

Effects of Glycerol and Moisture Gradient on Thermomechanical Properties of White Bread

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The thermomechanical properties of breadcrumb were investigated using dynamic mechanical analysis (DMA) and differential scanning calorimetry (DSC). The main transition (T_1 , near 0 °C) shifted to lower temperature with added glycerol due to freezing point depression. The low-temperature transition (T_3 , ~ -50 °C), found only in high-glycerol (8.8%) bread, suggested that of excess or phase-separated glycerol. The high-temperature transition (T_2 , 60–85 °C) appeared only in aged breadcrumbs; its temperature range was correlated well with the amylopectin melting transition (DSC) but its $\tan \delta$ amplitude did not correlate well with the amylopectin melting enthalpy ($r^2 = 0.72$). On the other hand, the change of E'' (viscous behavior) suggested that T_2 might be related to the change in the amorphous region. Domain-to-domain (amorphous) and crumb-to-crust moisture migrations are two critical phenomenological changes associated with aging and could lead to significant local dehydration of some amorphous regions contributing to mechanical firming during storage.

Keywords: Thermomechanical properties; moisture migration; white bread; glycerol

INTRODUCTION

Thermomechanical properties underlie some physicochemical changes in bread. Dynamic mechanical analysis (DMA) has been applied to characterize changes that occur in starch and bakery products (1–6). Glycerol is a humectant used in military shelf-stable [meal, ready to-eat (MRE)] bread. Glycerol is added to lower the water activity (a_w) to an intermediate moisture range (7) and is expected to plasticize and maintain softness of the bread over storage (1, 3). This leads to a freezing point depression and complex or multiple glassy–rubbery transitions (1, 3). MRE bread has been reported to show three transitions, ice melting around -12 °C, glycerol-rich glass transition from -90 to -50 °C, and fat melting from 8 to 14 °C (1).

The dependence of thermomechanical properties of bread polymers on some solutes has been reported (1, 3, 4, 8). However, this dependence and corresponding mechanisms may vary depending on the concentration of the added solute. For instance, in a case of excess addition, immiscible phases have been reported when glycerol was added to gluten as observed in multiple DMA transitions (3, 4). In cases when glycerol is added in excess, the loss factor or $\tan \delta$ amplitude of the glycerol-related peak (~ -60 °C) may change proportionally with glycerol content (3, 4).

Although glycerol application to bread is known to reduce a_w (7) and soften the bread (1), the mechanism underlying its effect on the staling process is not well understood. Berkowitz and Oleksyk (7) reported that decreased firmness of MRE bread could be achieved by the addition of antistaling agents, such as emulsifier

and poly(vinylpyrrolidone). The plasticizing effect of glycerol on wheat gluten is less effective than water at the same concentration (8). Furthermore, in water–glycerol–starch systems, multiple interactions among components, such as the presence of two competing plasticizers or a cosolvent (e.g., water and glycerol), can influence the ability of each to act as a plasticizer with respect to its relative concentration. For instance, when both glycerol and water are present, glycerol may act as an antiplasticizer below 12% and as a plasticizer above 12% (9). Similarly, when many polymers are present, one may serve as a structural component more than the others. This depends on the structural strength and microstructural arrangement in the continuous phase as well as cross-networking and interaction with nearby polymeric domains. Glycerol has an influence not only during the dough-mixing, fermentation, and baking stages but also during the aging of the polymers.

Among various factors, firming has been related to the degree of amylopectin retrogradation, although there are many other structural components predominantly in the continuous phase yet to be further explored (such as changes in gluten and amorphous starch). Bread stored in a hermetically sealed pouch can simultaneously increase in firmness and amylopectin recrystallization; the rates have been found to increase with the degree of moisture loss from the crumb to the crust (10). However, increasing the initial moisture of the bread cannot prevent the firmness increase. It is necessary to further investigate the role of water and glycerol on thermomechanical properties of bread during aging.

The objective of this work is to investigate the effects of storage with and without crust to evaluate the influence of crumb-to-crust moisture migration. The thermomechanical properties were studied as a function of aging and glycerol content.

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Table 1. Formulation of White Bread (in Grams)

component	control	2.6% glycerol (g of glycerol/100g of wheat flour)		8.8% glycerol (g of glycerol/100 g of wheat flour)	
		same water added ^a	more water added ^b	same water added ^a	more water added ^b
wheat flour	200.00	200.00	200.00	200.00	200.00
water	120.00	120.00	128.90	120.00	142.70
shortening	10.00	10.00	10.00	10.00	10.00
sugar	9.40	9.40	9.40	9.40	9.40
nonfat dry milk	6.00	6.00	6.00	6.00	6.00
active dry yeast	5.40	5.40	5.40	5.40	5.40
salt	4.50	4.50	4.50	4.50	4.50
calcium propionate	0.96	0.96	0.96	0.96	0.96
potassium sorbate	0.48	0.48	0.48	0.48	0.48
glycerol		5.28	5.28	17.60	17.60
moisture content of breadcrumb after baking (g of water/100 g of bread)	39.0 ± 0.1	38.0 ± 0.5	38.5 ± 0.5	35.7 ± 0.8	39.5 ± 0.4

^a Different initial moisture content of crumb from control bread after baking. ^b Same initial moisture content of crumb with control bread after baking.

