

# Differential Scanning Calorimetric Study on the Effects of Frozen Storage on Gluten and Dough

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ABSTRACT

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Water transport from the gluten to the starch paste during frozen storage of a dough was studied by analysis of the differential scanning calorimetry (DSC) peak shape for gluten and dough. The results indicate that in gluten stored at  $-15^{\circ}\text{C}$ , the water content in the gluten phase decreased by  $\approx 1\%$  over the first three weeks. An apparent steady state was attained over this period and the amount of ice did not increase

further. Such changes were not observed for samples stored at  $-25^{\circ}\text{C}$ . Qualitatively similar observations were made for dough stored at either  $-15$  or  $-25^{\circ}\text{C}$ . The greater sensitivity of dough to frozen storage is tentatively attributed to a slightly lower glass transition temperature in dough.

The quality of a bread product baked from a frozen dough decreases with increasing frozen storage time (Slade et al 1989; Inoue and Bushuk 1996; Nemeth et al 1996), and considerable effort has been spent on identifying the causes for this reduced quality. A possible explanation for the quality loss involves the changes in dough rheology as a result of water transport during storage from the hydrated gluten to the ice phase (Bot and de Bruijne 2003), especially at temperatures not far below the glass transition temperature ( $T_g'$ ) of the dough. For example, if a frozen dough is stored well below  $T_g'$ , it is expected to be relatively stable over storage time (Slade et al 1989). Unfortunately however,  $T_g'$  is usually not very much higher than the commercially relevant freezer temperature of  $-18^{\circ}\text{C}$ . Slow relaxation processes in dough could well be possible this near to  $T_g'$ . A potential cause driving relaxation is the increased hydration level of the stretched gluten after kneading compared to relaxed gluten. During storage, some of this hydration water may transport to the ice phase. During baking, the gluten does not rehydrate, and excess water may migrate to the starch paste, thus affecting the yield stress of the paste and compromising the baking performance of the dough (Bot and de Bruijne 2003).

In the present study, differential scanning calorimetry (DSC) was employed to study the effect of frozen storage on the water distribution in gluten and dough. DSC can distinguish between freezable and unfreezable water in a frozen biopolymer system such as hydrated gluten (Slade et al 1989; Slade and Levine 1991). Unfreezable water refers to water in the concentrated gluten phase, freezable water is the ice that separates out during freeze-concentration of the gluten phase. Freezable water is detected by DSC, unfreezable water is not detected. In thermodynamic equilibrium, the amount of freezable water depends on the frozen storage temperature only; the lower the storage temperature, the higher the amount of freezable water. In practical situations, however, equilibrium is not attained in concentrated gluten phases within realistic time periods. After rapid freezing, not only is a nonequilibrium system obtained below  $T_g'$ , but also in a considerable temperature range above this transition (Slade and Levine 1988).

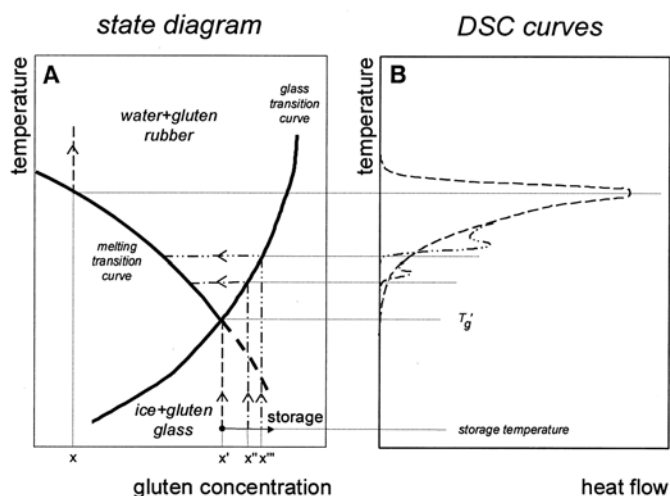
The nonequilibrium nature of the frozen system allows the possibility that small changes occur during frozen storage due to enthalpic relaxation, albeit at extremely slow rates (Slade and Levine 1988). This is illustrated in the state diagram in Fig. 1A. For example, transport of water from the concentrated gluten phase

