Differential Scanning Calorimetry, Water Activity, and Moisture Contents in Crumb Center and Near-Crust Zones of Bread During Storage

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ABSTRACT

Water content, water activity, and enthalpy changes (by differential scanning calorimetry [DSC]) were determined in the center of the crumb and the near-crust area of polyethylene-wrapped bread stored for up to 168 hr at 21 ± 2°C. Moisture content and water activity decreased little in the center of the crumb. In the near-crust area there was a large decrease in moisture content and in water activity during the first 24 hr after baking. For thermograms of melting amylopectin crystallites, at about 50–60°C, samples were tested by DSC after adding water to obtain a ratio of 1.5:1.0 of water to dry bread crumb. Mean temperatures of onset and maximum enthalpy changes were, respectively, 7.7 and 5.8°C higher for the center crumb than for the near-crust zone. The peak area of amylopectin crystallite melting increased during 168 hr of bread storage much more rapidly in the near crust than in the center crumb. The difference reached a maximum at about 48 hr after baking. In the crumb center and near crust, respectively, enthalpy changes were 0.27 and 0.66, 0.53 and 1.19, and 1.35 and 1.82 J/g after 24, 48, and 168 hr. Without added water (as-is basis), the mean onset and maximum peak temperatures for dissociation of the amylose-lipid complex (110–140°C) were, respectively, 10.1 and 17.0°C higher in the near crust than in the crumb center zones. Enthalpy values in the center averaged 0.52 J/g and changed little during storage; in the near-crust area, they averaged 1.04 J/g. When DSC thermograms were run with a 1.5:1.0 ratio of water to dry bread crumb, there were no significant differences in mean onset and maximum peak temperatures and enthalpy between crumb samples from the bread center and area near the crust zone. No consistent changes in enthalpy at 100–110°C were recorded as a result of bread staling.

Despite considerable work in the field, bread staling is of interest because many questions remain unanswered (Russell 1983; Pomeranz 1985, 1987; Ablett et al 1986; Siljestrom et al 1988; Eliasson and Ljunger 1988). According to Banecki (1982), gluten is the deciding factor in staling of bread. The conclusion was reached on the basis of firmness measurements of breads baked from doughs with starch-gluten ratios from 80:4 to 64:20 and dough water contents from 42.5 to 49.5%. The influence of gluten on elastic changes during regular bread staling was reported to be 6.7 times greater than that of starch. A subsequent study showed that there is no transport of water from starch to gluten in the first 72 hr after baking (Banecki 1983). Kay and Willhoft (1972) measured conductance and capacitance of center crumb and concluded that staling of bread is due to both retrogradation of starch and changes in the proteins. Fearn and Russell (1982) and Russell (1983) applied differential scanning calorimetry (DSC) to the measurement of thermal changes in bread during aging associated with alterations in the structure of the starch fraction. When stale bread stored at 21°C was heated in the DSC instrument, an endotherm was observed at around 60°C. This was absent in fresh bread and progressively increased with storage time.

DSC is uniquely suited to determine enthalpy of physical and chemical reactions that take place during temperature changes. The greatest use of DSC has been in phase changes during gelatinization and retrogradation of starches (Bushuk and Mehrotra 1977 a,b,c; Eberstein et al 1980). Some of the constraints of the DSC methods are inability to measure small caloric effects, limited sample size, effects of rate of heating, and concentration of tested components in an aqueous system. Some of the constraints have been overcome by improved instrumentation and methodology.

In studies of starch, there is a gelatinization or retrogradation peak at about 55°C, and a second peak at over 100°C is due to melting of an amylose-lipid crystalline complex. Enthalpy changes are unaffected by water levels above 60%; they decrease substantially at lower levels (Eberstein et al 1980). Temperature of enthalpy changes increases as water content of the system decreases from 75 to 35% (Eberstein et al 1980). Most of the studies that employed DSC to follow staling of bread were conducted on starch gels; a few were made on bread (Fearn and Russell 1982; Zeleznak and Hoseney 1986). To the best of our knowledge, none followed enthalpy changes in various zones of the bread and correlated those changes with water content and water activity. Earlier studies (Czuchajowska et al 1989) indicated that such changes may provide new information on staling taking place due to the retrogradation of amylopectin crystallites and the amylose-lipid complex in stored bread. Such studies are the subject of this publication.