MATERIALS AND METHODS

Bread. Wheat flour [unbleached, all-purpose, 10% protein, 73.3% carbohydrate (mostly starch), and 16.7% water], shortening, sugar, nonfat dry milk, active dry yeast, and salt were purchased at the local grocery store. Potassium sorbate was purchased from Sigma Chemical Co. (St. Louis, MO), calcium propionate from Pfizer Inc. (New York, NY), and glycerol from Fisher Scientific (Pittsburgh, PA).

White bread based on 0, 2.6, and 8.8 g of glycerol/100 g of wheat flour was made using an automatic bread-making machine (Bread Bakery model SD-BT51P, Panasonic, Secaucus, NJ) and a general baking method (20-min mixing time, 5-min resting time, 5-min kneading period, 160-min rising time, and 50-min baking time at 160 °C). The formulation of the white bread is shown in Table 1. Two water treatments were applied: (1) breadcrumbs with different initial moisture contents after baking (i.e., the same amount of water was used in the original formula with various glycerol contents and, after baking, the glycerol breads showed lower moisture contents than the control); and (2) breadcrumbs with the same initial moisture content after baking (i.e., more water was added to the original formula with increasing levels of glycerol in order to adjust the moisture content to the same level at 39.0% after baking [Table 1]).

Therefore, five types of bread were used in this experiment: (1) control; (2) 2.6% glycerol bread with different initial moisture contents after baking (lower than control); (3) 2.6% glycerol bread with the same initial moisture content after baking as control; (4) 8.8% glycerol bread with different initial moisture contents after baking (lower than control); and (5) 8.8% glycerol bread with the same initial moisture content after baking as control (Table 1). After baking, two storage methods were used. In the first method, each loaf of bread was packaged with the crust intact. In the second method, the 30-mm-thick crust was removed by cutting (with an electric knife) a loaf into a 13 × 13 × 60 mm (height × width × length) shape. Each bread sample was hermetically sealed in a trilaminated pouch (Cadillac Products Inc.) to prevent moisture loss to the atmosphere and then stored at 25 °C. Breads were baked in triplicate from three different machines for each treatment.

Moisture Content and Water Activity (a_w) Determination. The moisture content of breadcrumb was determined using a vacuum oven drying method at 70 °C and 29 in.Hg overnight (AOAC Method 925.09, 1973) and calculated from weight change.

Water activity (a_w) was determined using a modified isopiestic method (11).

Differential Scanning Calorimetry (DSC) Analysis. Sample (~15 mg) was placed in a stainless steel sample pan (Perkin-Elmer, Somerset, NJ) and hermetically sealed. A sample was analyzed by a DSC instrument (DSC100, Seiko Instruments, Torrance, CA), using an empty pan as reference. The sample was cooled to -40 °C using liquid nitrogen and then heated to 140 °C at 5 °C/min. Transition temperatures

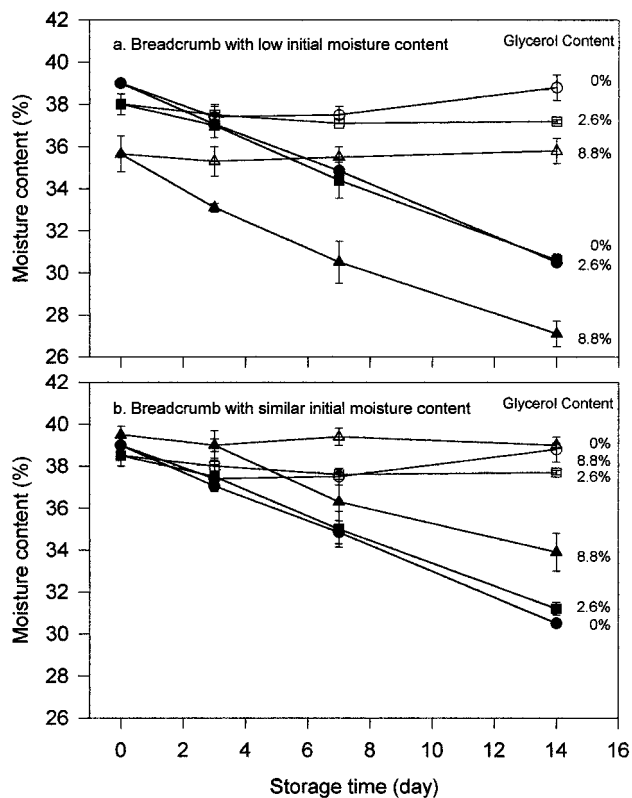


Figure 1. Effect of glycerol on moisture content of breadcrumb during storage. Open symbols represent breadcrumb stored without crust, and solid symbols represent breadcrumb stored with crust.

and enthalpy of ice and amylopectin melting transitions were determined as previously described (10).