initially at  $x'$  to the ice crystals can take place potentially during frozen storage. When the system is kept at freezer temperatures for a long period, it may crawl along the freezer iso-temperature line as a result of enthalpic relaxation. During this slow freeze-concentration process, the system may pass through the compositions  $x''$ ,  $x'''$ , etc. In practice, the rate of this process slows down tremendously when freezer temperature drops much below  $T_g'$ .

Consider the behavior of the three systems at concentrations  $x'$ ,  $x''$ , and  $x'''$  introduced above during a DSC heating experiment (Fig. 1B). All systems initially follow an iso-concentration path up to their respective  $T_g'$ , which increases with  $x'$ ,  $x''$ , and  $x'''$ , during which no ice melts.

The freshly frozen system, for which the freeze-concentrated gluten is still at  $x'$  at the start of the DSC experiment, shows a smooth DSC trace that reflects the melting of the ice crystals in the viscous concentrated gluten phase (Fig. 1B, dashed curve). Melting continues until all ice has disappeared, and the composition of the concentrated gluten phase is  $x$ . The DSC curve returns to the base line, and the system follows an iso-concentration path through the state diagram until the final temperature is reached.

The second system was stored for a considerable period, and the concentration of the concentrated gluten phase increased during storage to  $x''$  (dash-dotted curve). At the glass transition  $T_g(x'')$ , an excess amount of ice crystals suddenly melts, and the system jumps along an isotherm from the  $T_g'$  curve to a melting curve that is very close to that of the fresh system. As a result, an enthalpic



**Fig. 1.** State diagram and differential scanning calorimetry (DSC) curves for gluten and dough. Distances have been exaggerated for clarity. Melting of ice crystals in the viscous concentrated gluten phase:  $x'$  (---);  $x''$  (- · - · -);  $x'''$  (- · · - · -).

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relaxation peak in the DSC spectrum appears above  $T_g(x'')$ . When the system has joined the melting curve, the rest of its trajectory is almost identical to that of the freshly frozen sample.

The third system was stored for an even longer period, and the concentration of the concentrated gluten phase increased even further to  $x'''$  (dash-dot curve). Qualitatively, the path through the state diagram is the same as for the second system. However, the enthalpic relaxation peak in the DSC spectrum appears at still higher temperature because  $T_g(x''') > T_g(x'')$  and is more pronounced.

To first-order approximation, the enthalpic relaxation peak only involves a change in the shape of the DSC curve and not an increase in the area below the curve, because the factor by which the amount of freezable water increases when the composition of the concentrated gluten phase changes from  $x'$  to  $x'''$ ,  $(x'/x''') \cdot (x''' - x)/(x'' - x)$  [ $= 1 + (x''' - x'')/(x'' - x) \cdot (x/x''')$ ] will be very close to unity. The increase in DSC peak area will be discernible only through a very accurate determination of the peak area.

The present study was intended to use the technique described above to estimate the amount of water that is released from gluten and dough during frozen storage at  $-15$  and  $-25^\circ\text{C}$ .

### MATERIALS AND METHODS

The effect of frozen storage on the water distribution in gluten was investigated using a commercial preparation (Sigma, St. Louis, MO). Samples were prepared at two different nominal gluten concentrations, 33.0 and 27.1% by weight. The drier sample containing 33.0% gluten was prepared by kneading the gluten manually, the wetter sample containing 27.1% gluten was prepared by kneading using a farinograph (300-g mixer, 70 min at 300 BU).

