

The Role of Water in the Retrogradation of Wheat Starch Gels and Bread Crumb¹

K. J. ZELEZNAK and R. C. HOSENEY²

ABSTRACT

Cereal Chem. 63(5):407-411

The moisture content of a starch gel determines the extent to which that starch will retrograde. Maximum crystallinity occurs in gels of 50-60% starch. In this study, the effects on recrystallization of adjusting the water level during gelatinization and aging were examined. The degree of recrystallization was measured by differential scanning calorimetry. Retrogradation in wheat starch gels was controlled by the amount of water present during aging, regardless of the amount present during gelatinization. It was also found that solubilized amylopectin did retrograde, and as with starch, the extent of retrogradation was controlled by the amount of water present. Because the amylopectin used was not in

granular form, we concluded that the remnant of the granule structure did not affect retrogradation. Retrogradation in bread was also studied by differential scanning calorimetry. Bread baked with several antistaling agents was aged at various moisture levels to determine if these agents act by altering moisture availability to the starch and, thereby, the extent of starch retrogradation in the bread. The effect of moisture on recrystallization was not significantly different between the bread samples and the starch gels. It appears, therefore, that the action of these antistaling agents in bread is brought about by another mechanism.

The involvement of starch retrogradation in bread staling has been a major focus of research since Katz (1928) showed by X-ray diffraction that starch in bread returns to the semicrystalline state during staling. There is extensive work on the physical and chemical changes that occur in starch during baking and staling (Willhoft 1973, Maga 1975, Knightly 1977, Kulp and Ponte 1981). The semiquantitative method of X-ray diffraction was widely used as the most direct means of estimating the degree of recrystallization in starch (Zobel and Senti 1959, Dragsdorf and Varriano-Marston 1982), but the more quantitative thermal analysis techniques are now the preferred means of studying crystallinity. Differential scanning calorimetry (DSC) in particular has proved to be a valuable tool to quantify crystallinity, both in native starch and the retrograded starch of bread crumb and aged gels (Longton and LeGrys 1981, Fearn and Russell 1982). It enables us to directly measure the influence of various factors on the thermal properties of starch during gelatinization and recrystallization. Thermal analysis shows that storage time, storage temperature, and reheating after storage, which are known to influence the firming rate of bread, also affect the rate of starch retrogradation (Axford and Colwell 1967, Colwell et al 1969, Russell 1983, Ghiasi et al 1984). Longton and LeGrys (1981) reported that recrystallization in starch gels is profoundly influenced by gel moisture. Using DSC enthalpy values as the index of crystallinity in aged gels, they noted that crystallinity reached maximum development in 50% gels and disappeared altogether in very dilute (10%) or concentrated (80%) gels. This DSC data supports the X-ray diffraction studies of Hellman et al (1954), who found that 50% gels produce the most intense X-ray pattern but that pattern intensity decreases at higher or lower concentrations. These studies indicate that water plays an integral role in controlling retrogradation, and its involvement in the process bears closer scrutiny.

The goal of this study was to investigate how and when moisture content becomes influential in retrogradation. Does the amount of water present during granule swelling and gelatinization influence subsequent recrystallization? Or does water exert its effect only by mediating the formation and growth of crystallites during retrogradation? A starch model system was chosen for the main portion of this study, but the influence of water on retrogradation of starch in bread was also examined. DSC was used because it provides a reproducible means to quantify crystallinity in both starch gels and bread.

MATERIALS AND METHODS

The effect of moisture on the retrogradation in bread crumb, starch gels, and isolated amylopectin was examined by DSC. The instrument used was a Perkin-Elmer DSC-2 with FTS Systems Flexi-cooler and temperature controller. It was calibrated with indium, and enthalpy (ΔH) was determined by measuring the area of the endotherm, as described by Lund (1983), using a polar planimeter. In the calorimeter the temperature sensors are not in direct contact with the sample; therefore, the recorded temperature is that of the sample holders rather than the sample. The effect of this temperature lag can be corrected for, using the procedure described by Richardson (1972).

