Effect of Aging and Drying on Thermomechanical Properties of White Bread as Characterized by Dynamic Mechanical Analysis (DMA) and Differential Scanning Calorimetry (DSC)

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ABSTRACT

The effects of drying and storage on white bread was investigated using dynamic mechanical analysis (DMA), and the means for quantifying the results were explored. The "freezable" water content was obtained from differential scanning calorimetry (DSC) endotherm in ice melting region. A typical DMA thermogram of a high-moisture bread (>30%, w/w) resulted in a broad transition region, as evidenced by the tan δ curve and E' drop spanning a temperature range of 50°C in the ice melting region. DSC results show that freezable water was present at >33% moisture. With lower moisture, the observed transition appeared to move to a higher temperature range, and the tan δ curve decreased in height and broadened while the E' descending slope decreased. At <30%

moisture, the tan δ curves were best fitted with a single Gaussian curve, while at >30% moisture, best fit was obtained with a Gaussian overlapping with an asymmetric sigmoidal curve. Some broadening of the tan δ peak was observed in staled bread (seven months), but the temperature, in general, remained in the same range as fresh bread. The results also indicated that only extensive staling of bread (19 months) lead to a broad tan δ distribution (–70 to 100°C) of transitions which could be deconvoluted into at least two major, overlapping curves; while drying out of a fresh bread would only result in one major (–25 to 25°C) transition (although each of these major thermal events could arise from a distribution of multiple domains).

Bread staling is a much studied phenomenon involving a considerable deterioration of flavor, texture, and color. From a rheological standpoint, the increase in bread firmness is the most important indication of staling (Roewe et al 1982) To this end, factors such as gluten functionality changes (LeMeste et al 1992), and notably amylopectin crystallization or starch retrogradation (Longton et al 1981, Zeleznak and Hoseney 1987, Marsh and Blanshard 1988, Krog et al 1989, Cameron and Donald 1991, Slade and Levine 1991), have been most often implicated in bread staling. For example, Russell (1987) followed the staling of bread using differential scanning calorimetry (DSC) by observing an increase in the starch retrogradation or the so-called staling endotherm ≈60°C with storage, a phenomenon similarly observed in aging starch gels. Rogers et al (1988) have shown that retrogradation (due to amylopectin) could be influenced by the presence of water, which would result in varying firming rate. It was also suggested that bread firming increased as water migrated from crumb to crust in aging samples (Bushuk and Mehrotra 1977, Leung et al 1983, Ablett et al 1986, He and Hoseney 1990). Wynne-Jones and Blanshard (1986) showed that "bound" water slightly increased while "free" water decreased upon aging of bread. It has been proposed that as the bread ages, moisture from gluten is incorporated into the crystalline starch structure (Leung et al 1983, Wynne-Jones and Blanshard 1986, Slade and Levine 1991). Kim Shin et al (1991) using O^{17} NMR contested this theory, proposing instead that the redistribution of water occurs in the amorphous phase.

Recently, the cause of bread staling and the role of water have been attributed to the changes in molecular motion of polymer chains from a glassy (rigid) state to a rubbery (flexible) one characterized by a single temperature, the glass transition temperature, (T_g) (Slade and Levine 1991). The T_g of bread has been measured by various methods such as DSC (Slade and Levine 1991), a modified thermomechanical analysis (TMA) (LeMeste et al

1992), and dynamic mechanical analysis (DMA) (Hallberg and Chinachoti 1992). In each case, the T_g of the bread decreased with moisture because water was acting as a plasticizer. In addition, a single temperature was used to designate the transition region (tan δ peak in DMA, DSC peak temperature, and initial softening temperature in TMA). However, the transition region for bread spans a range of temperatures (which may indicate more than one transition) and, therefore, a single temperature is not adequate to describe the event taking place. In addition, as Hallberg and Chinachoti (1992) pointed out, the tan δ curve of fresh bread occurs concurrently with ice melting, thus the contribution of water to the transition needs to be ascertained.

In this work, DMA was used because it monitors textural changes and is theoretically 1,000× more sensitive in observing thermal transitions than is DSC (Wetton 1986, Rotter and Ishida 1992). The tan δ is often followed when using DMA because E' and E'' are highly sensitive to sample geometry (Hallberg and Chinachoti 1992, Rotter and Ishida 1992). Unfortunately, like other thermal analysis data interpretation, indication of the onset, midpoint, and final temperatures can be subjective, particularly in the case of DMA. The use of the tan δ peak temperature as the designation of T_g, for instance, has been suggested to be erroneous, particularly for biomaterials with a great degree of heterogeneity (Peleg 1995) when the entire transition is composed of a distribution of T_g values. To circumvent these problems, the entire thermal event should be accounted for and characterized. Charlesworth (1993) and Rotter and Ishida (1992) have used different curve-fitting models to describe the tan δ curve. When utilizing these techniques, some previous knowledge of the possible events that contribute to the curves is required (Charlesworth 1993), especially when dealing with complex heterogeneous systems such as bread. In addition, the designation of the number of deconvolution curves to be used must be done carefully (Rotter and Ishida 1992) because it is intuitively evident that a greater number of curves will improve the "goodness of fit" but may have no tangible meaning. The parameters obtained from these curves can be used to compare different treatments such as storage time or moisture contents.