MATERIALS AND METHODS

Doughs were made from commercial flour containing 12.2% protein (N × 5.7) and 0.45% ash on a 14% moisture basis. Optimum mixing time was 3 min 35 sec, and optimum water absorption was 66.5% (average of 10 determinations). Optimum mixing time and optimum water absorption were determined on the basis of mixograph determinations and by an experienced experimental baker. Mixographs were determined by the 10-g method as described by Finney and Shogren (1972). The breadmaking formula included 100 g of flour, water, 1.5 g of sodium chloride, 1.8 g of dry bakers' yeast, 4 g of nonfat dry milk, 6 g of sucrose, 0.5 g of 60°L malt syrup, 3 g of shortening, and 40 ppm ascorbic acid. A straight dough procedure with a 3-hr fermentation time at 30°C was employed. Panning and punching were done mechanically. Proof time was 55 min at 30°C. Optimum baking time was 24 min at 218°C (Finney 1984). Loaf volume was determined immediately after baking by the dwarf rapeseed displacement method.

Moisture content was determined by oven drying at 130°C for 4 hr. Water activity was determined by the Cx-1 Water Activity System (Decagon Devices, Inc., Pullman, WA).

A Perkin-Elmer differential scanning calorimeter DSC-4 fitted with a 3600 thermal analysis data station and a Perkin-Elmer Graphics Plotter 2 (Perkin-Elmer Corp., Instrument Div., Norwalk, CT) were used. Large volume capsules, LVC, no. 0319-0218, were used to increase sensitivity and reproducibility and decrease sampling error. Adding water increased the measured enthalpy of melting amylopectin crystallites and made their detection possible much earlier during bread storage than thermogram determinations without added water. Similarly, without added water, enthalpy changes could be determined in the center of the crumb but not in the near-crust region. Complete gelatinization of starch apparently cannot take place if the water content is too low (about 30%). Consequently, most determinations of changes around 55–60°C were made with water added to obtain a water/dry crumb ratio of about 1.5:1.0. For determinations of dissociation of amylose-lipid complexes in the 100–140°C range, determinations were made both with added

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water and on as-is samples. Pieces of bread crumb (about 20 mg), placed into preweighed sample pans, were weighed on a Cahn automatic microbalance; about 20 μl of water was added using a microsyringe, and the total weight was recorded. The pan was placed on the spacer insert and sealed by a Perkin-Elmer Quick Press. Within the next 20 min, the sample was scanned from 20 to 120°C or from 60 to 160°C at a 10°C/min heating rate, with an Al_2O_3 pan representing the reference sample.