To characterize the small transition occurring before ice melting, the ice melting transition was deconvoluted with PeakFit software (version 1.00, Jandel Scientific, San Rafael, CA) as earlier described (5).

DMA Analysis. Each loaf center was cut into a 13 × 13 × 60 mm (height × width × length) shape and then compressed with a Carver press at room temperature to obtain ~2.0 mm thickness and was then cut into a 50 × 10 mm bar. The sample was immediately analyzed by DMA using a three-point bending mode (DMA110, Seiko Instruments), as described by Hallberg and Chinachoti (1). The sample was heated from -100 to 120 °C at 2 °C/min. During the heating process, a sinusoidal stress was applied at 1, 5, 10, 50, and 100 Hz. Storage modulus (E'), loss modulus (E''), and $\tan \delta$ (E''/E') were recorded. DMA $\tan \delta$ curves were deconvoluted using PeakFit software (5).

Table 2. DSC Ice Melting Transition Temperature Range of Fresh Breadcrumbs^a

initial moisture content of crumb after baking	glycerol content (g/100 g of wheat flour)	transition temps (°C)		
		onset	peak	conclusion
different	0	-18.7 ± 1.4 a	-0.5 ± 0.3 e	14.6 ± 0.6 h
	2.6	-22.6 ± 1.0 b	-2.2 ± 0.8 f	13.6 ± 0.7 h
	8.8	-26.3 ± 0.5 d	-3.1 ± 0.6 g	8.6 ± 1.6 j
same	0	-18.7 ± 1.4 a	-0.5 ± 0.3 e	14.6 ± 0.6 h
	2.6	-21.8 ± 1.0 b	-2.0 ± 0.5 f	13.5 ± 1.0 h
	8.8	-24.8 ± 1.4 c	-2.5 ± 0.5 f	9.5 ± 3.0 i

^a Values in the same column followed by a different letter are significantly different at a level of $p \leq 0.05$.

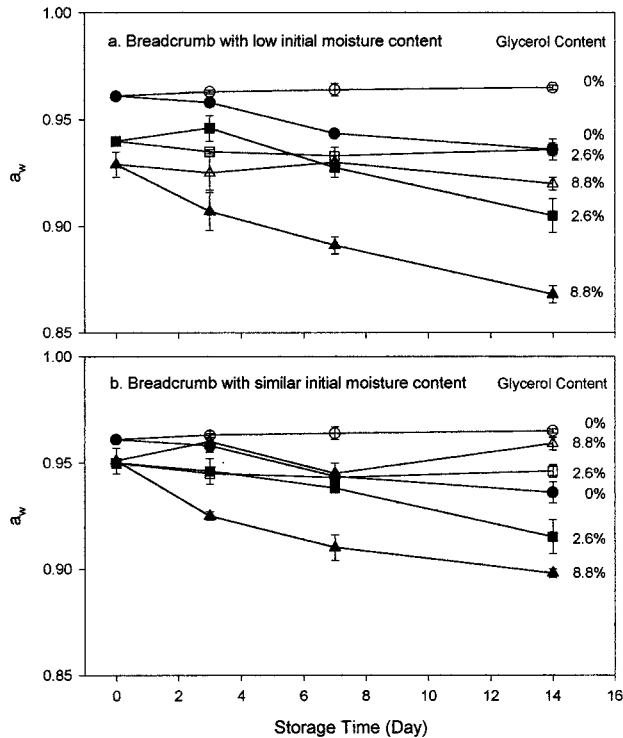


Figure 2. Effect of glycerol on water activity (a_w) of breadcrumb during storage. Open symbols represent breadcrumb stored without crust, and solid symbols represent breadcrumb stored with crust.

RESULTS AND DISCUSSION

Moisture Content and Water Activity (a_w). Moisture content of breadcrumb is shown in Figure 1. When glycerol was added without the addition of extra water, the initial moisture content (after baking) was lower than that of the control (no glycerol) bread (Figure 1a). Glycerol was also added with extra water to obtain the same moisture content after baking (Table 1; Figure 1b). In both cases (different and same moisture contents after baking), all breadcrumbs stored with crust lost their moisture but those stored without crust maintained their moisture during storage. Also in both cases, bread containing glycerol also lost moisture from crumb to crust. Moisture loss was controlled by the moisture gradient (between crumb and crust irrespective of glycerol added) (Figure 1).

The effect of glycerol on a_w of the bread is shown in Figure 2. Glycerol reduced the initial a_w of breadcrumbs (Figure 2) according to its colligative property due to its association with water. Over time, some decrease of a_w in both different and same initial moisture content breadcrumbs was observed (Figure 2). When bread was stored without crust, a_w remained relatively unchanged for all treatments during storage (Figure 2).