Water redistribution during frozen storage of dough was studied using a commercial wheat flour (Ibis wheat flour, Meneba, 15.2% protein, 15.3% moisture, 0.55% ash, Falling Number 308). The dough was prepared using 50 g of flour, 30 g of demineralized water, and 1 g of salt. No yeast was added. The dough was kneaded at  $20^\circ\text{C}$  for  $\approx 12$  min; slightly beyond the maximum torque in a farinograph using a small mixing volume.

The samples were loaded in an aluminum pan (0219-0062, Perkin Elmer) and filled with 5–20 mg of either dough or gluten,

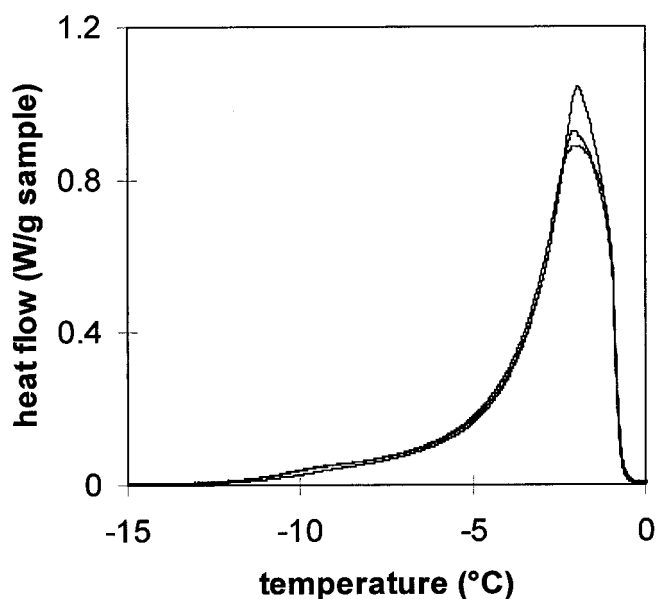
and the reference consisted of an empty pan. The pans were sealed using a standard crimper press. The samples were stored for the appropriate time in a freezer at either  $-25 \pm 1^\circ\text{C}$  or  $-14.5 \pm 1^\circ\text{C}$ . The higher temperature was chosen to mimic storage conditions in a regular  $-18^\circ\text{C}$  freezer, taking into account that the actual freezer temperature is often a few degrees higher than indicated. The samples were stored in a plastic bag to avoid condensation of ice on the pans.

Samples were removed from the freezer one at a time over the two-month storage time. A special container was prepared from a small plastic tube to avoid condensation of water on the cold sample pans during transport from the freezer to the DSC instrument ( $\approx 10$  m). The tube had the right circumference to hold the samples, and air flow through the tube could be reduced by placing small pieces of cotton wool at the bottom and at the top of the tube. The container was put in a 50-mL plastic box filled with cotton during transport from freezer to DSC instrument. The container was fitted through the air lock connecting the ambient atmosphere to the nitrogen atmosphere box around the DSC instrument. In this way, contact between the samples and the ambient atmosphere was reduced as much as possible.

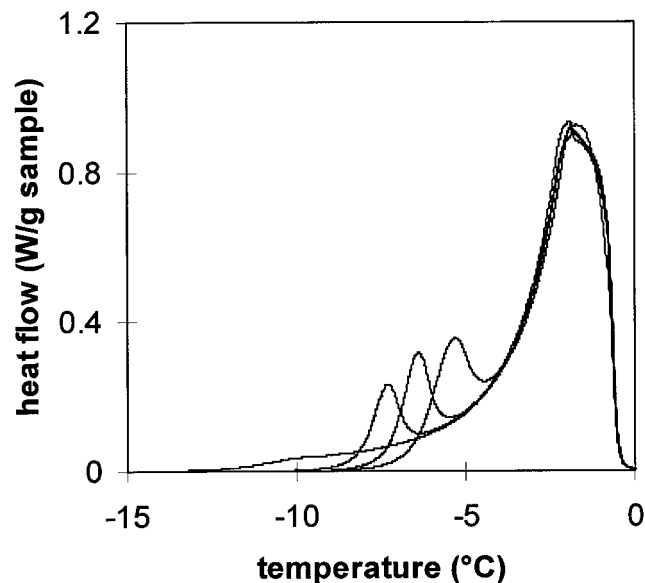
The experiments were performed using a thermal analysis system (1020 Series DSC 7, Perkin Elmer). This instrument allows experimentation under a dry nitrogen atmosphere, avoiding condensation of water vapor on the sample during the experiment.

