrease in G′ occurred during the first week of storage, and the storage from seven to 14 days did not produce any further changes ($P < 0.01$). This finding is consistent with measurements of the amount of liquid phase, as well as baking tests on prefermented frozen doughs (Räsänen et al 1997a).

In the baking tests, we discovered that water content should be reduced with longer frozen storage times to improve the gas holding properties of doughs (Räsänen et al 1997a). However, the rheological properties of dough should be dependent on the changes in polymers (especially gluten) of dough. Thus, as the amount of liquid phase increased and the water content of gluten decreased, higher G′ values would have been expected. One explanation for the difference between the observed results and theory could be that the ice crystals break the gluten network, or that dough rheology is not solely dependent on the properties of gluten. On the other hand, redistribution of water results in water-rich and dry areas and this might increase the softness of dough. Larsson and Eliasson (1996a) observed that with high water content, the rheology of dough is a linear function of water content, but at the same time the water content of gluten remains constant. The high linearity of dough rheology to total water content was also seen in our experiments, and although the G′ values decreased during frozen storage (1–7 days), the linearity between rheology and water content remained (Fig. 4).

The flour samples exhibited different G′ values, and reduced water content increased dough rigidity in all cases (Fig. 3) ($P < 0.01$). The dough rigidity decreased in the order: flours 3, 2, 1, and 4. The reverse order was seen in baking tests, flour 4 giving the greatest loaf volumes and flour 3 the smallest (Räsänen et al 1997a). Since the baking conditions were constant for all flours, the softest dough gave the highest loaf volumes. The case was different after frozen storage, where the dough with highest rigidity gave the smallest change in loaf volume. The dough with highest rigidity also better retained the form ratio (maximum height divided by width) during frozen storage. The increase of liquid phase during frozen storage correlated well with the rheological properties when the changes were compared for a single flour ($r = -0.78, -0.73, -0.80$, and -0.72 for flours 1–4, respectively). However, in good agreement with the results of Larsson and Eliasson (1996a), the absolute values of liquid phase could not be used to predict G′ values of water-flour mixtures ($r < -0.40$).

Effect of Ingredients

Amount of liquid phase. Effects of ingredients on the amount of liquid phase in dough were examined under five different conditions, first in water-flour mixtures and then in yeasted dough with all ingredients included (Fig. 5). Except with flour 3, use of warmer water (+37°C) slightly increased the amount of liquid phase. Evidently, the warmer water dissolves more water-soluble components from the flour. The most pronounced increase in the liquid phase occurred when salt and sugar, 1.5% and 2.0% as flour basis, were added to doughs ($P < 0.01$). The change in the liquid phase was monitored as percent by weight, and salt and sugar dissolved in the liquid phase of dough. Shortening appeared to have a greater effect on the amount of liquid phase than S-kimo, and it decreased the level from that with salt and sugar. However, when all the ingredients were included together with yeast, the values were closely similar to those obtained with just S-kimo. Thus, S-kimo normalized the effect of shortening, and yeast had no further effects. Larsson and Eliasson (1996b) report that adding ascorbic acid increases, and lecithin decreases, the liquid phase in water-flour mixtures. Their result was strongly cultivar-dependent, however. The effect of S-kimo and shortening on the amount of liquid phase can be explained by better dough-mixing properties and higher capacity to bond water.

The type of flour did not have as clear an effect as the above factors on the amount of liquid phase in water-flour mixtures with different ingredients (Fig. 5). After the addition of salt and sugar, other added ingredients had no effect on the liquid phase of flour 4 ($P < 0.05$). Although there was a highly statistically significant difference in the liquid phase of flour samples with different ingredients added, there was no clear pattern in the effect of the ingredients. Evidently the ingredients affected each flour uniquely.