All samples were encapsulated in aluminum pans and hermetically sealed to prevent moisture loss. They were heated at 10° C/min, using a sensitivity of 0.5 mcal/sec. The temperature range of the scan was typically 7-127° C, but the upper limit was adjusted according to the temperature necessary to allow gelatinization to be completed. All materials (starch, crumb, and water) were weighed directly into the calorimeter sample pan using a Cahn 21 automatic electrobalance. Enthalpy, the energy required to melt the crystalline material, was used as the index of crystallinity in the samples. It is reported as ΔH in calories per gram. All samples were run in duplicate or more. The standard deviation of ΔH values was 0.06 cal/g.

Preparation of Bread Crumb

The effect of sample moisture content on enthalpy values during DSC heating was studied on aged bread crumb. Bread without shortening was baked according to the standard pup loaf procedure (Finney 1984). It was aged for five days in plastic bags at 25° C, the crust was removed, and the crumb was frozen and lyophilized. The dried crumb was weighed into the calorimeter sample pan, and distilled water was added with a microsyringe to obtain 56, 39, 30, and 25% starch (determined by weighing pan plus sample) in the final mixture. The samples were equilibrated for 1-2 hr before heating in the calorimeter. Enthalpy calculations were based on the weight of starch (in milligrams) in the crumb sample. Crumb was estimated to contain approximately 65% starch.

DSC was also performed on bread to study the combined effect of moisture and various antistaling agents on starch retrogradation. Bread was baked with each of the following: 3% shortening (Crisco or Durkee D-10) and 0.5% monoglyceride (Dur-Em, Durkee). The control loaf contained none of these. Once cooled, the crumb was promptly frozen and lyophilized. A small sample (~5.0 mg) of lyophilized, fresh crumb was weighed into a calorimeter pan, and the desired moistures (15, 20, 30, 40, 50, 60, and 70%) were obtained as described above. All samples were prepared in duplicate, stored for 10 days at 25° C in the sealed calorimeter pans, and scanned. Both the concentration and

¹Contribution 86-203J, Kansas Agricultural Experiment Station, Manhattan 66506.

²Research associate and professor, Department of Grain Science and Industry, Kansas State University, Manhattan.

enthalpy calculations were based on the estimated weight of starch in the crumb.

Preparation of Starch Gels

Wheat starch (Midwest Solvent, Atchison, KS, 11.3% moisture) was weighed directly into aluminum sample pans. Water was added using a microsyringe, and the desired concentration was obtained by weighing the pan plus the starch/water suspension. Three series of samples were prepared in this manner. The protocol for post-gelatinization moisture adjustment in each of the series is described below. After aging, the effects of the moisture adjustments on the DSC thermogram of each gel of the three series were compared.

In the first gel series, each suspension was gelatinized and aged at the same moisture level. These suspensions of 80, 75, 70, 60, 50, 40, 30, and 20% starch were heated from 7 to 100 °C in the calorimeter. The more concentrated samples (60–80%) were not completely gelatinized at 100 °C; therefore, they were heated to the temperature of the completion of the endothermic transition. After this initial heating, the gels were cooled, stored at 25 °C for seven days and heated once again in the calorimeter from 7 to 127 °C to observe the melting of the recrystallized starch. All gels were heated, aged, and reheated in this manner.

In the second series of gels, the amount of moisture present in each sample during the initial heating (gelatinization) was 50% but was adjusted to five levels (70, 60, 50, 40, and 30% starch) for the seven-day aging period. The water content of the gels was changed by opening the sealed pan, lifting out the gel intact, and transferring it to a new, tared pan. Water was then added or allowed to evaporate until the desired concentration was obtained. The pans then were sealed and aged.

This procedure was reversed to prepare the third series of gels.

Samples of 60, 40, and 20% starch content were heated through gelatinization and then adjusted to the same moisture level (50%) before aging.

Preparation of Amylopectin

Amylopectin was isolated from starch according to the procedure of Schoch (1957). This solubilized amylopectin was examined microscopically under polarized light and in the calorimeter with excess water to determine if any crystallinity remained. There were no recognizable granules, birefringence, or phase transition to indicate the presence of crystallinity. Two mixtures of amylopectin and water were prepared at 30 and 50% solids. They were aged at 25 °C for 14 days and examined by DSC.