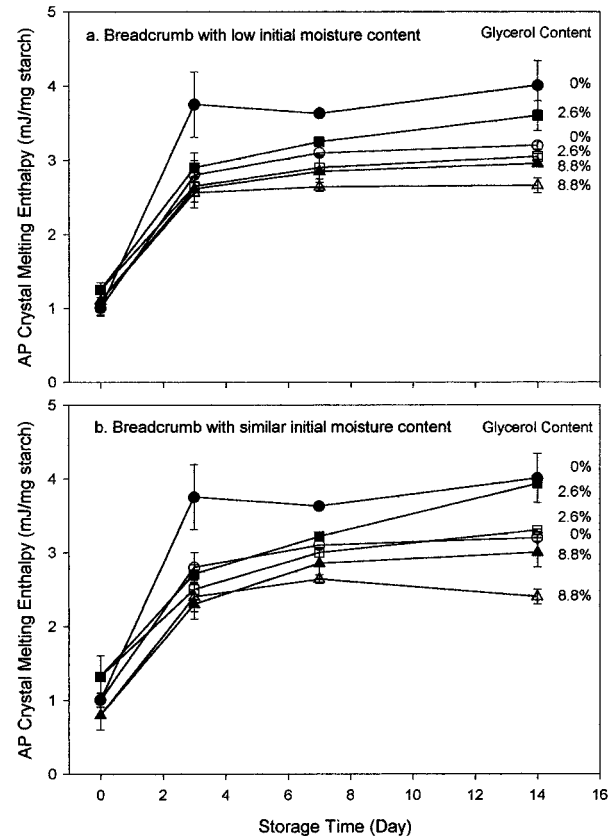


Figure 3. Effect of glycerol on amylopectin recrystallization of breadcrumb during storage. Open symbols represent breadcrumb stored without crust, and solid symbols represent breadcrumb stored with crust.

DSC Results. Amylopectin crystal melting enthalpy gradually increased during storage in all conditions, but the increase was dependent upon storage method and glycerol content (Figure 3). The breadcrumb stored without crust showed relatively less amylopectin recrystallization than that stored with crust. Because only the breadcrumb stored with crust intact lost a significant amount of moisture in a parallel fashion as the increase in amylopectin recrystallization, the two processes might be related. The presence of glycerol interfered with the recrystallization process in a concentration-dependent manner (Figure 3). The inhibition of amylopectin recrystallization by glycerol may be explained by molecular hindrance, or glycerol might also have penetrated the amorphous regions of amylopectin. The relatively larger (than water) glycerol molecule may lower the local mobility of the amylopectin chain, preventing it from reorienting into crystalline structure. Additionally, a small hygroscopic solute such as glycerol has a greater ability to associate with water as earlier shown by Chinachoti and Steinberg (12, 13) in solute-

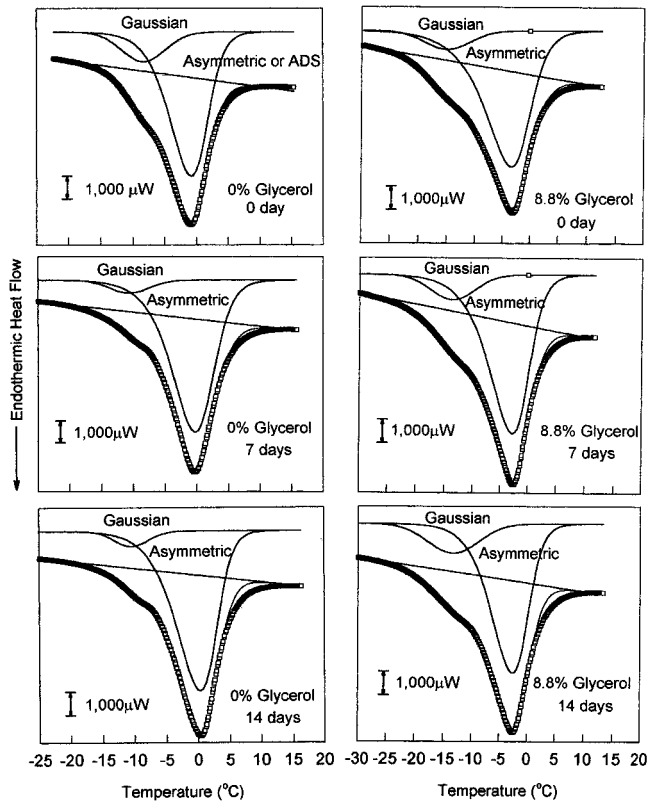


Figure 4. Deconvoluted DSC ice melting endotherm of breadcrumb stored without crust.