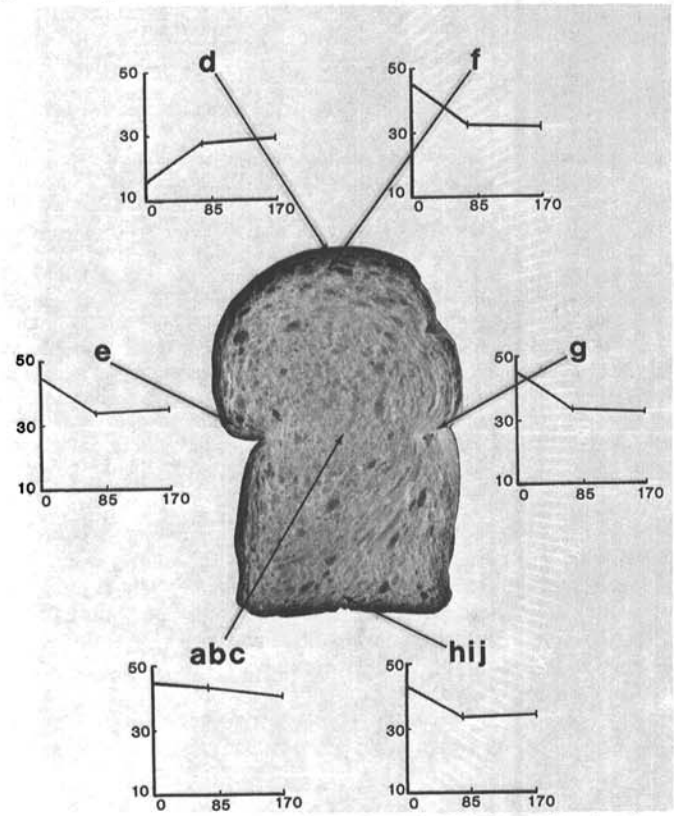

Fig. 1. Moisture (%) measured after 1, 72, and 168 hr in various locations in bread. In the simplified graphs, the x-axis is time (hours) and y-axis is moisture.

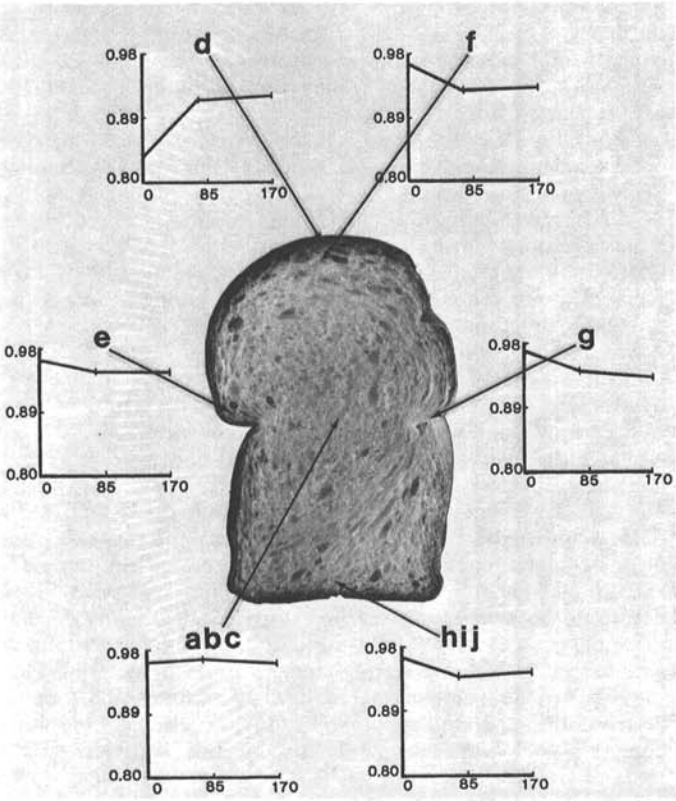

Fig. 2. Water activity (a_w) after 1, 72, and 168 hr in various locations in bread. In the simplified graphs, the x-axis is time (hours) and y-axis is water activity.

For each endotherm, onset (To), peak (Tp), and termination (Tm) temperatures were determined by a computerized system developed by Perkin-Elmer Corp. To and Tm temperatures were determined, in principle, as points at which there were deviations from the linear portions of traces before and after the endotherms, respectively. Endotherm enthalpies were computed, in Joules per gram of dry matter of bread, with reference to a straight baseline joining To and Tm.

Twenty loaves of bread were baked as described previously. Immediately after baking, each loaf was weighed, allowed to cool 1 hr, packed in a heavy polyethylene bag, and stored at 21 ± 2°C until measurements were made. Measurements were taken 1 hr after baking and continued at 24-hr intervals until the 168th hour. Each time, two separate loaves were examined. Before cutting, each loaf was weighed and the weight losses after baking and after storage were recorded. The loaves were about 12.0-cm high, 13.0-cm long, and 6.2-cm wide. For determination of moisture and water activity, slices were cut from 10 locations in the bread indicated in Figures 1 and 2. They included three sites in the center of the bread crumb (a, b, c), the top crust (d), the area immediately below the top crust (f), two areas near the crust in the middle of the loaf (e and g), and three locations near the bottom crust (h, i, j). Section a, from a slice cut from the center of the loaf, and sections b and c, from slices cut about 3 cm in from the ends of the loaf, were each 0.5–0.9 cm thick. The crust section, d, was 0.3–0.5 cm thick; section f (immediately below the crust) was 0.5-0.7 cm thick; sections e and g, about 0.7 cm thick, were cut 0.7 cm from the exterior surface; and h and j were 0.5–0.7 cm thick. From each slice, cylinders of 30 mm diameter were taken out with a cutter. The cylinders were placed into plastic cups of the instrument, and water activity (a_w) in bread crumb or bread crust was determined within 5 min. The cylinders were used afterwards for moisture determination. For determination of enthalpies by DSC, pieces from the center of the bread crumb and immediately below the center of the top crust were taken out, wrapped with Parafilm, and placed in small jars, which were closed immediately. DSC measurements were made as soon as instrument preparation and sample weighing were completed. Moisture, ash, and protein were determined by AACC approved methods (1983) at least twice. All results were averaged.