RESULTS AND DISCUSSION

A valid interpretation of the results of this study must be based on the prior knowledge that DSC accurately measures the degree of crystallinity in a given sample by recording the energy required to melt the crystals. A concern at the outset of this study was whether the amount of water present during melting of the retrograded starch in the calorimeter could alter the resulting enthalpy. In other words, when water is limited, will crystallite melting be complete and thus give an enthalpy representing the total crystallinity? To answer this question, a lyophilized crumb sample was prepared at several water-to-crumb ratios that were scanned in the calorimeter. It was found that, although endotherm shape was affected by moisture content, the enthalpy of melting was not significantly altered (Fig. 1). The same experiment was performed on aged starch gels at low water levels. Mixtures of 50, 60, 70, and 75% starch content were gelatinized by DSC and aged for seven days, at which time water was added in excess. The DSC scans showed that the enthalpy values of these gels aged in limited

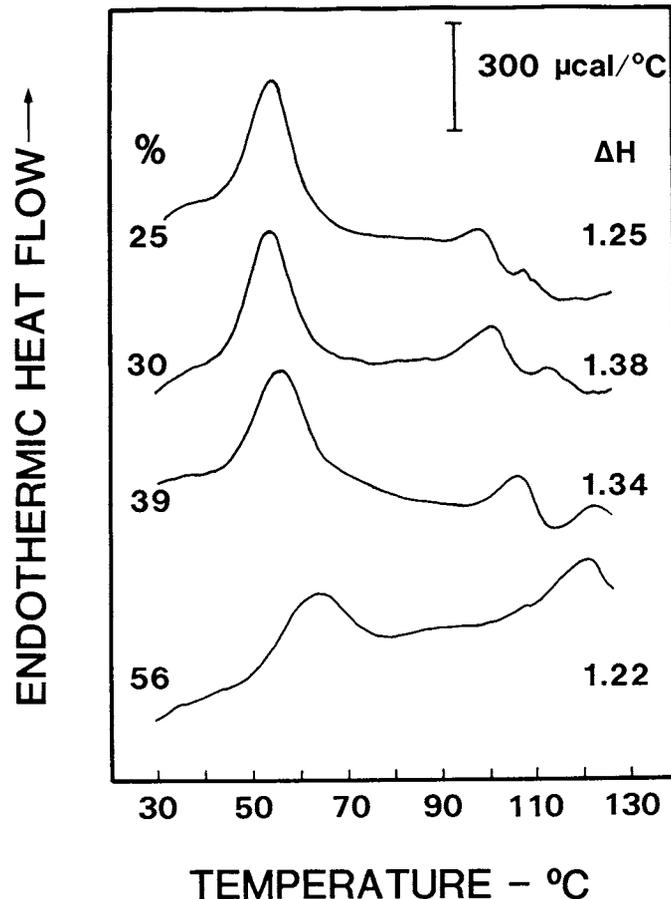


Fig. 1. Differential scanning calorimetry thermograms of bread crumb aged five days at 25 °C, lyophilized, and heated in the calorimeter at several moisture levels, reported as percent starch. Enthalpy (ΔH) of the starch recrystallization peak is in calories per gram of starch.