Rheological properties. The effect of the ingredients on dough rheology (G′ values) was examined in the same way as the effect on amount of liquid phase (Fig. 6). The type of flour was always significant, and G′ values decreased in the order: flour 3, 2, 1, and 4 ($P < 0.01$). Warmer water and salt and sugar had no statistical effect on the G′ values, even though they increased the amount of liquid phase in doughs. However, the doughs became softer when they contained S-kimo. Adding all ingredients further decreased the G′ values ($P < 0.01$). The addition of all ingredients also decreased the differences between flour samples. Shortening had a statistically significant effect in decreasing the rigidity of dough only for flour 3. No effect was observed with other ingredients after the addition of salt and sugar to flour 4 ($P < 0.01$), which was consistent with the results for the liquid phase. These experiments further confirmed that the absolute amount of liquid phase does not correlate with the rheological properties of fresh dough.

Yeasted Doughs

Amount of liquid phase. Table II presents the amount of liquid phase in fermented yeasted doughs, which was monitored in the same way as earlier in baking tests (Räsänen et al 1995, 1997a).

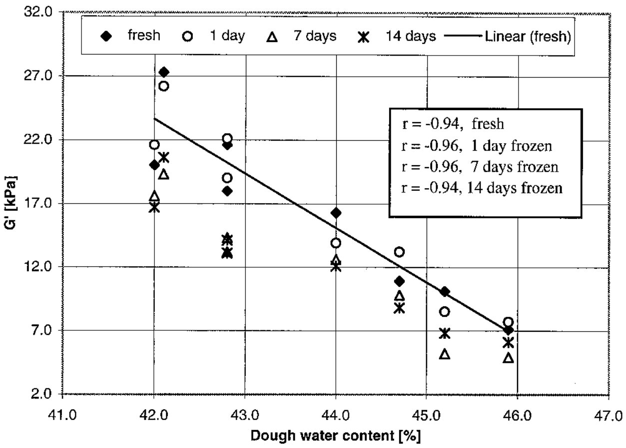

Fig. 4. Correlation between total water content of dough and the storage modulus (G′) of water-flour mixtures with different frozen storage times.

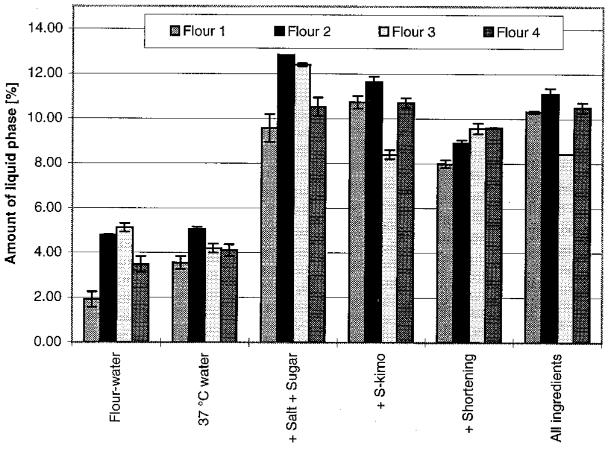

Fig. 5. Effect of ingredients on the amount of liquid phase in fresh water-flour mixtures. Ingredients listed were added to the previous sample, except that +shortening does not include S-kimo.

Effect of S-kimo was analyzed in 40-min prefermented samples, and different water contents in 25-min prefermented samples (optimal and –2 percentage units). The 25-min prefermented samples also included S-kimo. Although ultracentrifugation proved to be an unreliable method for analyzing the state of water in prefermented frozen doughs, there were some general trends in the observed results. Prefermentation time had no statistically significant effect on the amount of liquid phase on fresh doughs, but in frozen doughs, there was a general trend for a higher amount of liquid phase with a shorter prefermentation time (Table II). Czuchajowska et al (1989) pointed out that proofed doughs have a higher water content than freshly mixed doughs, owing to fermentation and to absorption of moisture from the fermentation cabinet. The results are consistent with those displayed in Fig. 6 and Table II, where fermentation increased the amount of liquid phase. A greater liquid phase in fermented doughs could also result from water separation from polymers during extension. Czuchajowska et al (1989) did not observe any statistically significant effect of reduced water content on the moisture content of dough. However, Table II shows that the amount of liquid phase was smaller ($P < 0.01$) when no S-kimo was added (except flour 3) or the water content was reduced. Reduced water content had a similar effect on water-flour mixtures. S-kimo had the greatest effect on flour 1. When S-kimo was not used, flour 1 had the smallest amount of liquid phase, and when it was added, flour 1 had the greatest amount of liquid phase. (Note that the concentration of liquid phase after frozen storage of seven days was exceptionally high for flour 2, and this was eliminated from statistical analyses.)