The DSC instrument was set to the storage temperature of the frozen sample at least 5 min before loading the sample to avoid any annealing of the sample before measurement. The samples were given 15 min to equilibrate after transport from the freezer to the DSC instrument. The scanning range was chosen from the frozen storage temperature to  $+10^\circ\text{C}$ . The heating rate was chosen as  $1^\circ\text{C}/\text{min}$ , which is a compromise between short duration of the experiment and sharp features of the DSC peak shape.

The accuracy of the calibration of the DSC instrument is insufficient to guarantee an absolute temperature scale. As a result, all experimental curves are shifted with respect to each other. As our interest concerned primarily peak area and peak shape, the experimental curves were shifted to achieve superposition near the melting point, using the water curve as a reference.



**Fig. 2.** Effect of frozen storage of hydrated gluten at  $-25^\circ\text{C}$  after 4 hr, 9 days, and 56 days for samples containing 33.0% gluten. Horizontal axis represents relative temperatures.



**Fig. 3.** Effect of frozen storage of hydrated gluten at  $-15^\circ\text{C}$  after 4 hr and 6 days, 14 days, and 56 days for samples containing 33.0% gluten. Enthalpic relaxation peak in the low-temperature tail of differential scanning calorimetry (DSC) curve shifts upward in temperature with increasing frozen storage time. Horizontal axis represents relative temperatures.

## RESULTS AND DISCUSSION

### Pure Water

The shape of the DSC curve for pure water was almost symmetrical and had a full width at half maximum of  $\approx 2.3^\circ\text{C}$ . This curve was considered to be the instrumental profile at the current heating rate.

The accuracy of the DSC method to determine the melting enthalpy  $\Delta H$  was tested by a number of experiments on samples containing different amounts of pure demineralized water. The maximal difference between the literature value of 333.5 J/g (Lide 1962) and the present experimental value is  $\approx 2\%$ . It was assumed that the melting enthalpy of ice always has the same value and depends neither on biopolymer content of the gluten phase nor on depressed melting temperature. This assumption was held for an acceptable first-order approximation, and deviations from this assumption are discussed elsewhere (Ablett et al 1992; Johari and Sartor 1996; Sartor and Johari 1997).

### Effect of Frozen Storage on Gluten

DSC curves for gluten samples were much more asymmetric than the curve for water. Their shape reflects approximately the freezing point depression curve of water as a function of biopolymer content (Chen 1985), except near and below  $T_g'$ . The curve shows that the freezing point depression curve decreased much faster at low water content than at high water content,

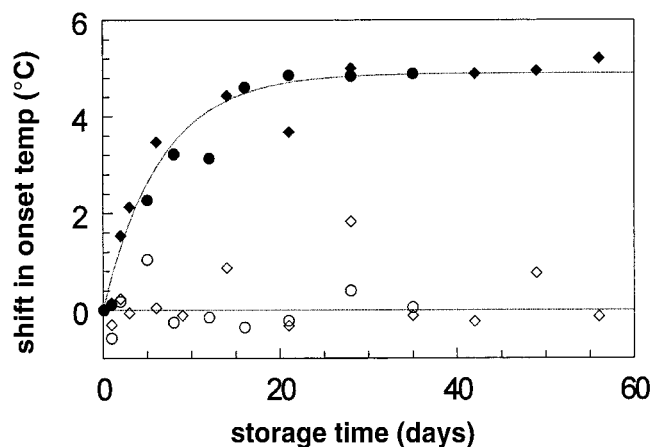


Fig. 4. Shift of onset temperature of enthalpic relaxation peak in gluten as a function of frozen storage period. Gluten samples 33.0% (●, ○), 27.1% (◆, ◇). Samples stored at  $-15^\circ\text{C}$  (filled symbols), samples stored at  $-25^\circ\text{C}$  (open symbols).