The objective of this study was twofold: 1) to characterize the white bread thermal transitions depicted by the tan δ curve in

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DMA using a deconvolution curve fitting program; 2) to examine the thermal transition arising from drying and aging and comparing the DMA results.

MATERIALS AND METHODS

Materials

White bread was formulated according to the straight-dough formula (Table I). The rapid bake method (Bread Bakery model SD-BT51P, Panasonic, Secaucus, NJ) was used. The mixing time was 20 min, the rest time was 5 min, and the knead period was 5 min. The dough was allowed to rise for 50 min, followed by a second rise period of 40 min, and then it was baked at 160°C for 45 min. For storage experiments, bread was made by a straight-dough method (Nussinovitch et al 1991) with 0.25% potassium sorbate and 0.15% calcium propionate, both on flour basis (added as mold inhibitors). All loaves were left to cool for 1 hr before further analysis. The bread showed moisture content of 38 ± 1%. Because the two methods were somewhat different in time and temperature used during preparation, the two breads might be different. The greatest concern was the degree of starch gelatinization. DSC runs after baking showed no sign of crystalline starch present, indicating that all the starch was gelatinized. The DMA thermograms were similar.

Methods

Each loaf of bread was sliced to 1.3 cm thickness with a rotary meat slicer. The three center slices were used for analysis. After removing the crust, the crumb was compressed with a Carver press at room temperature to 1.5 mm thickness using a metal guide or spacer. For the storage experiments, the bread was stored in polyethylene bags at 25°C for up to 19 months before slicing. At least three replicate samples were used for each analysis and the data were averaged as described below.

DMA Analysis

DMA applies stress in a sinusoidal wave pattern as the sample temperature is increased. The responding strain frequency is either in phase with the stress (ideal elastic material) or out of phase (viscous material). For viscoelastic material, stress and strain are out of phase by a sinusoidal angle delta (δ); tan δ can be calculated as the ratio between the loss modulus (E'') and storage modulus (E').

The pressed sample (1.5 mm thick) was cut with a die into a 50- × 12-mm rectangle. The moisture content of the bread samples was adjusted with a desiccant (Drierite) for 0, 1, 4, 8, 12, and 24 hr at ambient temperature. For some samples of higher moisture content (28–38%), the moisture content was adjusted by placing the sample in a vacuum oven (3.05 KPa absolute or 98.25 KPa vacuum) for 0–2 hr at room temperature. For samples with lower moisture content, adjustment of moisture was done slowly by placing them in a desiccator (with Drierite) to avoid drying in a vacuum oven, which resulted in case hardening and easily cracked samples. The samples were immediately placed in hermetically sealed glass cylinders until testing ($\approx$2 hr) to avoid moisture loss.

TABLE I
Straight-Dough Bread Formula

Ingredients Added	% Wet Basis
Flour	55.9[a]
Water	34.9
Sugar	4.59
Shortening	2.6
Yeast	1.06
Salt	0.95

[a] 12% Moisture.

A sample (50- × 12- × 1.5-mm) was inserted into the sample holder with a three-point bending head of a DMA instrument (DMA 110, Seiko Instruments, Torrance, CA) and locked down with thumb screws. Cooling rate was $\approx$10°C/min. When the sample reached $\approx$ –40°C, the screws were tightened only to sufficiently hold the sample in place. The sample was then further cooled to –80°C before it was heated to 180°C at a rate of 2°C/min. During the heating process, a sinusoidal force was applied to the bar over a range of preselected frequencies (1, 5, 10, 50, and 100 Hz). The results were recorded as the storage modulus (E'), the loss modulus (E''), and the tan δ (E''/E'). A more detailed description of this analysis for bread can be found elsewhere (Hallberg and Chinachoti 1992). At least three replicates were tested.

Characterization of DMA Transitions

The tan δ curve obtained (1 Hz) showed the possibility of multiple transitions and thus was deconvoluted using PeakFit (Jandel Scientific, San Rafael, CA). The peak height, width at half height, and % area were recorded and compared for the different moisture contents. The degree of fit was determined by r^2. Average thermogram of the replicates was obtained manually from E' and tan δ at a given temperature. Standard deviations for a value of tan δ and E' were 0.018 and 337 Pa, respectively.