Table 3. Change of the Peak Area of Gaussian Component after Deconvolution of DSC Ice Melting Endotherm^a

storage time (days)	enthalpy (mJ/mg of sample)	
	control bread	8.8% glycerol bread
0	7.8 ± 1.2	5.4 ± 0.4
3	5.1 ± 0.6	7.5 ± 0.5
7	3.1 ± 0.1	10.2 ± 3.1
14	3.6 ± 0.1	10.0 ± 1.2

^aBreadcrumb with same initial moisture content after baking and stored without crust.

polymer systems. Local osmotic dehydration of starch and gluten due to glycerol could reduce their hydrated state and, in the case of amorphous starch, reduce its molecular ability to form a more ordered structure as observed from the DSC enthalpy. Schiraldi et al. (14) suggested that the water-binding compounds, such as glycerol, compete with large biopolymers for water and reduce a_w , thus hindering moisture redistribution and starch retrogradation.

Table 2 shows that glycerol had a significant freezing point depression effect (lower in the melting temperature, T_m) at the 8.8% glycerol level but not at the 2.6% glycerol level ($p < 0.05$).

DSC ice melting endotherms were deconvoluted into asymmetric double sigmoidal (ADS) and Gaussian curves ($r^2 > 0.99$) as shown for control and 8.8% glycerol (Figure 4). The ADS enthalpy did not change significantly during storage, whereas the Gaussian enthalpy

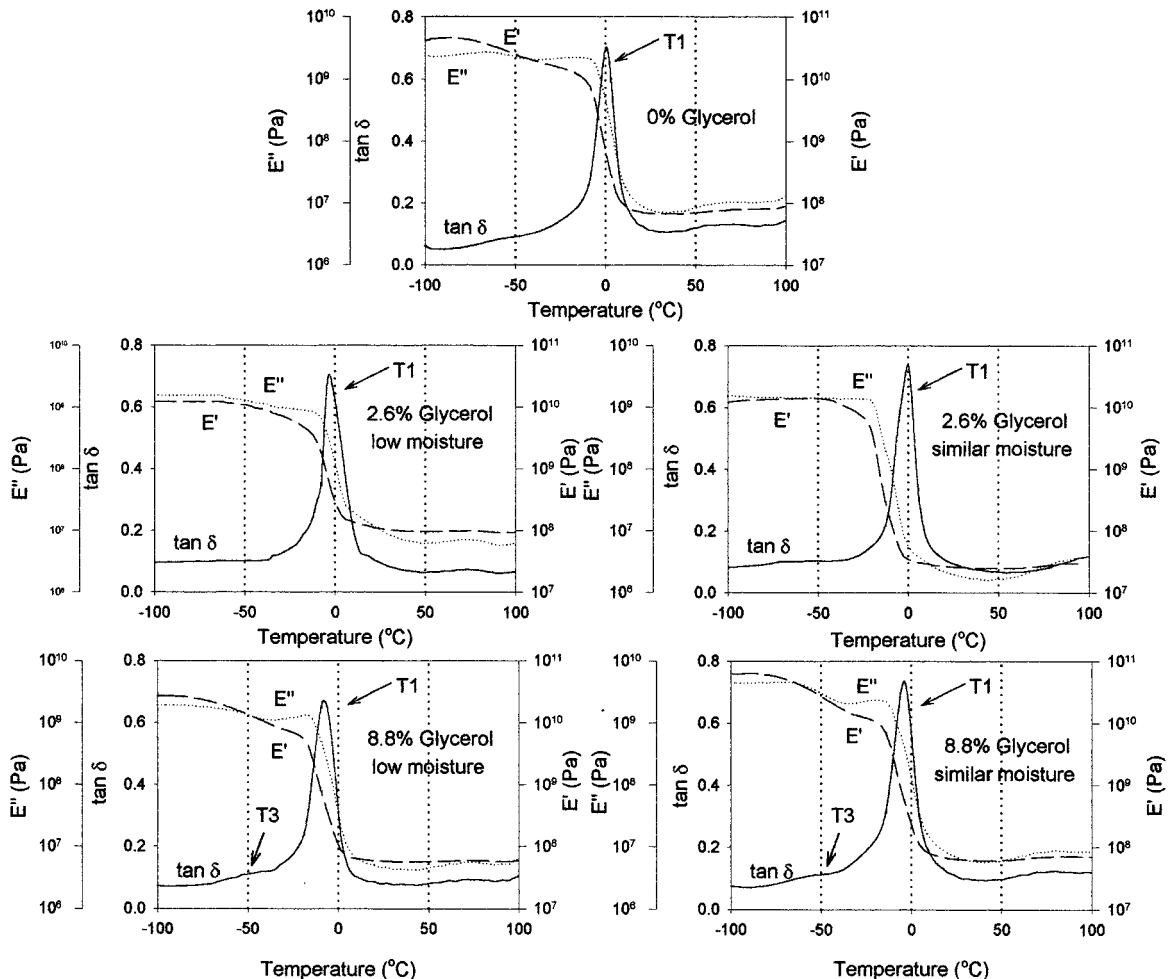


Figure 5. DMA thermograms of fresh breadcrumbs containing 0, 2.6, and 8.8% glycerol (wheat flour basis).