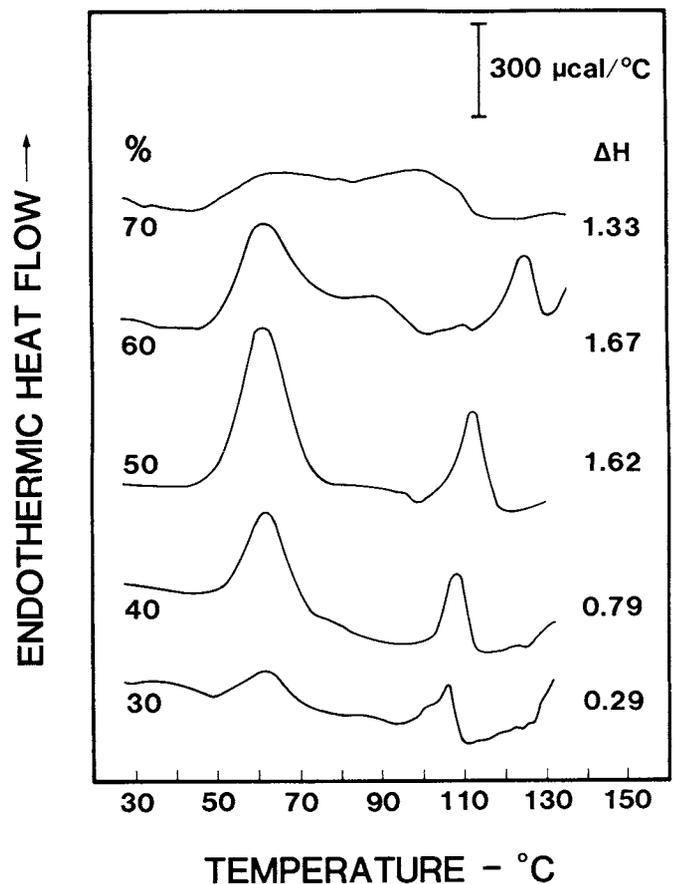


Fig. 2. Differential scanning calorimetry thermograms of aged starch gels in which the concentration (percent starch) of each was maintained at one level throughout the first heating, aging, and second heating in the calorimeter. ΔH is in calories per gram of starch.

water did not increase in the presence of excess water. This evidence was convincing that enthalpy values obtained at the levels of moisture used in this study are an accurate measure of crystallinity. Therefore, the differences in enthalpy could be interpreted as resulting from variability in the gelatinization moisture or the aging-period moisture of the sample.

Aging of Wheat Starch Gels

Three series of starch gels were examined to determine the effect of moisture during gelatinization and aging on recrystallization. The energy required to melt the retrograded crystals, ΔH , was calculated from the DSC thermograms and compared. In the first series, samples at eight concentrations were gelatinized and aged at the same moisture level. The endotherms obtained from heating the aged gels (Fig. 2) indicated that the enthalpy values and the shape of the peak changed as water level changed. There was little crystallinity present in the dilute or very concentrated gels. The ΔH values were greatest in the 50–60% starch gels. Figure 3 shows the bell-shaped response of enthalpy to moisture of the gels. These results are similar to those of Longton and LeGrys (1981). In order to identify whether this observed effect was because of the water present during gelatinization or during aging, two additional series were examined.

In the second series, each suspension of starch and water was gelatinized at a starch concentration of 50% but then adjusted to five different levels for aging. The thermograms (Fig. 4) obtained after aging showed enthalpy values and peak shapes to be essentially identical to those obtained in the first series of gels, despite differences in gelatinization moisture. The moisture present during aging was the same in both treatments and thus seemed to be the factor determining the enthalpy. One further step was necessary to clarify whether variation in gelatinization moisture was contributing to differences in enthalpy. This was accomplished in the third series.

In this series, the starch and water suspensions were gelatinized

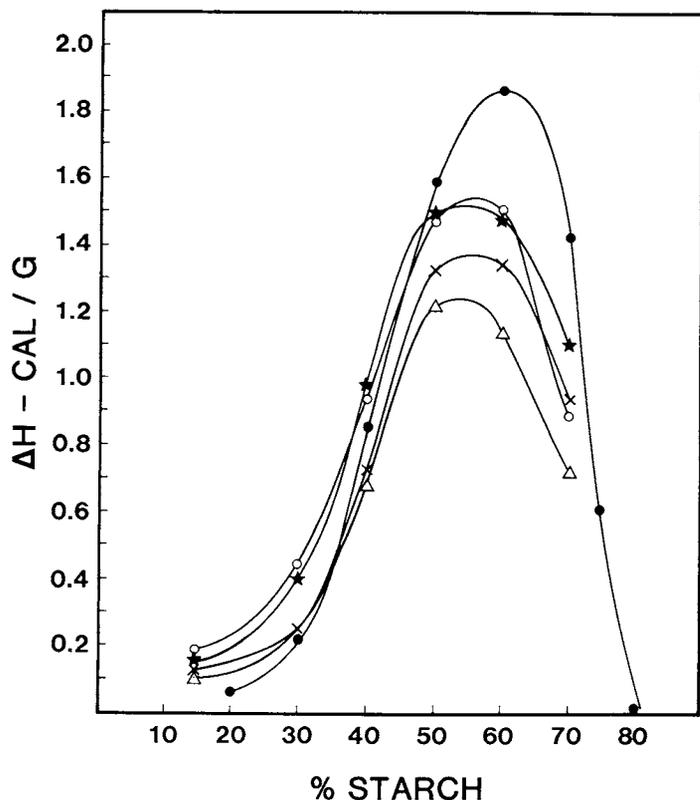


Fig. 3. Effect of the moisture present during aging on the enthalpy (ΔH in calories per gram of starch) of retrograded starch in starch gels and in bread baked with antistaling agents. Starch gels (●—●), control bread (★—★), bread with Crisco shortening (○—○), bread with Durkee D-10 shortening (Δ — Δ), bread with Durkee, Dur-em monoglyceride (×—×).