RESULTS AND DISCUSSION

Volume and weight of loaves baked from 100 g of flour and stored up to 168 hr are given together with standard deviations in Table I. The results point to rather uniform loaf volumes and weights of individual loaves, even after storage for seven days. The weight loss after baking ranged from 2.87 to 3.65 g, averaged 3.20 g, and was independent of the length of storage.

The manual separation, shortly after baking, of two loaves into crumb and crust showed the ratio was 53.4:46.6, respectively, on a dry matter basis. This indicates a low crumb percentage in the small (100 g of flour) and crusty loaves.

Changes in moisture content in various areas of the loaf are summarized in Figure 1. For simplicity of presentation, only data for 1, 72, and 168 hr are given. Corresponding changes in water activity are summarized in Figure 2. More detailed pictures of changes in moisture content (Fig. 3) and water activity (Fig. 4)

TABLE I
Volume and Weight of Loaves Baked from 100 g of Flour and Stored up to 168 hr

Parameter	Mean	Standard Deviation
Loaf volume, cm^3	857	12.7
Wt. of freshly baked loaf, g	152.3	0.8
Wt. of bread before cutting, g	149.1	0.7

are given for three bread areas only: center of the crumb (a, b, and c, averaged), the area immediately below the crust (f), and the top crust (d) of bread stored for up to 168 hr. There was a gradual, consistent decrease in moisture content in the center of the bread crumb. Moisture in the area immediately below the top crust decreased substantially during the first 24 hr after baking and very little thereafter. Almost the opposite took place in the crust; moisture increased most rapidly during the first 24 hr, somewhat more slowly during the next 48 hr, and very slowly thereafter. The changes in water activity basically paralleled the changes in moisture content. Still, as the total loaf weight loss was small and did not vary consistently with length of storage, the results seem to indicate that the ratio of "free" to "bound" water was not changed to a significant extent during storage. The only exception seems to be that free water increased during the first three days more rapidly in the crust than it decreased in the crumb beneath the crust. Leung et al (1983) found that during storage of bread there is an increase in water binding and decrease in water mobility as determined by an NMR deuteron relaxation method. The studies were conducted on 10-g flour loaves. According to Wynne-Jones and Blanshard (1986), however, there was no change in levels of bound water in crumb of bread stored for seven days in sealed glass tubes. An increase in bound water in crumb of bread stored in sealed plastic pouches was attributed to loss of free water from the crust-free bread. The measurements were made by proton magnetic resonance.

DSC data for crumb of bread from the center and below the top crust of loaves stored for up to 168 hr are summarized in Table II. Mean temperatures of onset and maximum were, respectively, 7.7 and 5.8°C higher for the center crumb than for the zone below the crust. At first glance, this is somewhat surprising because, generally, temperature of enthalpy changes increases as water content of the system decreases (Eberstein et al 1980). It would, therefore, seem that the differences in enthalpy and temperatures between the center and near crust crumb are due, in part at least, to differences in extent of organization of amylopectin crystallites. The temperature at which gelatinization was complete (i.e., at which complete loss of birefringence occurred) was shown to increase as the water content was reduced (Burt and Russell 1983). A correlation was established between the loss of birefringence and melting of retrograded amylopectin crystallites. Lower temperatures of enthalpy changes in the zone below the crust suggest that the extent of organization that develops during the retrogradation of amylopectin is less advanced than that existing in the center of the loaf (Stevens and Elton 1971). Less perfected amylopectin crystallites (with lower Tm and ΔH) may result from retarded annealing during storage, caused by lower moisture content (and resulting decreased mobility for diffusion-limited recrystallization) during storage. Moreover, water is an internal part of amylopectin crystalline regions and is required to be incorporated during crystal growth. Similarly, Soulaka and Morrison (1985) found that storing bread at a low temperature (at which retrogradation is more rapid but less complete) resulted in a Tp of about 52°C compared with about 60°C in bread stored at a high temperature (at which retrogradation is slow but complete).