DSC Analysis

The moisture content of the samples was adjusted as for the DMA samples. Eight to ten milligrams of sample were placed in a stainless steel hermetic sample pan (Perkin Elmer, Somerset, NJ). The sample and an empty reference pan were positioned in the DSC4 (Perkin Elmer, Norwalk, CT), and the chamber was cooled to –30°C at $\approx$25°C/min. The chamber was then heated to 100°C
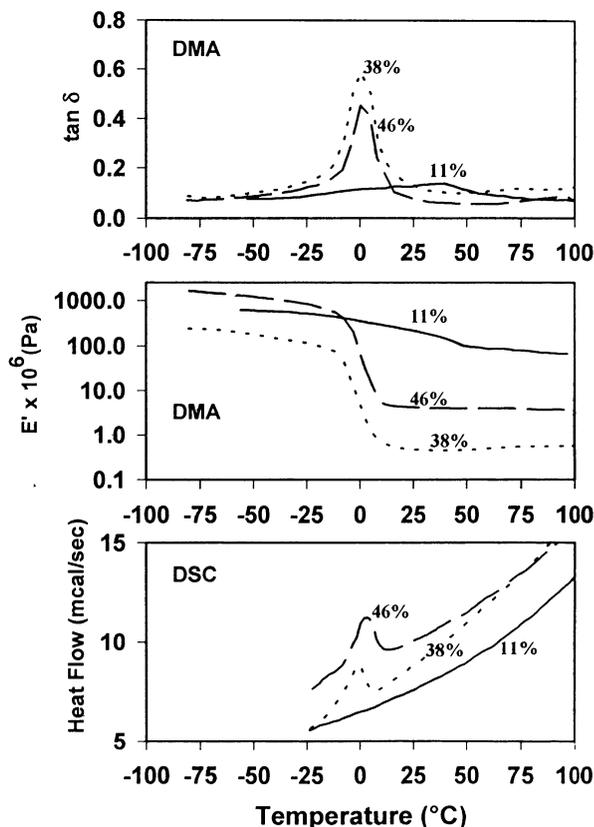
Fig. 1. Dynamic mechanical analysis (DMA) and differential scanning calorimetry (DSC) thermograms of fresh white bread conditioned to 11, 38, and 46% mc, indicating a broadening of the transition (DMA) once freezable water has been removed (11% mc).

at 10°C/min, and the ΔH of the endothermic transition recorded. The DSC instrument was calibrated using indium. Average thermogram of three replicate samples was calculated manually from heat flow at a given temperature (standard deviation 0.6 mcal/sec/g).

Moisture Content Determination

The moisture content of the bread samples subjected to above treatment (drying and storage over the period stated) was obtained from weight loss upon drying following the vacuum oven method (AOAC 1973).

RESULTS

Effect of Moisture

The averaged DMA and DSC results of white bread samples at different moisture content are shown in Figure 1. DSC measurements of white bread resulted in a single melting endotherm ranging from −25°C to 14°C (Fig. 1c) whose area decreased with moisture and therefore was attributed to ice melting. Below 33%, w/w, water remained unfrozen during DSC, while at >33% moisture, the ice melting increased linearly with an increase in moisture content (Fig. 2). According to Slade and Levine (1988), a moisture content of ≈27% is sufficient to fully plasticize starch after gelatinization and, therefore, moisture above this is phase-separated and freezes easily upon lowering of temperature to sub-zero. The rest of the water, because of freeze concentration, freezes at a much lower temperature (Mannheim et al 1957).

The E' values decreased with increasing temperature, particularly at 38 and 46% moisture. This degree of decline in E', or the slope, decreased with decreasing moisture content (Fig. 1b). The abrupt drop in E' in the high-moisture samples occurred in the ice melting region and, therefore, includes the contribution from the phase-separated ice which undergoes the change from a rigid solid to a flowing liquid once melted. LeMeste et al (1992) and Hallberg and Chinachoti (1992) also attributed part of the transition region for bread at >25% moisture to the melting of ice. As the moisture content decreased to <33% moisture (e.g., the 11% moisture sample in Fig. 1b), ice was absent and the existing water resulted in a broad transition at higher temperature range. In this case, E' became a relatively flat line (Fig. 1b, 11% moisture).

Fig. 2. Enthalpy of endotherm in ice melting region for standard white bread at different moisture contents obtained from differential scanning calorimetry (DSC).

Biliaderis and Gallway (1989) and Zeleznak and Hoseney (1987) found that the minimum moisture content sufficient to completely plasticize starch was ≈30%. Thus, from the E' and DSC data, full plasticization by water results in a rather broad E' spread of transition temperatures with no ice melting, as compared to the sharper E' transition temperature when there is ice melting (Fig. 1).