at three concentrations but were brought to one level (50%) before aging. The second heating of these aged gels produced endotherms (Fig. 5) similar to all previous 50% gels, despite the differences in gelatinization moisture. Using all the data from the three series, the effect on enthalpy of aging-period moisture resulted in a bell-shaped curve (Fig. 3). This curve clearly illustrates that enthalpy is strongly dependent on moisture during aging only. This study confirms that the magnitude of the amylopectin melting peak, i.e., its crystallinity, is controlled by the water present during retrogradation and that gelatinization moisture has little or no effect.

Aging of Amylopectin

The concentration of a starch and water suspension during gelatinization does not control the degree of recrystallization, but it may still exert some influence through an effect on granule remnant structure. Thus, the possible role of granule structure in this water-mediated control of recrystallization was examined further using solubilized amylopectin, in which granule structure had been destroyed. The amylopectin exhibited no crystallinity after solubilization. However, after aging for 14 days, at 50% and 70% water, both had recrystallized. The 50% amylopectin peak clearly had a much larger melting enthalpy (Fig. 6) than the sample in 70% water. Both occurred at a temperature typical of granular starch. Several conclusions may be drawn from these findings:

- 1) Amylopectin will retrograde when not in the granular form.
- 2) The major endotherm observed in both bread crumb and starch gels does represent the melting of retrograded amylopectin.
- 3) The retrogradation is controlled by the water present during aging.
- 4) The remnant of the granule has no influence on retrogradation.

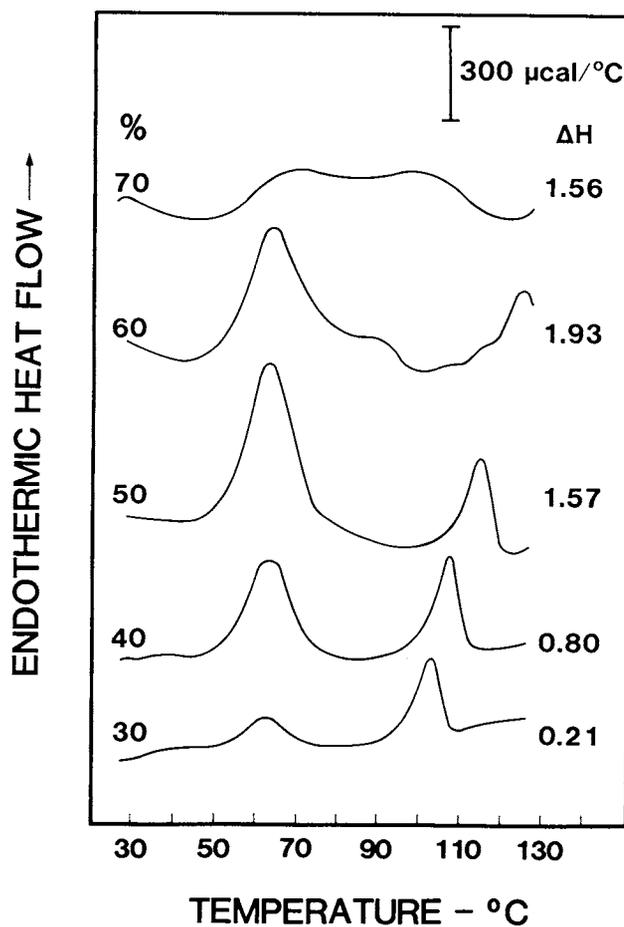


Fig. 4. Differential scanning calorimetry thermograms of aged starch gels in which the concentration at gelatinization was 50% but adjusted to 30, 40, 60, and 70% starch before aging. ΔH is in calories per gram of starch.