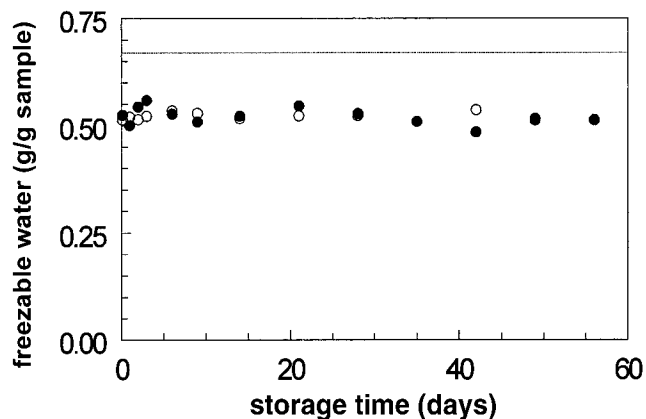


Fig. 5. Effect of storage period at either  $-15^\circ\text{C}$  (○) or  $-25^\circ\text{C}$  (●) on the amount of freezable water in gluten as derived from differential scanning calorimetry (DSC) peak area for 33.0% gluten samples. Line indicates nominal amount of water in gluten.

implying that much less ice could melt at low temperatures than at high temperatures.

Figure 2 shows that the DSC curves for different storage periods at  $-25^\circ\text{C}$  did not differ much after background subtraction. There was no trend in either peak area or peak shape, except the appearance of a small feature near  $T_g'$  at approximately  $-10^\circ\text{C}$ , which is consistent with reported literature values for  $T_g'$  in the range  $-5 < T_g'/^\circ\text{C} < -10$  (Slade et al 1989).

A completely different result was obtained for samples stored at  $-15^\circ\text{C}$  (Fig. 3). Under these conditions, an enthalpic relaxation peak developed in the low-temperature tail of the DSC curve, which shifted upward in temperature with increasing frozen storage time. The relaxation peak seems to have developed because the  $T_g'$  of the sample shifted considerably upward during storage, in line with the mechanism outlined above. One way to quantify this enthalpic relaxation peak is by monitoring its onset temperature. There are various ways to determine the onset temperature, but for the present purpose, it seemed the most convenient to draw a tangent through the inflexion point in the low-temperature wing of the enthalpic relaxation peak, and determine its intersection with the base line. The increase of the onset temperature with frozen storage time was determined relative to the onset temperature of a sample that was stored for only a few hours to avoid any problems with the accuracy of the temperature calibration of the DSC instrument (Fig. 4). The shift in the onset temperature could be reinterpreted semiquantitatively in terms of a freeze-concentration process in the gluten phase from  $x'$  to  $x''$  using the slope  $dT_g(x)/dx$  of the glass transition curve for glutenin near  $T_g'$ , which was reported to be  $\approx 4.9 \cdot 10^2$   $^\circ\text{C}$  (Kokini et al 1994). This suggested that the  $\approx 5^\circ\text{C}$  shift in onset temperature represented an increase of the gluten concentration in the gluten phase by  $\approx 1\%$  over the first three weeks of storage at  $-15^\circ\text{C}$ .