In a complex composite material, such as bread, it might be possible that components other than water are responsible for the thermomechanical changes observed above. For instance, some small amount of sugar present in the bread would have had some additional freezing point depression effect. Evidently, freezing or ice crystal formation of water has been reported to continue to occur even at temperatures as low as −50°C (Mannheim et al 1957). Thus, it is possible that ice melting transition could start at a very low temperature (< −25°C). Additionally, presence of other components such as fat or shortening, although not plasticized by water and considered phase-separated from most components, can undergo a melting transition. Shortening (Crisco) showed two melting transitions at ≈−13 and 48°C (peak temperatures), and it might be possible that the −13°C peak was partly responsible for the tan δ peak and E' drop around what we assigned as the ice melting transition. It is highly unlikely that this was the case. First, shortening was present in such small amounts that its contribution (calculated from the endothermic melting energy) would be 0.16 cal/g of sample as compared with the total endothermic energy observed, 11.4 cal/g of sample for fresh bread at 39% moisture, or ≈1.4% of the total. Additionally, the endothermic melting peak at 48°C in bread was not observed at all, and recrystallization of a higher T_m (melting point) fat is theoretically faster than that of a lower T_m fat at room temperature. Thus, in the case of bread stored at room temperature, absence of higher T_m fat endothermic peak means that it was highly unlikely that the lower T_m fat would be present. In drier samples, such as the 11% moisture sample in Figure 1, absence of ice melting confirms that no fat melting peaks due to shortening was present.

Figure 3 represents the tan δ of fresh bread as affected by moisture. Three replicates (solid line) and their average results (closed symbols) are shown. It is clear that there was some degree of variation among samples; the temperature range for the transition differed slightly more among replicates with lower moisture content.

At >30% moisture, the tan δ curve (Fig. 1a) ranged from −25°C to 25°C, which could be considered to be mainly due to ice melting. At moisture well below 30% (Fig. 1a), samples resulted in a very broad transition (100°C spread in 11% moisture sample) at much higher temperatures (peak temperature far above 0°C). At intermediate moisture, ≈25-30%, the tan δ peak was similar to higher moisture samples (Fig. 3b). The increase in the span of the transition region with decreased moisture content was also observed in bread by Hallberg and Chinachoti (1992) and by LeMeste et al (1992), and in synthetic polymers by Brinke et al (1983). Therefore, in the presence of ice (phase separation), the transition observed by DMA was a sharp and relatively rapid process within the time frame of the experiment. We observed a single E' drop and tan δ curve slightly skewed on the left (low temperature) side of the peak (Fig. 1a, also see peak deconvolution below). It was thus concluded that this transition contributed mostly to ice melting. The skewed peak to the left needed to be further characterized.

It has been mostly agreed that a small but significant amount of water remains unfrozen at lower temperatures. Mannheim et al (1957) reported that this water in bread continued to freeze at subzero temperature and did not completely freeze until −60°C. Thus, this could lead to the skewed tan δ peak to the left (lower temperature side). Recently, it has been proposed by many (Simatos et al 1989, Goff 1992, Simatos and Blond 1992) that freeze-concentrated matrices showed a glassy-rubbery transition with temperature T_g or T_g' (for maximally freeze-concentrated
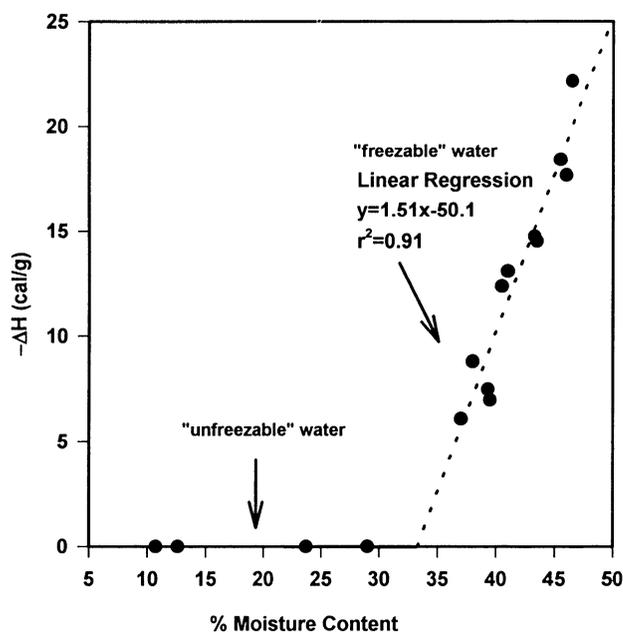
solution or matter). Thus, it was possible that this T_g fell at subzero temperature before the main ice melting transition. But any plasticization by the unfreezable water or so-called plasticizing water (Slade and Levine 1991) must have been relatively small and overlapping with the ice melting transition. The peak deconvolution exercise below shows our attempts to characterize the left shoulder (skewed) of the tan δ peak as this might shed some light on this issue.

On the other hand, in absence of ice melting, the transition observed was broad. DMA measures the segmental mobility of polymer chains (Murayama 1978) and, therefore, an increased temperature range for a transition signifies an increase in the heterogeneity of segmental mobilities. Thus, our low moisture data indicated a great degree of domain heterogeneity, each hydrated and plasticized differently. Since bread is complex and the microenvironment of each domain is not known, no specific conclusion can be made without further evidence on the molecular level. It is also interesting to note that the extent of the E' change observed in bread (in the absence of ice) is less than one order of magnitude (Fig. 1b, 11% moisture), not as speculated earlier (Slade and Levine 1991). Note that the absolute value for E' was not comparable among samples because the values are highly dependent on sample geometry and density; thus, such comparisons are not recommended (Hallberg and Chinachoti 1992, Rotter and Ishida 1992).