In general, neither freezing nor frozen storage had a statistically significant effect on the liquid phase when flour 4 was used (Table II). However, with other flour samples freezing had, generally, a more dramatic effect than frozen storage on the amount of liquid phase in fermented doughs. As described above, the amount of liquid phase increased in water-flour mixtures subjected to freezing and frozen storage. The increase was explained by the growth of ice crystals, and on this basis, the amount of liquid phase would have been expected to increase during frozen storage of fermented doughs as well. Possibly the explanation of this discrepancy is that the phase separation monitored by ultracentrifugation is dependent on the rheology of the dough. Ultracentrifugation can break dough structure, and fermented doughs, especially after freezing, are more sensitive to structural changes than water-flour mixtures are. The difference between the rheological properties of water-flour mixtures and fermented doughs can be seen in Figs. 3 and 7. The temperature of the dough can also affect the phase separation. When fresh fermented doughs were analyzed, the temperature of the dough was ≈+28°C, whereas when thawed doughs were analyzed, the temperature was ≈+5°C. Clearly, ultracentrifugation should not be used to analyze the water state in fermented doughs.

The only statistically significant effect of flour quality on the liquid phase of fermented doughs was seen with flour 3, which resulted in the lowest liquid phases (Table II). When the effect of total water content of dough on the amount of liquid phase was analyzed, the result was similar to that for water-flour mixtures: there was little correlation ($r < 0.60$). However, longer frozen storage improved the correlation, and after 14 days storage "40 min + S-kimo" samples showed a correlation as high as $r = 0.78$.

Rheological properties. Yeast-fermented doughs are difficult to study because the dimensions and physical properties of the dough change with time. Thus, possibly the only published article on the small deformation rheology of yeasted doughs is Kaufmann and Kuhn (1994). In our studies, the StressTech rheometer was equipped with a gas cylinder that allows the dough to expand during measurement. Expansion potential (ΔGap) and G' of yeasted doughs are reported for the same conditions as the amount of liquid phase with frozen storage times up to 14 days (Fig. 7).

In fresh doughs, the G' values were highest for the samples without S-kimo (except flour 3), whereas in frozen doughs, G' values were as high as or higher for the samples with reduced water (Fig. 7a). In water-flour mixtures, S-kimo and higher water content led to decreased G' values. In the fermented samples, those with the highest amount of liquid phase ("40 min + S-kimo" and "25 min + S-kimo") (Table II) exhibited the lowest G' values ($P < 0.01$), when compared flour by flour. In general, prefermentation time presented no statistically significant effects on G'. However, Kaufmann and Kuhn (1994) have reported lower elasticity at longer fermentation times. Figure 7b shows the effect of fermentation time on the expansion potential (ΔGap) of yeasted doughs. The findings are in agreement with those of Kaufmann and Kuhn (1994). Although there were no clear differences for the fresh doughs, for frozen stored samples, ΔGap values were higher with reduced water content or shorter prefermentation time ($P < 0.01$). In most cases, ΔGap values were smaller for the samples with S-kimo.