A clear shift in onset temperature of the enthalpic relaxation peak was observed for samples stored at  $-15^\circ\text{C}$ , whereas little changed for samples stored at  $-25^\circ\text{C}$  (Fig. 4). The nominal amount of water in the systems seemed to have little influence. Within experimental accuracy, there was no difference in the shifting rate of the onset temperature with frozen storage time between the drier and wetter samples. The DSC peak area was constant within experimental accuracy as a function of storage period (Fig. 5). The results for pure water and the DSC data implied that the increase in amount of freezable water is  $< 0.02$  g/g of sample. This is consistent with the semiquantitative interpretation of Fig. 4, which indicated that, even at  $-15^\circ\text{C}$ , the increase of the DSC peak area is expected to be only  $\approx 1\%$ . This implies that the changes due to freeze-concentration could not be extracted from the peak area under the study conditions. Clearly, the peak shape is much more sensitive.

### Effect of Frozen Storage on Dough

An advantage of the experiments on dough over those on gluten was the clear freezing point depression of the water due to the presence of salt in the system,  $\Delta T = -1.6^\circ\text{C}$  for 1.0 g of NaCl in 30 g of water. This led to a separation of the DSC signal originating from ice in the dough and ice that condensed elsewhere (e.g., in the headspace on the inside of the sample pan). The condensate is pure ice, which does not show any freezing point depression. The condensate contributed a very small peak in the DSC curve at exactly  $0^\circ\text{C}$ , which could be used as an internal calibration. Therefore, the temperature scale for the experimental results (Figs. 6 and 7) can be considered correct in an absolute sense. This differs from the gluten experiments, where the temperature scale was shifted.

Figures 6 and 7 show the effect of frozen storage on the shape of the DSC curves. In contrast to the results for pure gluten, an enthalpic relaxation peak developed at both storage temperatures for dough. However, the effect of frozen storage on dough was less pronounced than for gluten. Furthermore, there was much more scatter in the data for dough than for gluten.

The scatter in the data is also apparent from the onset temperature of the enthalpic rearrangement peak (Fig. 8). Some of the samples that were stored for several weeks showed an onset temperature and DSC peak shape that is characteristic of a freshly frozen sample. This is probably because dough is more heterogeneous than gluten, which may lead to considerable sample-to-sample variation where only tiny amounts of sample were introduced in the DSC sample pans.

Nevertheless, Fig. 8 shows that higher onset temperatures were achieved faster for storage at  $-15^{\circ}\text{C}$  than for storage at  $-25^{\circ}\text{C}$ . The guiding lines represent our bias toward the effect of frozen storage on dough.

Figure 9 displays the amount of freezable water in the dough as a function of storage time. There is a slight tendency toward an increase in the amount of freezable water with frozen storage time (dashed line fit through all data points). Over a period of 40 days, the amount of freezable water went up from  $\approx 0.255$  to  $0.270$  g/g of sample. At  $-15^{\circ}\text{C}$ , the increase was slightly higher; at  $-25^{\circ}\text{C}$ , the increase was slightly lower.

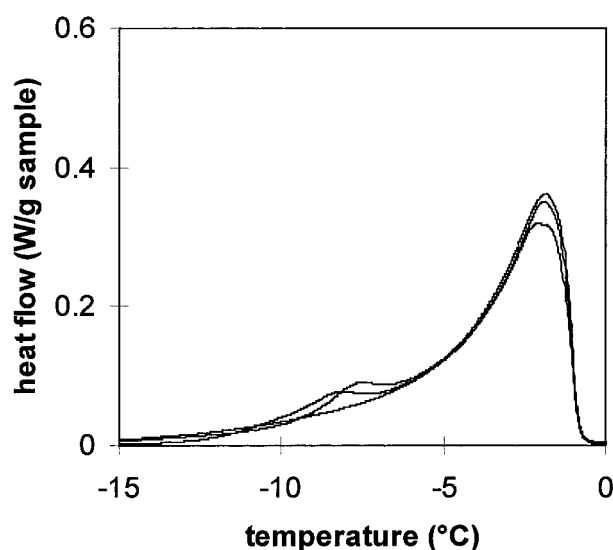


Fig. 6. Effect of frozen storage on dough without yeast stored at  $-25^{\circ}\text{C}$  after 2 hr, 7 days, and 23 days. Enthalpic relaxation peak in the low-temperature tail of differential scanning calorimetry (DSC) curve shifts upward in temperature with increasing frozen storage time.