Due to the broad range of temperatures involved, the thermomechanical transition observed cannot be adequately represented by a single parameter such as the tan δ peak temperature. Therefore, to better characterize the transition region, deconvolution of the tan δ curve was performed. Although it might be speculative and some guesswork may be involved for picking the appropriate deconvolution curves, various combinations were explored using the least number of curves before accepting a particular set. In addition, thermal transitions (such as glassy to rubbery transition) are expected to be symmetrical, e.g., Gaussian or Lorentzian (Rotter and Ishida 1992, Charlesworth 1993). However, the high moisture (>30%) tan δ curves could not be properly deconvoluted with one or two symmetric curves, but instead required an asymmetric contribution on the left (low temperature) side of the tan δ peak (Fig. 4a). The symmetrical Gaussian and the asymmetric double sigmoidal curve (Fig. 4a) were found to best fit the experimental tan δ values. For those samples with moisture content <30%, the tan δ curves were found to be of a symmetrical Gaussian shape and deconvolution was not necessary (Fig. 4b).

For the case of high moisture content (>30%), two deconvoluted tan δ peaks were evaluated. The parameters of peak amplitude, width at half amplitude, and area of the tan δ were plotted for each of the curves: the two for high moisture (symmetric Gaussian and asymmetric curves) and the one for low moisture (Gaussian). The high-moisture and low-moisture deconvoluted curves were treated separately as the high-moisture curves had a contribution from ice melting (as was seen with the DSC data). Figure 5a–c shows the results obtained for the deconvoluted Gaussian curve of the high-moisture samples (see Fig. 4a). The width at half amplitude was small (<20°C wide) when compared to the Gaussian peak in the low-moisture samples. The peak amplitude and area (Fig. 5b and c, respectively) showed no relationship with moisture content. This was somewhat unexpected because this Gaussian curve corresponded to the main ice melting process (–20 to 10°C) found by DSC to increase with moisture content (>30% mc range). Bread is a heterogeneous system consisting of starch and gluten polymers in structurally diverse domains (LeMeste et al 1992). As ice melts, E' is expected to drop accordingly, and the magnitude should be proportional to
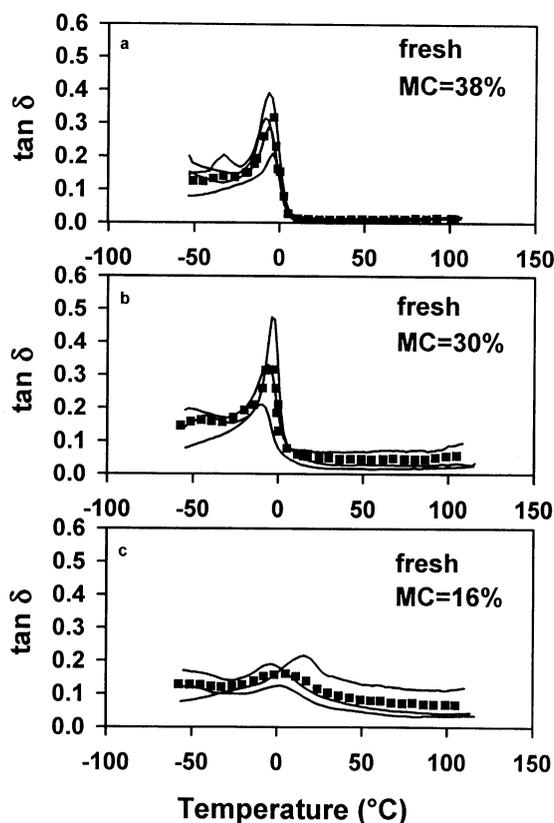
Fig. 3. Dynamic mechanical analysis (DMA) tan δ results of fresh white bread at different moisture contents. Solid lines represent individual experiments; squares represent averaged results.
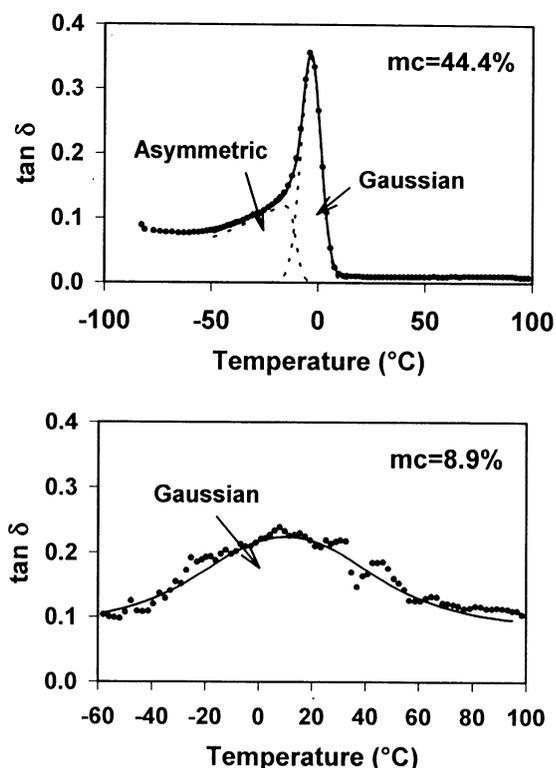
Fig. 4. Deconvolution of tan δ curves of fresh white bread conditioned to 44.4 and 8.9% mc. Circles represent experimental data points; solid line is the resultant curve of the deconvoluted (dotted line) curves. Resulting tan δ curves with moisture range above freezable water (44.4%) were best fitted with an asymmetric double sigmoidal curve and a Gaussian curve, while those below freezable water content were deconvoluted by a single Gaussian curve.