TABLE II
Effect of Frozen Storage on the Amount of Liquid Phase in Yeasted Doughs

Frozen Storage Time (days) and Sample Preparation	Amount of Liquid Phase (%)							
	Flour 1		Flour 2		Flour 3		Flour 4	
40 min[a] + no S-kimo		±		±		±		±
0	7.11	0.28	9.90	0.02	9.86	0.11	9.05	0.13
1	6.69	0.32	8.49	0.53	7.13	0.10	9.11	0.12
7	5.97	0.27	9.83	0.34	6.88	0.48	9.31	0.30
14	7.91	0.16	8.82	0.31	6.52	0.12	8.82	0.66
40 min[a] + S-kimo								
0	12.31	0.33	12.45	0.23	9.68	0.14	10.04	0.21
1	10.14	0.04	9.11	0.10	6.70	0.05	8.47	0.13
7	9.86	0.00	10.32	0.35	6.36	0.24	10.58	0.65
14	9.69	0.03	8.82	0.28	5.92	0.16	10.33	0.03
25 min[a] + S-kimo								
0	11.35	0.20	13.10	0.19	9.61	0.25	10.31	0.02
1	10.39	0.53	11.31	0.06	9.04	0.14	10.66	0.00
7	11.11	0.22	12.93	0.03	6.95	0.22	10.47	0.25
14	10.75	0.26	11.35	0.35	6.78	0.10	10.67	0.29
25 min[a] + S-kimo + red. water								
0	10.21	0.62	9.95	0.18	7.69	0.01	8.01	0.00
1	9.63	0.28	7.82	0.19	5.29	0.29	6.69	0.16
7	8.44	0.27	12.08	0.24	6.50	0.03	8.56	0.41
14	8.53	0.15	9.08	0.30	6.65	0.47	8.81	0.91

[a] Prefermentation time.

Although ΔGap values of all flours were strongly dependent on frozen storage time, frozen storage clearly decreased only the *G′* values of flour 3 (Fig. 7). In part, this was because one-day storage increased *G′* values (flours 1 and 2). Flours 1 and 4 withstood well the effects of frozen storage on *G′* values (small deformation rheology). However, ΔGap values (large deformation rheology) were strongly affected by frozen storage. Thus, the loaf volume decrease of frozen prefermented doughs during storage was better predicted by large than small deformation rheology. When no S-kimo was used, ΔGap values decreased regularly over one to 14 days ($P < 0.01$), except for flour 2 at seven days and flour 4 at 14 days. Lower water content reduces the negative effects of frozen storage, as can be seen in the almost constant *G′* values and ΔGap values at reduced water content. This is consistent with our earlier baking tests, in which, after 14 days frozen storage, the dough with reduced water content gave a bread with fresh-like baking quality (Räsänen et al 1997a). Where S-kimo was added, the most dramatic decrease in the expansion potential (ΔGap) occurred between one and seven days of frozen storage (except flour 2). A comparable result was reported in our earlier studies (Räsänen et al 1995, 1997a).

In general, statistically significant differences were associated with flour quality, especially in the case of fresh doughs and samples with reduced water content (Fig. 7). *G′* values were always lowest and ΔGap values highest ($P < 0.01$) for flour 4. Differences between other flour samples were less pronounced, and no clear order could be observed. However, it was obvious that a more rigid dough exhibited lower expansion potential, lacking the potential to return to the shape before compressing. There was strong evidence that the rheology of yeasted doughs is dependent on the total water content of the dough (Table III). As noted above, this was also the case for water-flour mixtures. However, when the correlations between the amount of liquid phase, *G′* values, and ΔGap values during frozen storage were investigated flour sample-by-flour sample and all together, the only meaningful correlation was for flour 1 ($r = -0.88$). Thus, the good correlations between the different flours and different conditions for water-flour mixtures disappeared when doughs were prefermented and frozen.

^{1}H NMR

The self-diffusion coefficients (*D*) of water-flour mixtures were measured for three different water contents (optimal, +3, and –3 percentage units) at +40°C. Frozen samples were measured after frozen storage up to 19 days, until the self-diffusion stabilized.