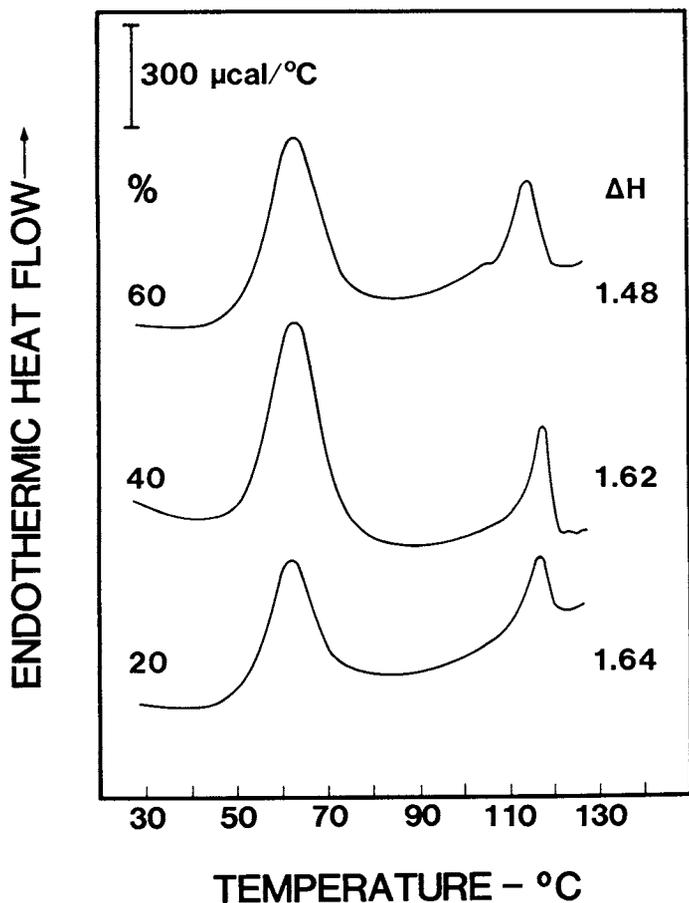


Fig. 5. Differential scanning calorimetry thermograms of aged starch gels where the concentration at gelatinization was 60, 40, and 20% starch, but adjusted to 50% before aging. ΔH is in calories per gram of starch.

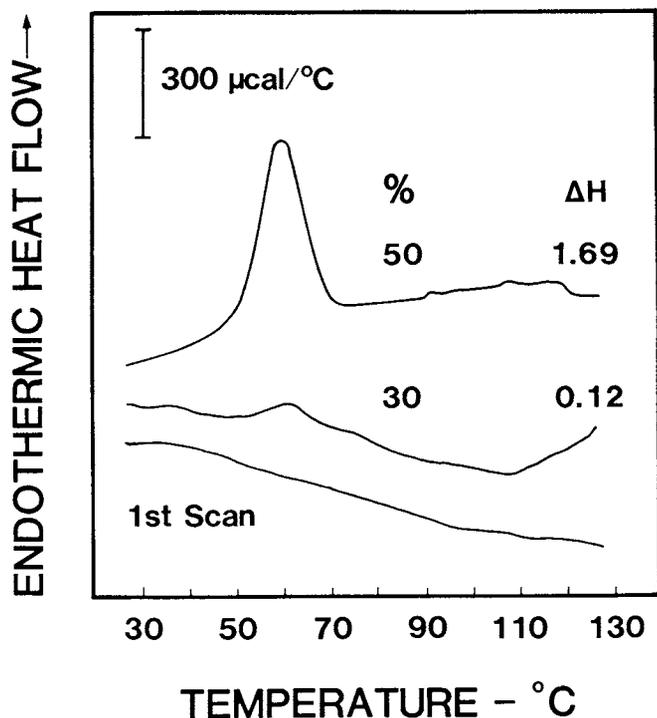


Fig. 6. Differential scanning calorimetry thermograms of solubilized amylopectin in excess water (first scan), and samples aged 14 days at concentrations of 30 and 50% solids. ΔH is given in calories per gram of amylopectin.