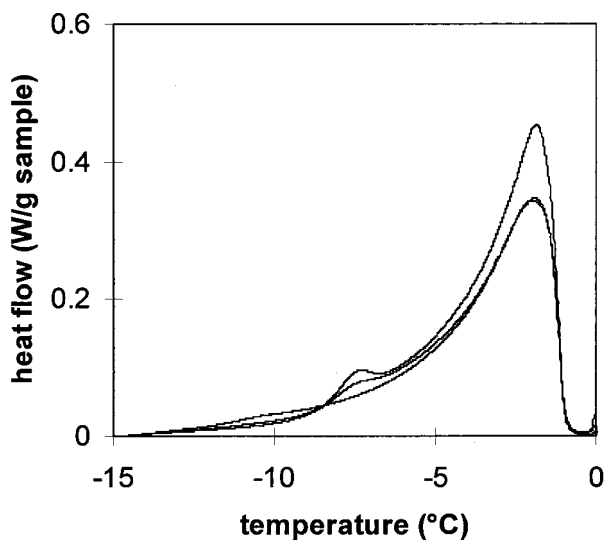


Fig. 7. Effect of frozen storage on dough without yeast stored at  $-15^{\circ}\text{C}$  after 2, 3, and 11 days. Enthalpic relaxation peak in the low-temperature tail of differential scanning calorimetry (DSC) curve shifts upward in temperature with increasing frozen storage time.

The magnitude of the effect for dough clearly suggests that, for dough, not only water from the gluten phase is involved in water redistribution because 1 g of dough contains only  $\pm 0.1$  g of gluten; the increase of free water from the gluten phase during frozen storage should be much smaller than in Fig. 5. This amount is far too small to be observed in Fig. 9, especially for storage at  $-25^{\circ}\text{C}$ . This also suggests that dehydration of the starch phase occurred, which might explain why the qualitative difference between the curves for  $-15$  and  $-25^{\circ}\text{C}$  frozen storage is much smaller for dough than for gluten.

A quantitative evaluation similar to the one for gluten would be desirable for the enthalpic relaxation processes in dough. However, at present, data on the  $T_g'$  curve and melting point depression curve are lacking. The data in Fig. 8 suggested that  $T_g'$  was lower in dough (in line with results obtained by Slade et al [1989]), and that the transition between rubbery and glassy state was much smoother than in gluten, mainly because the effect of frozen storage at lower temperature was less dramatic than for gluten. Furthermore, the data suggest that additional dehydration of the starch phase occurred. Otherwise, the magnitude of the change in peak area in Fig. 9 cannot be explained satisfactorily. This might also explain why the qualitative difference between the curves for  $-15$  and  $-25^{\circ}\text{C}$  frozen storage was much smaller for dough than for gluten.

### CONCLUSIONS

The present results indicate that DSC is a valuable technique for following the effect of frozen storage on gluten and dough.