the amount of melted ice. However, the phase-separated water or ice in a discontinuous phase (as a filler) might be located and distributed in the matrix differently, depending on the nucleation and growth kinetics. In the case where nucleation is promoted, the presence of a large number of small ice crystals may have a different thermomechanical impact upon melting as compared with the case when growth is encouraged and the ice crystals present are larger in size but smaller in number. The scatter in the tan δ height and area may indicate differences in the ice distribution as well as the intrinsic differences among samples. We noted that tan δ peak amplitude in the 35–40% moisture range varied greatly among samples (Fig. 5b). Additionally, the structural components (starch and gluten) are expected to serve as the continuous phase with phase-separated ice crystals embedded within serving as a discontinuous phase. Perhaps when the ice melted, these pockets of water turned from solid to liquid, which could contribute to E' and E'' differently (E' would approach zero while E'' would tend towards a maximum due to viscous flow). However, the instrument continued sensing the structural continuous phase (polymers).

In these high-moisture samples, the left shoulder of the tan δ peak was deconvoluted as an asymmetric double sigmoidal curve (data not shown, see Fig. 4a for example). The origin of this part of the curve is likely to be ice melting, but other possibilities have not yet been ruled out. It is known that polymers normally exhibit a β transition before an α transition; the former is due to the increased mobility of the side chain motion (Aklonis and MacKnight 1983). One might also speculate that this transition range may be attributed to T_g of a freeze-concentrated system (Slade and Levine 1992). If so, this transition is expected to be Gaussian or Lorentzian in shape (Charlesworth 1993, Rotter and Ishida 1992) but the curve was not. It is possible that as the onset of ice melting began, the transition was disrupted and terminated abruptly. However, the possibility exists that this transition could also be due to relatively imperfect ice crystals that melted and formed a more perfect crystal of a higher melting point. As the sample was heated, because of the melting and recrystallizing

into a more stable crystal form (higher T_m), there was a redistribution of melting points. As a result, the first melting process ended abruptly as the onset of melting of the more stable crystal began. In addition, the entire (undeconvoluted) tan δ curves at various moisture contents (10–46%) were found to be frequency independent (Fig. 6), which seems to indicate a melting phenomenon rather than a glassy-rubbery transition. This is because a first-order transition (such as melting) is frequency independent while a second-order (glass transition) thermal event is highly frequency dependent (Murayama 1978). Thus, we concluded that it is more likely that both transitions were dominated by (if not entirely due to) the ice melting phenomena. However, further studies need to be done on a molecular level (such as solid state NMR) to further verify this explanation.

For the low-moisture content samples, the Gaussian curve parameters were plotted against moisture content (Fig. 5d–f). As the moisture content decreased from ≈30%, the tan δ curve width increased and its amplitude decreased (Fig. 5d and e, respectively) as a result of tan δ broadening at lower moisture. As discussed earlier, this was probably due to an increase in the degree of heterogeneity in the transition temperatures in different microenvironments. It is not clear what information is related to the area (Fig. 5f) but it was interesting to observe an increase in the peak area with decreasing moisture content. It should be noted that this transition was also found to be frequency independent (10% mc) (Fig. 6) unlike any typical glassy-rubbery transitions. There is no explanation to this phenomena except that the samples were highly heterogeneous and interpretation of such data with up to 100°C spread is impossible without additional information from other analytical methods, such as NMR, ESR, or X-ray diffraction.