The peak area of amylopectin crystallite melting increased rapidly during the first 72 hr and slowly thereafter for the center and near crust regions (Fig. 5). During the first 48 hr there was an increasing difference in peak area between the center and the zone beneath the top crust. The difference remained constant thereafter (Fig. 6).

The effects of storing bread for up to 168 hr on the dissociation of the amylose-lipid complex are summarized in Table III. These samples were tested by DSC without (as-is) or with added water. Without added water, the mean onset temperature was 10.1°C higher, and the mean peak temperature was 17.0°C higher in

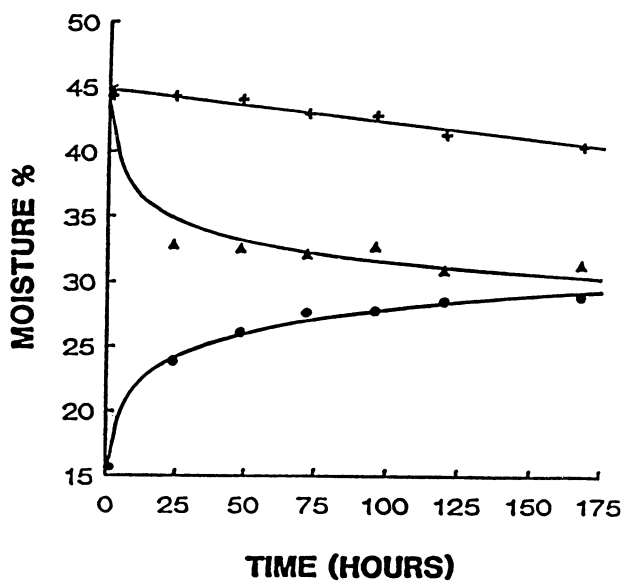

Fig. 3. Changes in moisture content in crust (•), center crumb (+), and near crust (▲) zones of 100-g flour loaves stored for up to 168 hr.

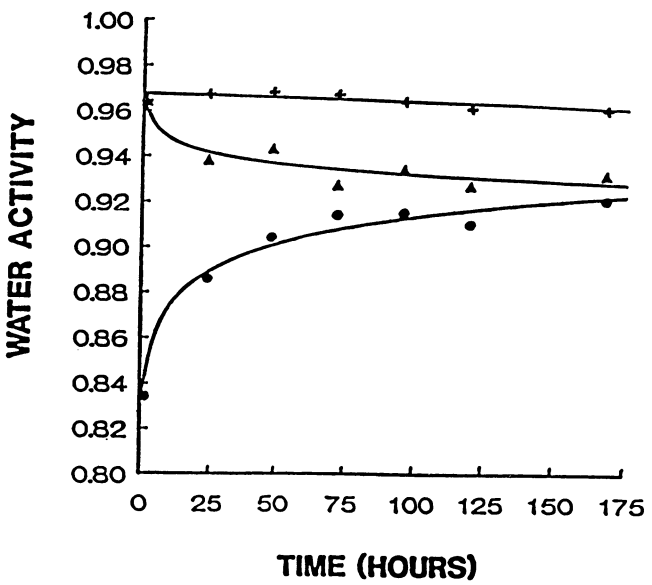

Fig. 4. Changes in water activity in crust (•), center crumb (+), and near crust (▲) zones of 100-g flour loaves stored for up to 168 hr.

