



## Probing molecular and structural thermal events in cereal-based products

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### Abstract

Thermal and molecular analyses of cereal food systems have been considered as a new approach to clarify these complex systems. The roles of glass transition, crystal melting, phase separation, and molecular mobility and interaction, are of great interest. The starch melting and glass–rubber transition, for instance, are complex, biphasic phenomena. NMR, X-ray diffraction, and many other methods have brought out new evidence and arguments for different models. Glass–rubber transitions of gluten and its components have been reported by various techniques. Thermal events in bread at various moisture levels observed by DSC, DMA and TMA are compared in terms of rheological properties, volume expansion, and melting behavior. The staling of bread is described as a function of storage time and moisture loss.

*Keywords:* Cereal; DMA; DSC; Glass transition; Melting; Model; Starch; TMA

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### 1. Introduction

In recent advances in the study of cereal-based food systems, thermal events, such as melting and glass transition, have been of much interest to food scientists in both industry and academia. The structural components responsible for these products (mostly starch and gluten) are the key factors that determine the desired textural quality, e.g. crunchiness, softness/moistness, and hardness, etc. In addition, deterioration of texture is often a common subject for study in terms of molecular mechanisms so that it can be minimized or inhibited. Thus, characterization of cereal biopolymers by thermal analysis is an important approach to the understand-

ing of the functionality of starch and gluten on a structural and molecular level. Much of the background theories of various thermal analyses have been reviewed, see, for example, Refs. [1] and [2]. Detailed component functionality in cereal systems can be found elsewhere [3,4].

Because most cereal systems are complicated, with combinations of many functional ingredients, the study of problems such as starch gelatinization and retrogradation, and gluten functionality on structural and molecular levels, has been difficult and inconclusive. In many cases, this is due to an inability to exploit a multiple approach including the techniques of thermal analysis, microstructure and texture analysis, and molecular spectroscopy. If used and interpreted appropriately, such application of a variety of techniques would lend itself to a far better understanding of the problems. This is a rather common practice in materials research, in which various techniques are used, including X-ray diffraction, nuclear magnetic resonance (NMR), thermal analyses, electron microscopy, electron spin resonance (ESR), and mechanical testing (e.g. Instron).

This paper describes examples of studies in which starch and gluten were investigated by thermal analyses, molecular spectroscopy, and other techniques. The results of such an approach have allowed scientists to begin to understand the molecular contribution and consequences of these biopolymers with respect to cereal products. The conclusions in this field are far from complete and much is to be done before we understand fully the relationships between molecular conformation (and its change), microscopic and macroscopic structure, and mechanical properties, as well as other important functionalities, such as barrier properties, puffing ability, and pro-/anti-staling quality.

## 2. Thermal and molecular events

The most common analytical methods used to characterize melting or the glass–rubber transition are thermal analysis techniques. In cereal-based products, differential scanning calorimetry (DSC) has been used extensively, mostly to determine the degree of melting of starch upon heating. The study of starch gelatinization by this technique has been well documented [5] in terms of the effects of moisture, sugars, lipids, starch granule size, etc. Yet, the molecular mechanism of starch melting is still subject to debate, partly due to conflicting results. The DSC melting endotherms of starch upon gelatinization and the supporting evidence from loss of birefringence and crystallinity are inconclusive.

Starch melting endotherms have been reported to be biphasic and the change in heat capacity ( $c_p$ ) indicates a glass transition followed by a melt [6]. According to some, the glass transition that occurred prior to the melt is a glass–rubber transition of the amorphous regions of the granule; completion of the glass transition allows the crystalline regions to undergo a non-equilibrium melting, resulting in the second peak [7,8]. Therefore, by observing molecular order using X-ray diffraction or birefringence, it would be expected that starch molecules maintain most of their crystalline order during the first thermal event and are

totally destroyed upon completion of the second. However, there seems to be some contradiction in the X-ray diffraction results. While Maurice et al. [7] reported little loss in ordered structure after the first transition, Liu et al. [9] reported that melting accounted for both transitions and that the thermal expansion derived by thermomechanical methods was due to the increasing amount of amorphous starch rather than to a glass transition followed by a melt. It is plausible that the biphasic behavior follows the model suggested by Evans and Haisman [10], i.e. due to the moisture gradient, starch fraction(s) with higher moisture might melt at a lower temperature as compared to the fraction(s) with lower moisture content, resulting in double melting peaks. Nevertheless, some mobilization of amorphous regions has been suggested to precede melting [11]. However, it has also been suggested that the description of the regions, where individual chains or two or more chains form ordered structure, as being either amorphous or crystalline is not appropriate [12]. In addition, it has recently been pointed out by Slade and Levine [13] that penetration of water into starch granules does not necessarily mean plasticization.