Effect of Aging

The tan δ curves of bread stored for up to 19 months (with moisture loss) are shown in Figure 7. The tan δ increased in temperature, decreased in amplitude, and increased in breadth with aging similar to the loss of moisture as discussed above. Aging of
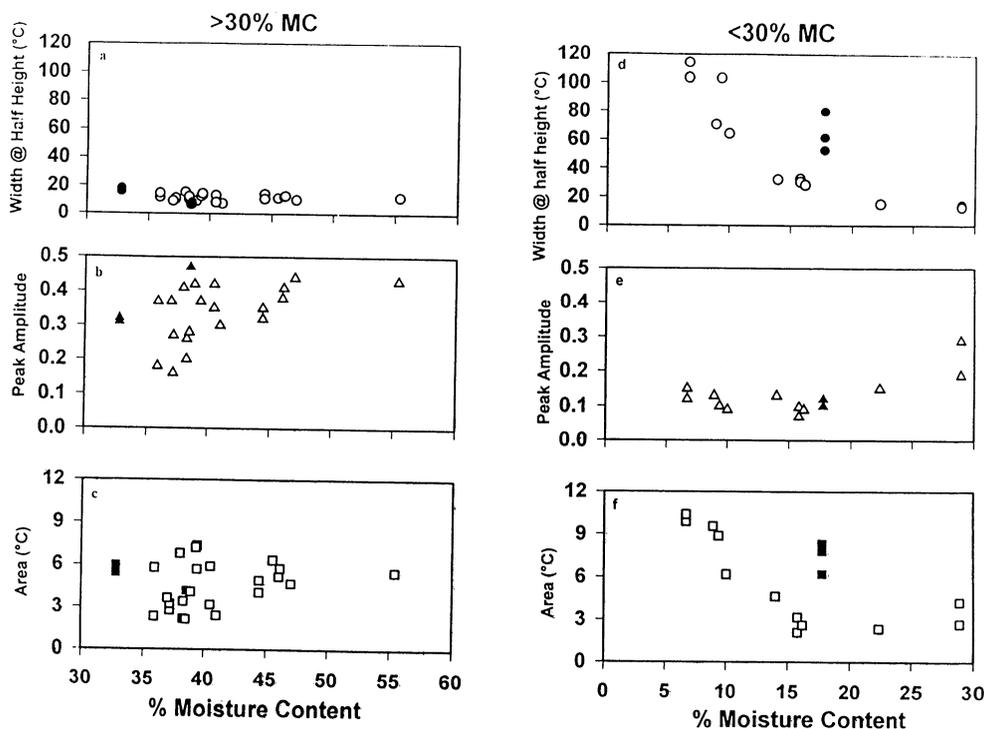
Fig. 5. Fitted parameters of width at half magnitude, peak amplitude, and area of both stored and fresh, dried standard white bread obtained from analysis of the tan δ deconvoluted Gaussian curve for high-moisture (>30%) and low-moisture (<30%) samples. Fresh bread (open symbols); aged bread (closed symbols).

bread resulted in somewhat broader curves, particularly at lower moisture (19 months, Fig. 7c). The sample stored seven months (33% moisture, Fig. 7b) showed a larger transition temperature range than the fresh sample but fell in the same general range of temperatures (Fig. 7b).

It has been postulated by some researchers (reviewed by Kulp and Ponte 1981) that aging of bread involved the formation of a network or structured gel that reached a maximum resulting in the highest mechanical strength. Additionally, Slade and Levine (1992) proposed that bread polymers (mainly starch) played a critical role in bread T_g, and that during aging or staling of bread, network formation in the amorphous domains (during starch retrogradation) resulted in an increased T_g (Slade and Levine 1992). One of the reasons for such an extended aging period used here was to allow time for such a phenomenon. In this work, samples were measured every other day during the first week, then once every week for a one-month period (data not shown). During this first month of aging, no significant change in thermograms was observed, and only slight broadening was detected during the first six-month period. This is beyond the normal shelf-life of bread. Yet, based on DMA results, no network formation was observed. After an extended storage, broadening of tan δ was detected only when moisture in the sample was depleted below the freezable water range as seen in the transition for the samples stored 19 months (18% moisture, Fig. 7c) with a span of >150°C. In addition, the deconvolution parameters of width at half amplitude and area showed a significant deviation of the stored samples from dried, fresh sample of the same moisture. The very drastic shift to the broad tan δ in the aged sample occurred at moisture below freezable water range and when aged very extensively.

There might be a question raised whether the broadening in the tan δ peak at lower moisture content was partially due to moisture loss driving off the plasticizer and raising the transition temperature. We ran a separate experiment to measure the moisture loss at various operating temperatures (by weight loss) and found that
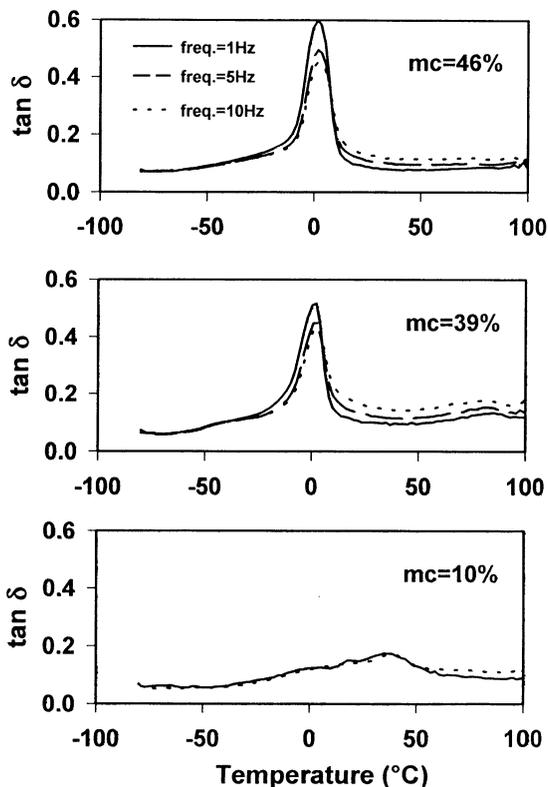
for low moisture (<30% moisture), the weight loss started to become significant when temperature was raised >50°C. It is possible that a transition occurring >50°C could be influenced by drying of the samples, resulting in an increased transition temperature. Therefore, further work should be done to confirm the value before it is used.