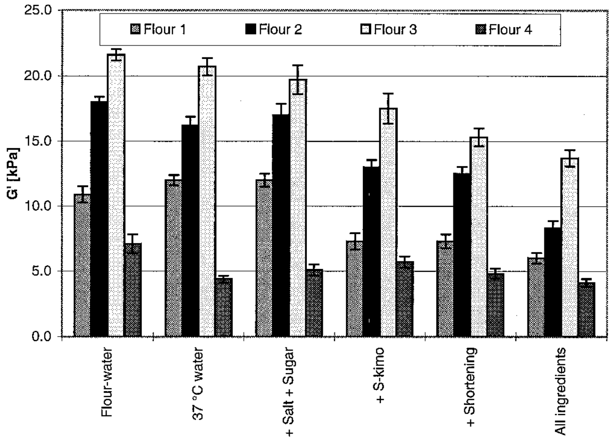

Fig. 6. Effect of ingredients on the storage modulus (*G′*) of fresh water-flour mixtures. Ingredients listed were added to the previous sample, except that +shortening does not include S-kimo.

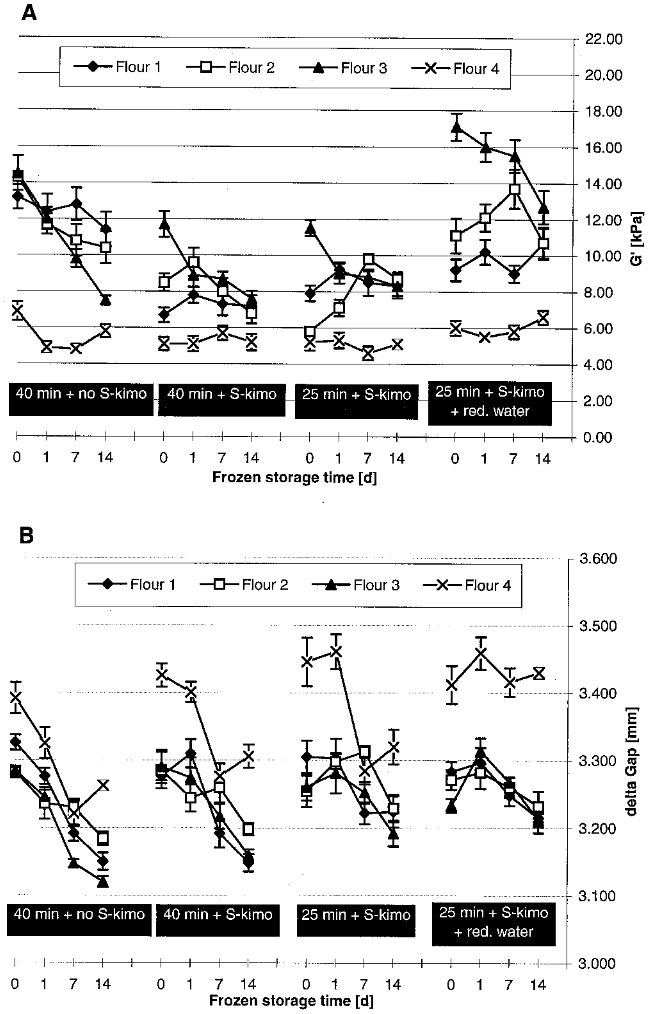

Fig. 7. Effect of frozen storage time and different conditions on storage modulus (*G′*) (**A**) and expansion potential (ΔGap) of fermented doughs (**B**). Time indicates the fermentation time before freezing. Effect of S-kimo was investigated for 40-min prefermented doughs and effect of water content for 25-min prefermented doughs (red. = –2% reduced water content).