TABLE II
Differential Scanning Calorimetric Data for Melting Amylopectin Crystallites in the Crumb of Bread Stored for up to 168 hr

Length of Storage (hr)	Peak (°C)		Enthalpy (J/g)
	Onset Temperature (To)	Peak Temperature (Tp)	
Loaf center			
1	...	...	below 0.10
24	49.4	55.0	0.27
48	52.8	60.3	0.53
72	51.0	59.3	0.90
96	52.9	61.8	0.88
168	49.9	59.8	1.35
Mean	51.2	59.2	...
SD	1.61	2.57	...
Near crust			
1	...	...	below 0.10
24	43.7	56.4	0.66
48	41.4	50.5	1.19
72	42.6	51.6	1.29
96	45.8	55.4	1.50
168	44.0	53.3	1.82
Mean	43.5	53.4	...
SD	1.66	2.49	...

dissociation of the complex in the crumb near the crust than in the center of the loaf. Enthalpy values in the center averaged 0.52 J/g. Those in the near-crust area were much higher, averaging 1.04 J/g. In samples tested by DSC in the presence of added water, there were no significant differences in either onset or peak temperatures and enthalpy values between crumb from the center or near crust area. When tested, there was no significant, consistent change in the onset and peak temperature or enthalpy during bread storage, either on an as-is basis or in the presence of added water. It is obvious that both the number and organization of the amylose-lipid complexes, unlike the amylopectin crystallites, are not changed during bread staling (and therefore probably are formed during or immediately after baking). Their dissociation, however, is moisture dependent. More energy is required to dissociate the complexes in the absence of added water. In the center of the bread crumb, apparently, enough water is present so as not to require additional water to facilitate dissociation.

The standard deviation for mean enthalpy for the dissociation of amylose-lipid complexes was much higher for the near-crust samples tested as-is (Table III) than for any of the other samples. The possibility of several amylose-lipid complexes varying in strength and capacity to form and reform cannot be excluded (Soulaka and Morrison 1985).

Zeleznak and Hoseney (1986) found that retrogradation in wheat starch gels is controlled by the amount of water present during aging, irrespective of the amount present during gelatinization. Changes taking place in a baked bread are more complex as they involve, among others, interchanges between crumb and crust, starch and gluten, and spatial populations of water with different mobilities. Thus, for instance, changes in

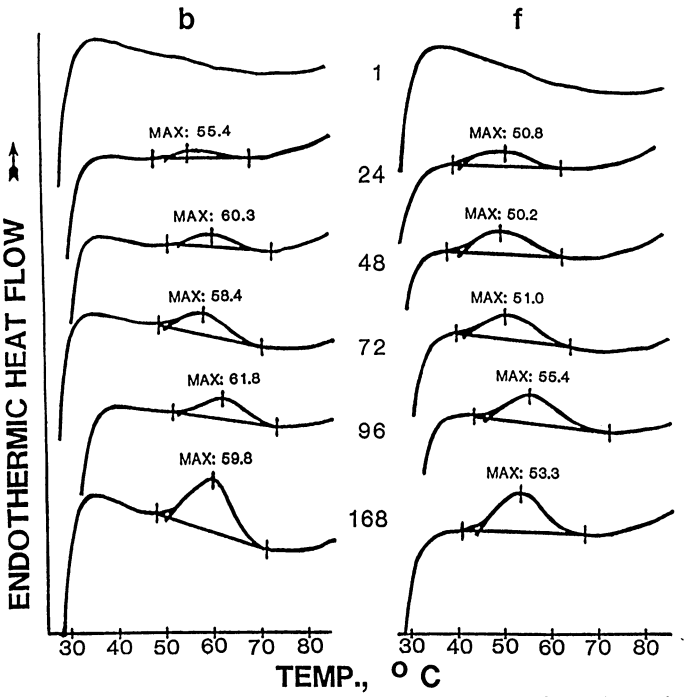

Fig. 5. Differential scanning calorimetric thermograms of bread crumb from **left,** the center of the loaf (b in Figs. 1 and 2); and **right,** the area near the crust (f in Figs. 1 and 2) during storage of bread for 1, 24, 48, 72, 96, and 168 hr (from top to bottom).