It should be mentioned that dehydrated samples were subjected to a short resting time (6 hr) before each DMA analysis. Therefore, there could be a considerable lack of equilibration among regions in the sample. This and possible moisture loss discussed above could have attributed to the broad transition observed in a low moisture range. Consequently, we ran samples at different resting times after desiccation. Resting times of 0–4 hr gave irreproducible results. Resting times of 6–10 hr were more or less reproducible (Fig. 3). The narrower distribution in tan δ values of dried fresh bread as compared to that of aged bread of the same moisture (Fig. 7) could have been due to many differences among samples, but this is unlikely due to the lack of equilibration.

It could be concluded here that the different domains in the amorphous phase (rather than the lack of water equilibration) caused the observed additional broadening of tan δ. It was noted that individual tan δ replicates for the samples stored 19 months (18% moisture, Fig. 7c) showed an indication of overlapping double peaks (although the averaged tan δ curve did not show this), possibly suggesting additional events, distributions of microenvironments, or heterogeneity. There could be other factors, such as amylopectin recrystallization and gluten crosslinking, etc. These changes monitored by DMA need to be further studied.

CONCLUSION

In conclusion, the characterization of the thermal transitions occurring in bread, as depicted by the tan δ curve, using deconvo-

Fig. 6. Tan δ curves of varying frequency for standard white bread conditioned to 46, 39, and 10% mc.
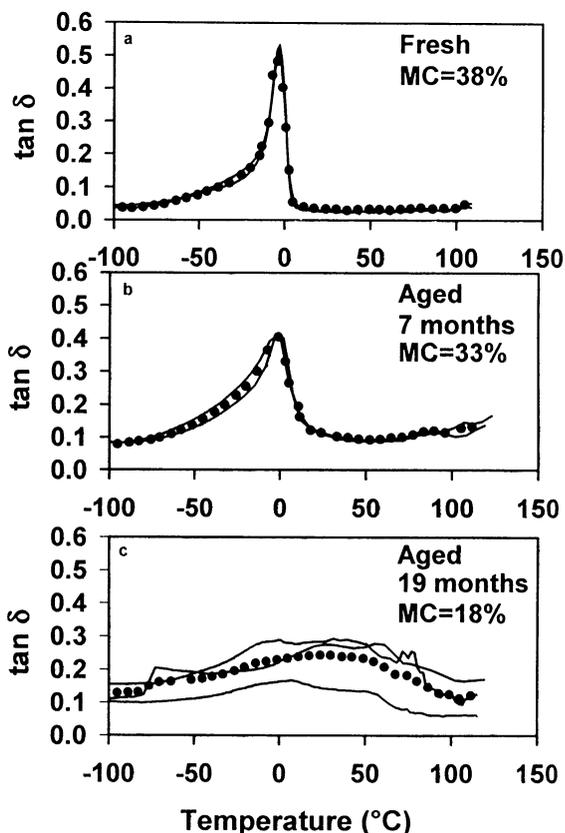
Fig. 7. Dynamic mechanical analysis (DMA) tan δ results of fresh white bread stored for different lengths of time. Solid lines represent individual experiments; circles represent averaged results.

lution techniques to give a range, width, and area of the tan δ curve, provided more information about the nature of the transitions. Ice melting transitions seemed to dominate the thermomechanical events at higher moisture range (>30%) when freezable water was present. In absence of freezable water, the transition became broad and relatively small in magnitude, showing a great degree of heterogeneity. Upon aging, an extremely broad (up to 150°C) transition was observed. Further work on a molecular scale is obviously required to identify the origin of the transition regions. None of the DMA transitions observed here were highly frequency dependent. In lower moisture samples, E' drop was very small (less than one order of magnitude); no change in Cp under DSC and a great degree of heterogeneity was observed. This could be due to many complicated factors relating to the samples, the experimental conditions, and the nature of starch and gluten. It is clear, however, that a glassy-rubbery transition in such biopolymers does not exist as a single event but rather a distribution of many minor events. This is expected, given the heterogeneous microenvironments in starch (amylose, amylopectin) and gluten (gliadin and glutenin), and the knowledge that starch and gluten are hardly miscible with one another based on microscopic image analysis (Vodovotz and Chinachoti, *unpublished*) and NMR data (Li, *unpublished*).

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