TABLE III
Correlation between Total Water Content of Dough and Rheology of Yeasted Doughs

Frozen Storage Time (days) and Sample Preparation	**Correlation Coefficient (*r*)**							
	Storage Modulus (*G′*)				**Expansion Potential (ΔGap)**			
Frozen storage time (days)	0	1	7	14	0	1	7	14
40 min[a] + no S-kimo	–0.89	–0.76	–0.55	–0.36	–0.97	–0.98	–0.37	–0.72
40 min[a] + S-kimo	–0.89	–0.95	–0.95	–0.77	–0.76	–0.95	–0.23	–0.66
25 min[a] + S-kimo	–0.53	–0.52	–0.88	–0.84	–0.93	–0.85	–0.20	–0.84
25 min[a] + S-kimo + reduced water	–0.84	–0.91	–0.98	–0.85	–0.88	–0.80	–0.75	–0.78

[a] Prefermentation time.

Figure 8 shows the self-diffusion coefficient D compared with D_0, the self-diffusion coefficient of water. The self-diffusion coefficient increased during frozen storage with optimal and +3 percentage units water content, but remained constant with reduced water content ($P < 0.01$). Self-diffusion was always higher at the higher water content. The self-diffusion in water-flour mixtures correlated well with the amount of liquid phase. Thus, the self-diffusion as well as the amount of liquid phase increased during frozen storage because of the growth of ice crystals. This, in turn, resulted in drying of polymers and in large water patches. Reduced water content can be used to control the growth of ice crystals and the negative changes in dough rheology.

Autoradiography

Macroscopical water distribution in frozen prefermented doughs was analyzed with a method based on autoradiography. The most dramatic improvement in water distribution was seen when S-kimo was included (Fig. 9 and Table IV). Table IV shows a more even water distribution, reflected as a decrease in the percentage area of dark pores. Background presents the proportion of light areas up to the adjusted threshold value. The effect of S-kimo can be explained by the water-binding properties: S-kimo contains wheat flour, gluten, and glucose, all of which improve water-binding properties. When the water-binding properties of dough improve, the amount of "free" water and the number of ice crystals, decreases. The analysis of the liquid phase had, in fact, suggested an increase rather than a decrease in the free water on addition of S-kimo (Table II). Although, all four fermented doughs were examined in the liquid-phase analysis, only flour 3 was used here. It may be that the ultracentrifugation is dependent on the rheological properties so that a more viscous dough gives a better phase separation and more liquid phase. A similar result was noted in reference to experiments of Eliasson and Larsson (1996a,b). A further explanation for the effect of S-kimo on the macroscopic water distribution could be the porosity of dough. S-kimo also contains DATEM and ascorbic acid, which promote the formation of small air bubbles in dough after mixing (Junge et al 1981, Abd El-Hady et al 1995).

TABLE IV
Image Analysis of Autoradiographs

Sample	Area (%)			
	Dark Pores		Background	
		±		±
40 min[a] + no S-kimo	40.5	0.5	32.0	2.0
40 min[a] + S-kimo	28.4	1.7	25.0	1.6
25 min[a] + S-kimo	22.9	1.3	26.9	1.3

[a] Prefermentation time.

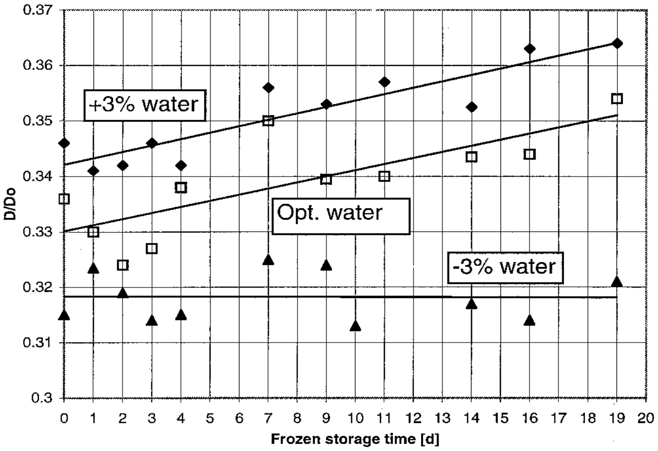

Fig. 8. Self-diffusion of water-flour mixtures (D) with different water contents and frozen storage times. Measured at +40°C (opt. = optimal water content).