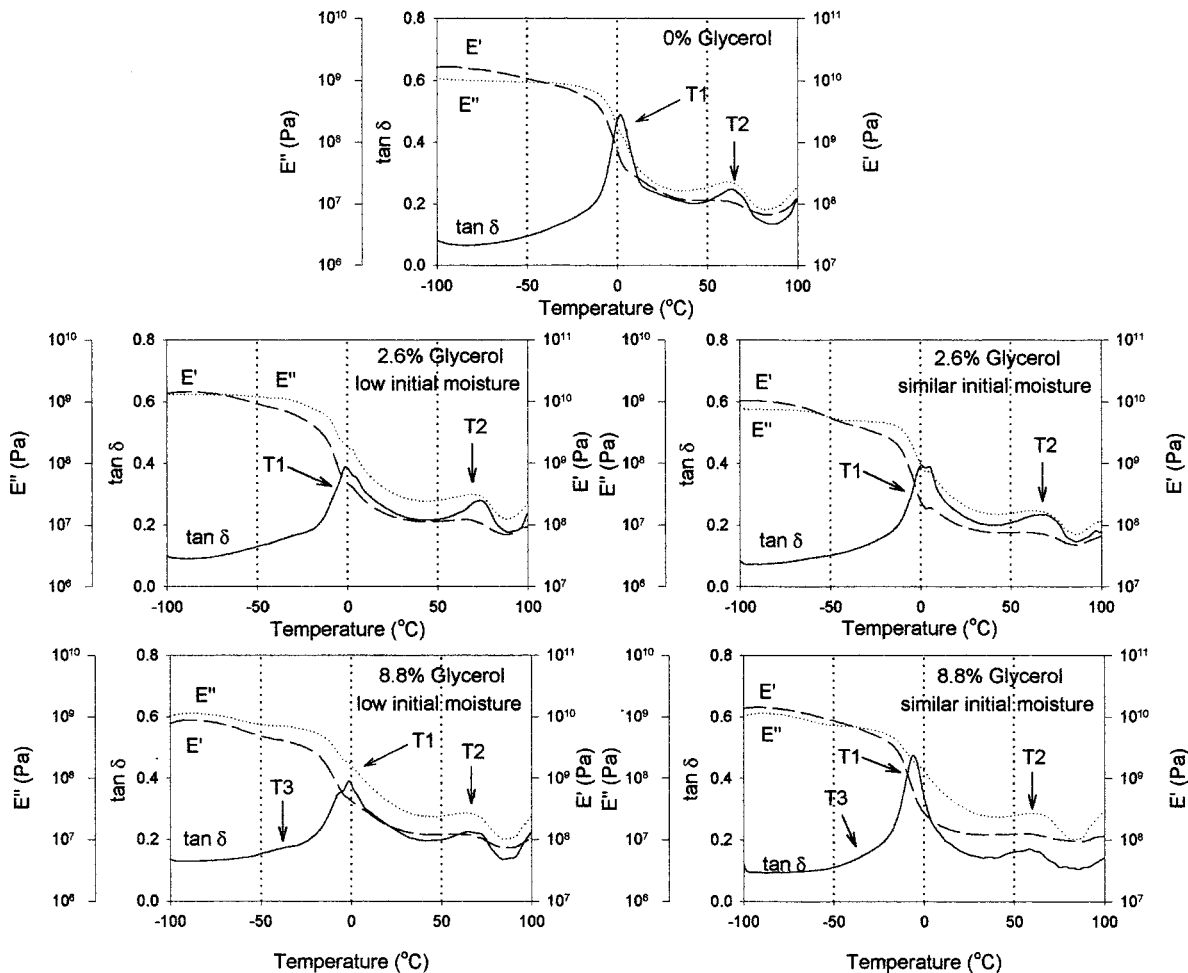


Figure 6. DMA thermograms of aged breadcrumbs (stored with crust for 7 days at 25 °C) containing 0, 2.6, and 8.8% glycerol (wheat flour basis).

significantly decreased in the case of the control bread but increased in the case of the 8.8% glycerol bread during storage (Table 3). It has been suggested earlier that a glass transition might occur at a temperature right below the main ice melting transition in high-moisture starch gels and that its temperature (T_g) might be related to the tendency for amorphous starch to recrystallize (15–19). However, arguments have also been made that some water population in a freeze-concentrated state with lower freezing points (than bulk) could also exhibit a melting transition in the same temperature range (20, 21). If the Gaussian component represents melting of the water population in the freeze-concentrated domains, such water in the freeze-concentrated domains exists in a close proximity to bread polymers. As the control bread aged, this water portion might easily be incorporated into more rigid amorphous and crystalline domains, leading to a decrease in the amount of freeze-concentrated water of the bread system. With glycerol phase separated or excess, glycerol might have pulled the water from the bread polymer matrices; this process might proceed over storage, resulting in a Gaussian component. This may be one possible explanation for the decrease in the Gaussian component of the control bread and the increase of the Gaussian component of the 8.8% glycerol bread during staling. In excess water, the glass transition (if it exists) overlaps the ice melting range (in freeze-concentrated phase), and the characterization or interpretation of this overlapping transition is nonconclusive (22).