It has been speculated that a thermally induced rearrangement of the hydrogen bonding may be involved in the endothermic event at 50–70°C [14] but infrared spectroscopy data before and after a 70°C treatment (of potato starch, guar gum, and kappa carrageenan) showed no difference in spectra, indicating no significant hydrogen bonding rearrangement [12]. This is because infrared spectroscopy detects short-distance (atomic) changes but DSC is a long-distance measurement of polymer connectivity and cooperativity.

In this area of research, it is important to appreciate the fact that most granular starch shows a high degree of heterogeneity, e.g. granular size, varying starch composition, etc., which is a potential problem in quantitative analysis of the data. It is, therefore, necessary to undertake a multiple technique approach. Unfortunately, the various techniques described above measure different physical properties. For example, combining X-ray diffraction and CP/MAS <sup>13</sup>C NMR (cross polarization/magic angle spinning carbon-13 nuclear magnetic resonance) techniques may be a powerful approach to investigate molecular arrangement or rearrangement which DSC cannot do. However, these two methods of analysis detect changes in different regions of starch, in that NMR spectroscopy detects a short-range organization level of molecules, e.g. the double helix content, while X-ray diffraction detects double helices that are packed in arrays. As a result, the degree of ordered structure has been reported to be higher when observed by NMR than when observed by X-ray diffraction [15]. Utilizing the capability of X-ray diffraction to measure long-range configuration of retrograded starch, Cameron and Donald [16] applied small-angle X-ray scattering (SAXS) to measure the size and development of starch crystal nucleation. Interestingly, they compared SAXS and DSC results and concluded that the driving force for nucleation was higher at 4°C (by DSC endotherm) so that starch crystallized faster. However, the relatively lower melting temperature indicated that the crystals were less organized than those developed from starch aged slowly at room temperature. It should be kept in mind, however, that DSC is more sensitive to amylopectin crystalline melting than amylose [17] and X-ray diffraction measures the crystalline structure of amylose and amylopectin combined [15].

Translation of the molecular events observed under NMR, X-ray diffraction and DSC into the net rheological impact is often difficult with respect to quantitative correlation of the measured parameters. It has been pointed out [5,18] that starch recrystallization observed under X-ray diffraction and DSC does not necessarily imply an increase in firmness of bread during storage.

In addition, while much emphasis has been placed on starch and its complexes with other ingredients, the role of gluten during bread staling has not been much addressed, even though it contributes to the amorphous components forming the continuous phase or matrix in which starch granules are embedded. An example of the importance of the amorphous components has been discussed by Levine and Slade [19]. They reported a possible role of gluten in the freeze–thaw stability and baking performance of frozen bread dough, suggesting that, by selecting specific flour fractions or by blends, one can improve the freeze–thaw stability and baking functionality of frozen dough.

Thus, methodologies have been developed in bakery science to characterize the glass transition temperature of the amorphous counterpart in dough systems so that it would be possible to clarify its functionality and the role of ingredients, such as surfactants, lipid and humectants. Most of these methods are thermal analysis techniques, such as DMA (dynamic mechanical analysis), DSC, TMA (thermomechanical analysis) and molecular spectroscopic techniques, such as NMR and ESR (electron spin resonance). Theoretically, these techniques should complement each other in data interpretation and their combination offers a powerful way to identify unambiguously and to characterize a thermal event. For example, an endothermic, melting reaction observed by DSC may be used to explain a drop in storage modulus observed under DMA, and NMR may help to investigate the molecular mobility change during such an event. Combined use of DSC and DMA can be found useful in differentiating a glass transition phenomenon from a melting event. In addition, unlike DSC, DMA can measure rheological changes although it is generally limited to extremely low strain levels and to a specific sample geometry.

With so much recent interest in thermal events in baked goods and dough systems, considerable attention has been paid to thermal analysis techniques, e.g., DSC, DMA, TGA and TMA, but very little to events on the molecular level. Because various interpretations of thermal analyses always imply molecular changes accompanying a glass transition or a melting event, developments in molecular spectroscopy to observe molecular changes during such events would be useful. In fact many of the approaches discussed above have already been utilized routinely in other fields, such as polymer science and materials research. Because of the complexity of dough systems, NMR and ESR have mostly been limited to model systems with specific interactions in mind. Therefore, the following discussion covers mostly the thermal analyses of cereal food systems.