The macroscopic water distribution was also dramatically improved by use of a shorter prefermentation time (Fig. 9 and Table IV). The fact that most of the dark areas in the autoradiographs coincided with pores of the dough, is partly explained by the different range of β-energy of tritium in air (5 mm) and in biological material (2 μm). On the other hand, the pores appeared larger in the autoradiographs than they were in the dough, which suggests that water diffuses from the dough matrix to the wall of air bubbles. Gan et al (1990) also report that with scanning electron microscopy (cryo-stage), the surface of gas cells is completely covered with ice crystals. In earlier experiments, we found that, as with additives, the number of small air bubbles was greater at shorter than at longer prefermentation time (Räsänen et al 1995). Thus, shorter prefermentation time resulted in more even water distribution and improved baking quality.

CONCLUSIONS

Frozen storage increased the amount of liquid phase and decreased G' values in water-flour mixtures. These negative effects of ice crystals could be controlled by reducing the water content. Frozen storage also increased the self-diffusion of water-flour mixtures when water content was optimal or higher than optimal. Monitoring of the liquid phase by ultracentrifugation showed that frozen storage had no statistically significant effect on fermented doughs. The phase separation measured by ultracentrifugation was observed to be strongly dependent on the rheological properties of the dough. Thus, ultracentrifugation should not be used to monitor the state of water in fermented doughs. The autoradiographic method proved to be a valuable method for analyzing the changes in the distribution of macroscopical water in fermented doughs. Autoradiography studies showed addition of S-kimo, as well as shorter prefermentation time, resulted in more even water distribution. This result can be explained in terms of the greater amount of small air bubbles and more even pore size distribution. In rheological experiments, frozen storage was consistently observed to decrease dough rigidity, and this kind of change was best withstood with reduced water content. In the case of prefermented frozen doughs, large defor-

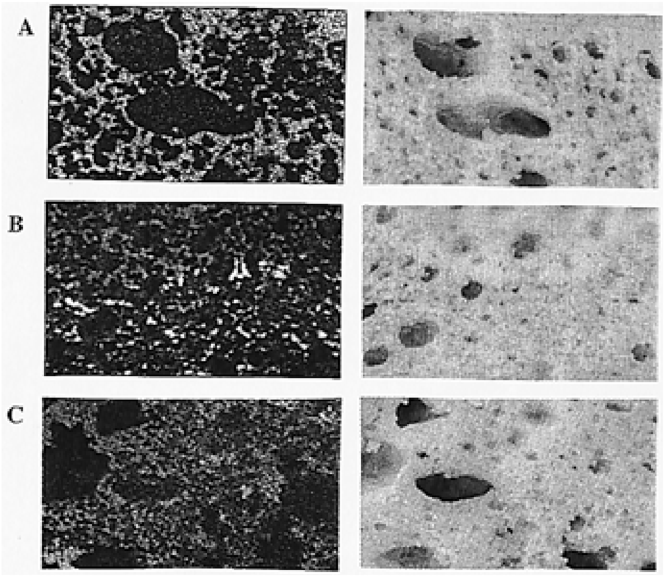

Fig. 9. Effect of prefermentation time and S-kimo as seen in autoradiographs (left) and photographs (right) of dough sections for sample flour 3. **A,** 40-min prefermentation and no S-kimo; **B,** 40-min prefermentation with S-kimo; **C,** 25-min prefermentation with S-kimo.

mation rheology (expansion potential) correlated better with the results of baking tests than did small deformation rheology (oscillation*)*. In all experiments, dough rheology was strongly dependent on the total water content of dough. However, there was no correlation between the amount of liquid phase and total water content.

ACKNOWLEDGMENT

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