DMA Results. DMA thermograms of fresh breadcrumbs are shown in Figure 5. The main (ice melting) transition (T_1) was observed at ≤ 0 °C. An additional small transition near -50 °C (T_3) was noted only in the 8.8% glycerol bread and was previously described as a glycerol-dominant phase transition (1, 3). This glycerol transition temperature has been reported to vary with glycerol and moisture contents and was also found in this work. The existence of this transition indicates that glycerol, when present in a high concentration, may become phase separated.

As found in the DSC results, the addition of glycerol led to a depression of the ice melting transition (T_1) temperature (Table 2), which was earlier described as a plasticizing effect of glycerol (3). In MRE bread, the addition of 12.5% glycerol (flour basis) resulted in a T_1 temperature decrease from -2.5 to -9.7 °C (1).

Figure 6 shows the corresponding DMA thermograms of breadcrumb stored with crust intact after 7 days of storage. When aged, breads stored with crust showed a decrease in T_1 amplitude and simultaneous emergence of an additional transition near 60 °C (T_2). This transition has been earlier reported (10, 23), but its origin remains unclear. Unlike T_1 , which involved a 1–2 order of magnitude drop in E' , T_2 involved very little change in E' but more change in E'' (Figure 6). The observed change in the loss modulus reflected possibly an increase in viscous behavior (24).

During the storage, some of the water is redistributed among various heterogeneous domains (25–29). In

Table 4. Moisture Remaining in Breadcrumbs after Heating in DMA to Various Temperatures^a

glycerol content (g/100 g of wheat flour)	storage time (days)	moisture remaining (% of initial moisture)			
		25 °C	50 °C	68 °C	88 °C
0%	0	100	88.5 ± 0.8 a	78.5 ± 3.0 b	63.4 ± 7.3 c
	7 (with crust)	100	86.5 ± 1.0 a	79.1 ± 2.6 b	57.2 ± 4.4 d
	7 (without crust)	100	89.4 ± 1.2 a	78.5 ± 0.7 b	66.0 ± 1.1 c
8.8%	0	100	88.7 ± 0.8 a	77.5 ± 3.2 b	68.4 ± 3.9 c
	7 (with crust)	100	88.3 ± 0.2 a	77.3 ± 1.8 b	64.5 ± 2.3 c
	7 (without crust)	100	88.1 ± 0.3 a	76.0 ± 0.9 b	66.0 ± 0.5 c

^a Values in the same column followed by different letters are significantly different at a level of $p \leq 0.05$.

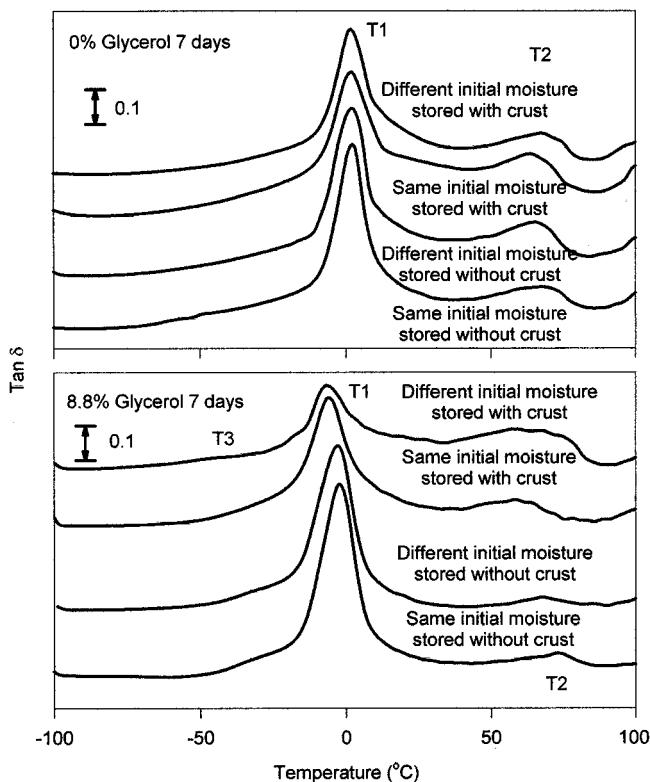


Figure 7. Comparison of DMA $\tan \delta$ between breadcrumb stored (7 days at 25 °C) with or without crust.

breadcrumb stored with crust, moisture migrates from the crumb to the crust according to the moisture gradient (29, 30), leading to physicochemical changes such as shrinkage during storage.