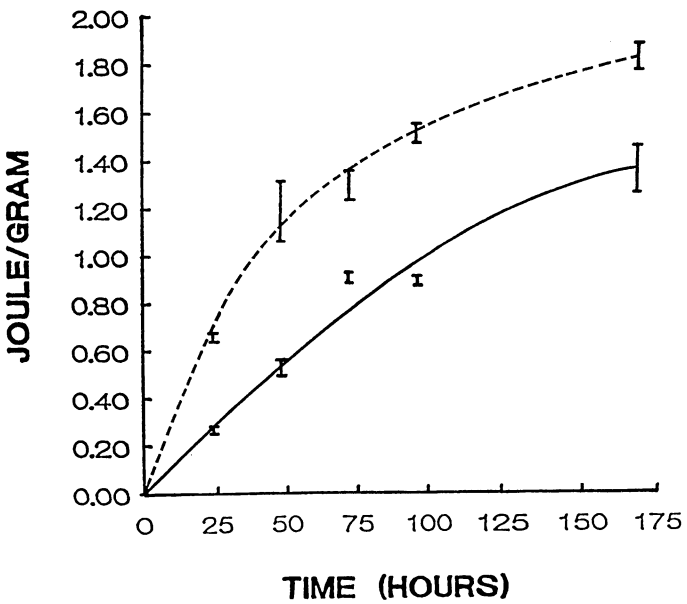

Fig. 6. A plot of enthalpy changes (J/g) in the center of bread crumb (——) (b in Figs. 1 and 2) and area near the top crust (- - - -) (f in Figs. 1 and 2) during storage of bread for 168 hr. Vertical lines denote range of values.

TABLE III
Differential Scanning Calorimetric Data for Dissociation of Amylose-Lipid Complexes in the Crumb of Bread Stored for up to 168 hr

	As-Is Basis			With Added Water		
	Peak (°C)			Peak (°C)		
Length of Storage (hr)	Onset Temperature (To)	Peak Temperature (Tp)	Enthalpy (J/g)	Onset Temperature (To)	Peak Temperature (Tp)	Enthalpy (J/g)
Loaf center						
1	100.7	109.2	0.53	92.0	103.2	0.44
24	109.6	115.0	0.43	...	...	...
48	110.5	119.9	0.52	91.5	101.4	0.50
72	111.3	119.6	0.56	91.7	102.6	0.50
96	112.6	119.2	0.56	...	...	...
168	114.1	123.9	0.50	92.8	102.9	0.55
Mean	109.8	117.8	0.52	92.0	102.5	0.50
SD	4.73	5.07	0.05	0.57	0.79	0.05
Near crust						
1	115.6	121.2	0.43	91.7	103.2	0.51
24	124.4	139.9	1.03	...	...	...
48	120.6	134.8	1.20	93.0	103.9	0.52
72	120.2	136.9	1.42	95.7	105.0	0.50
96	118.2	139.9	1.02	...	...	...
168	120.6	136.3	1.13	95.1	104.2	0.59
Mean	119.9	134.8	1.04	93.9	104.1	0.53
SD	2.92	6.98	0.33	1.86	0.75	0.04

DSC enthalpy cannot be eliminated by wrapping bread or storing crumb under conditions that prevent loss of moisture. In the center of the crumb there was little change in total moisture; still increases in DSC peaks were substantial.

The differences in DSC enthalpy at about 60°C between the crumb center and near-crust zones of bread are of interest. Loss of moisture is known to be most pronounced in the crumb region just beneath the crust (Neukom and Rutz 1981). The white ring formed in frozen bread in the near-crust is considered a zone of weakness that may affect bread quality, including staling (Pence 1961). Whether the amylose-lipid complex in the near-crust area contributes to this weakness is yet to be determined. Whatever the explanation, our findings may have implications in relation to the best method of slowing down staling. Thus, the formation of a thick crust would be conducive to a significant transfer of water from near crust zone. Commercial bakers in the United States bake bread in a manner that avoids the formation of such a crust in white pan bread: short time at high temperatures. Another means to reduce the rate of staling could be to produce bread in which the transfer of moisture from the near crust to the crust is minimized. In such a bread not only would crispness of the crust be retained, but rate of crumb staling would be slowed down. Research on means of achieving that objective is urgently needed.

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