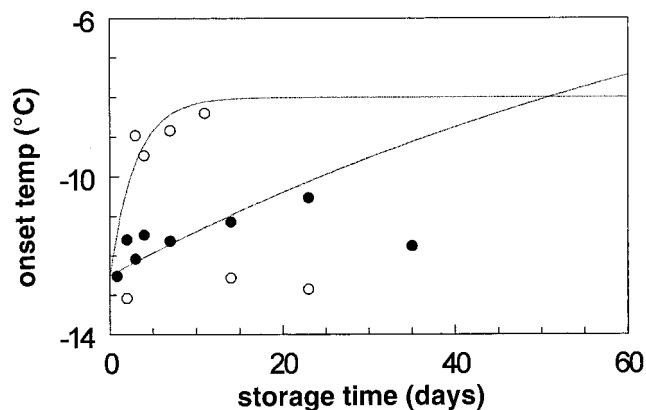


Fig. 8. Onset temperature of enthalpic relaxation peak as a function of frozen storage period. Samples stored at  $-15^{\circ}\text{C}$  ( $\circ$ ) and  $-25^{\circ}\text{C}$  ( $\bullet$ ). Lines represent expected trends in the data.

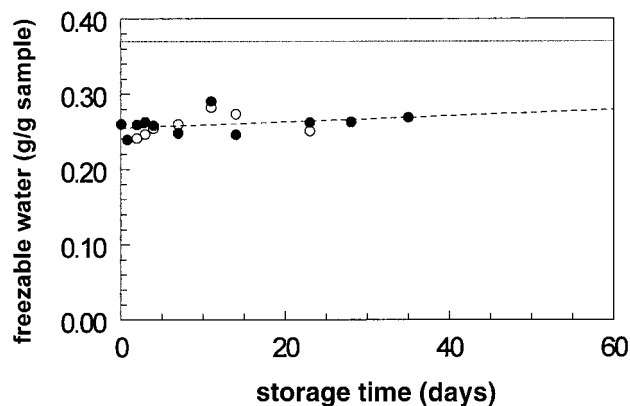


Fig. 9. Effect of storage period at either  $-15^{\circ}\text{C}$  ( $\circ$ ) or  $-25^{\circ}\text{C}$  ( $\bullet$ ) on amount of freezable water in dough derived from differential scanning calorimetry (DSC) peak area. Lines represent a linear fit through all data points and the nominal water concentration in the dough.

The current use of DSC differs from earlier applications to frozen doughs where the technique was insufficiently accurate to detect  $T_g'$  (Laaksonen and Roos 2000).

For gluten, the present results enabled a quantitative analysis of the data. This analysis showed that the shape of the DSC peak is a much more sensitive probe of the freeze-concentration process than the peak area. By taking larger samples, however, it should be possible to achieve sufficient sensitivity to use the DSC peak area also to monitor the progress of the freeze-concentration process.

The results indicated that in gluten stored at  $-15^\circ\text{C}$ , the gluten concentration in the gluten phase increases by  $\approx 1\%$  over the first three weeks. No such change is observed for samples stored at  $-25^\circ\text{C}$ . This result confirms the suggestion that the effect of frozen storage for pure gluten can be suppressed rather straightforwardly by decreasing the storage temperature.

The analysis of the experiments on dough was more qualitative than for gluten because the situation was complicated by the presence of a starch phase that also seems to lose water during storage. However, it was clear that, qualitatively, the same changes occur in dough stored at either  $-15$  or  $-25^\circ\text{C}$  as in gluten at  $-15^\circ\text{C}$ . The fact that changes occurred at lower storage temperature for dough than for gluten seemed consistent with the lower  $T_g'$  for dough.

The results indicate that the present technique is a promising method to study freeze damage in dough. There are basically two routes that will have to be explored further. First, to determine whether the starch phase can indeed lose water during storage by studying the behavior of frozen starch dispersions. Second, to increase the sensitivity of the method using two aspects: 1) larger samples, which may help to determine the DSC peak accurately enough to use it as a measure for the total amount of freezable water in the dough; 2) more homogeneous samples, which may reduce the sample-to-sample variation in the DSC experiments. The latter variability, however, may be intrinsic to dough and unavoidable.

Such an approach, supplemented with other promising techniques like cross-relaxation NMR (Esselink et al 2003) and biaxial compression tests (Nicolas et al 2003), will help to identify the mechanism causing freeze damage to dough during frozen storage.

#### ACKNOWLEDGMENTS

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