When bread was stored without crust, relatively large T_1 and small T_2 transition $\tan \delta$ amplitudes were observed (Figure 7). This remained relatively unchanged over the storage period. However, the T_1 transition intensity of breadcrumb stored with crust (moisture gradient) was found to decrease over time significantly, regardless of glycerol level. However, the T_2 transition temperature appears to increase slightly over time (Figure 7). Previously, Baik and Chinachoti (10) postulated that the $\tan \delta$ peak intensity of T_2 might correspond to the change of amorphous region rather than the amylopectin melting transition. Here we found the DMA T_2 transition $\tan \delta$ peak intensity and DSC amylopectin melting transition did not show a good correlation ($r^2 = 0.72$, data not shown), supporting the hypothesis.

T_2 could also be a result of moisture loss during the DMA measurements. To determine its contribution, moisture loss of breadcrumb samples was measured during DMA runs (Table 4). T_2 was observed only in

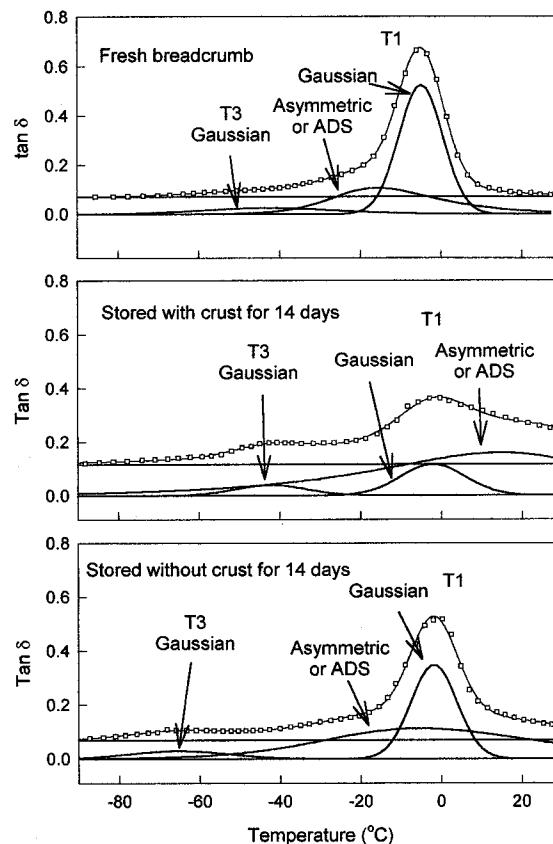


Figure 8. Deconvoluted DMA $\tan \delta$ peak (T_1 and T_3) of 8.8% glycerol breadcrumb.

aged breads and not in fresh breads. If T_2 was due to moisture loss during the DMA run, fresh and aged breads, which lost moisture at similar rates (insignificantly different, $p \leq 0.05$), should show the same T_2 change. Therefore, T_2 is likely to be caused by aging of the amorphous domains in bread.

But what could have caused a change? In an earlier study, it was suggested that moisture transfer from one domain to another could occur without affecting the overall moisture content (25). This could lead to some thermomechanical changes. As mentioned earlier, T_2 may still be partially related to amylopectin recrystallization, but evidence shows that it is more related to aging of the amorphous domains, leading to exchanging of water among them. More work is needed in this area to further investigate the origin of this transition.

The peak area and temperature range of the ADS component were found to increase with storage time (Figure 8), which supported earlier findings (6, 31). Aging leads to a more closely packed starch network with decreased polymer chain mobility, but the decrease in hydration in some domains with the increase in

another could contribute to an increase in system heterogeneity and consequently a broader glass transition temperature range (31). This broadening was found in all cases (Figure 8) but was greater when bread was stored with crust (with macroscopic crumb-to-crust moisture gradient), suggesting that moisture loss during aging may be one of the factors affecting the heterogeneity of domains, some of which might have a strong contribution to mechanical firmness.

Conclusion. Addition of glycerol and moisture loss from crumb led to three general thermomechanical transitions: T_1 , the main transition (ice melting); T_2 , the transition at 60–85 °C; and T_3 , the transition of glycerol-rich regions at ~-50 °C. The T_1 transition could be deconvoluted into bulk ice melting (Gaussian) and ADS transitions. The latter was associated with freezing of water in a freeze-concentrated phase and possibly a glass transition. Differences in initial moisture did not affect thermomechanical transitions. Glycerol shifted both the DMA main transition (T_1) and the DSC ice melting endotherm to a lower temperature (freezing point depression). For DMA $\tan \delta$, glycerol transition was not found in 2.6% glycerol bread but was detected in 8.8% glycerol bread at ~-50 °C, and this glycerol transition was more distinguishable with aging and moisture loss. T_2 might be more related to moisture loss among domains, and less to amylopectin recrystallization. Domain-to-domain (amorphous) and crumb-to-crust moisture migrations are two critical phenomenological changes associated with aging and could lead to significant local dehydration of some amorphous regions contributing to mechanical firming during storage.

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