Aging of Bread Crumb with Antistaling Agents

Starch retrogradation is thought to be involved in the mechanism of bread staling. In light of this and the findings presented here, it was postulated that the agents that retard staling in bread do so by altering moisture relationships during aging and thereby decrease the rate of recrystallization. The moisture level in bread (35–40%) provides what we now recognize as optimum conditions for starch recrystallization. Antistaling agents may retard starch recrystallization by either raising or lowering water availability to a level outside the optimum range. To test this, bread was baked with various agents, lyophilized, and aged for 10 days at several moisture levels, ranging from dilute to concentrated. The enthalpy of the amylopectin endotherm at each moisture level for each agent and the control were compared (Fig. 3). The effect of moisture level on retrogradation was the same as that found in starch gels. Maximum crystallinity developed in the intermediate range (50–60%) just as in the gels. The curve was shifted only slightly in the bread samples compared to the starch gels. This indicated that the mechanism by which these agents retard staling does not alter moisture availability during the retrogradation of starch. These results also confirmed that the characteristics of starch revealed by studying a simple model system are an accurate reflection of the behavior of starch in the more complex bread system.

ACKNOWLEDGMENT

We gratefully acknowledge the assistance of Debi Rogers in the baking portion of the study and the financial support of Continental Baking Company.

LITERATURE CITED

- AXFORD, D. W. E., and COLWELL, K. H. 1967. Thermal investigation of bread staling. *Chem. Ind. London* 6:467.
- COLWELL, K. H., AXFORD, D. W. E., CHAMBERLAIN, N., and ELTON, G. A. H. 1969. Effect of storage temperature on the aging of concentrated wheat starch gels. *J. Sci. Food Agric.* 20:550.
- DRAGSDORF, R. D., and VARRIANO-MARSTON, E. 1980. Bread staling: X-ray diffraction studies on bread supplemented with α -amylases from different sources. *Cereal Chem.* 57:310.
- FEARN, T., and RUSSELL, P. L. 1982. A kinetic study of bread staling by differential scanning calorimetry. The effect of specific loaf volume. *J. Sci. Food Agric.* 33:357.
- FINNEY, K. F. 1984. An optimized, straight-dough, bread-making method after 44 years. *Cereal Chem.* 61:20.
- GHIASI, K., HOSENEY, R. C., ZELEZNAK, K., and ROGERS, D. E. 1984. Effect of waxy barley starch and reheating on firmness of bread crumb. *Cereal Chem.* 61:281.
- HELLMAN, N. N., FAIRCHILD, B., and SENTI, F. R. 1954. The bread staling problem. Molecular organization of starch upon aging of concentrated starch gels at various moisture levels. *Cereal Chem.* 31:495.
- KATZ, J. R. 1928. Gelatinization and retrogradation of starch in relation to the problem of bread staling. Page 68 in: *Comprehensive Survey of Starch Chemistry*. Vol 1. R. P. Walton, ed. Chemical Catalog Co.: New York.
- KNIGHTLY, W. H. 1977. The staling of bread. A review. *Bakers Dig.* 51(5):52.
- KULP, K., and PONTE, J. G. 1981. Staling of white pan bread: fundamental causes. *Crit. Rev. Food Sci. Nutr.* 15:1.
- LONGTON, J., and LEGRYS, G. A. 1981. Differential scanning calorimetry studies on the crystallinity of aging wheat starch gels. *Stärke* 33:410.
- LUND, D. B. 1983. Applications of differential scanning calorimetry in foods. Page 129 in: *Physical Properties of Foods*. M. Peleg and E. B. Bagley, eds. Avi Publishing Co.: Westport, Conn.
- MAGA, J. A. 1975. Bread staling. *Crit. Rev. Food Technol.* 5:443.
- RICHARDSON, M. J. 1972. Precision differential calorimetry and the heat of fusion of polyethylene. *J. Polym. Sci. Part C.* 38:251.
- RUSSELL, P. L. 1983. A kinetic study of bread staling by differential scanning calorimetry. The effect of painting loaves with ethanol. *Stärke*

35:277.

SCHOCH, T. J. 1957. Preparation of starch and starch fractions. Page 5 in: Methods of Enzymology. Vol. 3. S. P. Colowick and N. O. Kaplan, eds. Academic Press: New York.

WILLHOFT, E. M. A. 1973. Mechanism and theory of staling in bread and

baked goods and associated changes in textural properties. J. Texture Stud. 4:292.

ZOBEL, H. F., and SENTI, F. R. 1959. The bread staling problem. X-ray diffraction studies on breads containing a crosslinked starch and a heat-stable amylase. Cereal Chem. 36:441.

[Received December 9, 1985. Revision received April 28, 1986. Accepted May 9, 1986.]