Glass transitions in cereal polymers were first detected using DSC [20,21]. In both studies, the glass transition temperatures ( $T_g$ ) were reported to be within the same range, decreasing from approximately 160°C at 1% moisture to approximately 20°C at 16% moisture. A number of investigators later reported similar values with some variations; the values can deviate by as much as 40°C, depending on the

methods used (DSC or DMA) and the parameters used to indicate  $T_g$  [22,23]. Discrepancies in  $T_g$  values due to variation in measurement techniques, parameters, and conditions of the measurement, will be discussed later in this paper. Using DSC, Kalichevsky and Blanshard [24] reported that mechanical mixtures of starch and gluten (moistened, pressed at  $T < 50\text{--}60^\circ\text{C}$ ) showed distinctive transitions after a first scan; after a second rescan, they became miscible and a single transition was observed. Because the second scan was poor in resolution, it was debatable whether one or two transitions were present. This may have made a difference in answering the question whether, under certain circumstances, the two components can become miscible with respect to each other. In nature, the starch–gluten mixture that occurs in flour may not react in the same way as the mechanical mixture. The fact that only one transition has been reported means either that starch and gluten in flour coincide in their glass–rubber transition temperatures and cannot be differentiated by DSC, or that the two components are completely miscible. It would be rather difficult (although possible) to prove conclusively which is the case and it might require a specific experimental design and a more sophisticated molecular spectroscopic technique, such as NMR and ESR combined with some thermal analysis techniques.

Gliadin and glutenin have been compared in terms of their  $T_g$  values (data obtained from the same DSC method in same laboratory [25]; the transition temperature seemed to agree with an exception in the lower moisture range (3–7% moisture), when glutenin was  $20^\circ\text{C}$  higher in  $T_g$  than gliadin [25]. However, the transition temperatures observed by NMR showed more similarity among gluten, glutenin, and gliadin, and the values agreed with the DSC data [22]. While gliadin (a mixture of proteins of 30 000–100 000 daltons) is known to have sticky, extensible characteristics, glutenin (20 000–200 000 daltons) is resilient but not very extensible due to disulfide bonding [26]. Specific changes in these rheological properties as related to the glass–rubber transition have received much interest of late because it has been proposed that an understanding of these processes may help to solve problems, for instance relating to the functional properties of dough, e.g. the freeze–thaw stability of frozen bread dough, and microwave toughening and staling [13,19,27].

Because a typical glass transition method of analysis cannot differentiate starch from gluten, it is difficult to correlate quantitatively any glass transition temperatures to rheological or other physical functionalities of baked products. Much is yet to be investigated in the area of micro structures and the role of ‘fillers’, such as lipids and starch granules; most scientists believe that these components do not simply fill the volume but interact with other components. The presence of any phase-separated entities and their impact on the final food quality are also unclear. Because thermomechanical analyses allow scientists to observe thermal events from a mechanical point of view, their application to bakery products is relevant when food texture is of interest. The most beneficial application is probably when they are used in combination with other techniques.

DMA is one form of thermomechanical analysis where a sinusoidal stress of small amplitude (within the linear elastic range) is applied to a sample at a set

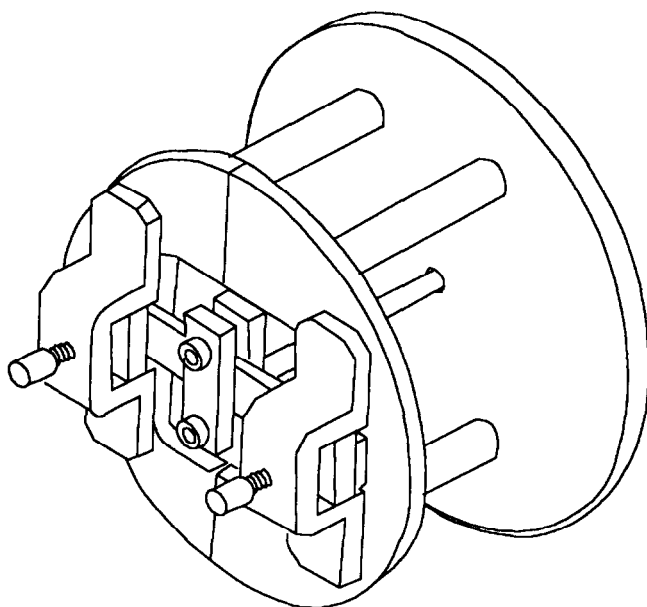


Fig. 1. DMA sample holder (courtesy of Seiko™ Instruments Inc.).

frequency or frequencies. The stress and strain are measured and recorded as the sample is heated at a given rate. The recorded parameters are stress, strain, time, temperature, and frequency. Fig. 1 is a schematic diagram of the DMA three-point bending module in which a bar-shaped sample is clamped at both ends and a sinusoidal stress is applied to the mid-point. Fig. 2 demonstrates the sinusoidal function of stress and the resulting strain over time. The phase difference is described as the phase angle,  $\delta$ . An out-of-phase response with a  $\delta$  of  $90^\circ$  represents viscous flow behavior and an in-phase response represents a truly elastic polymer. In viscoelastic materials, the  $\delta$  angle is between 0 and 90 degrees. Food systems show plastic, irreversible deformation if the elastic limit is exceeded. Thus, DMA

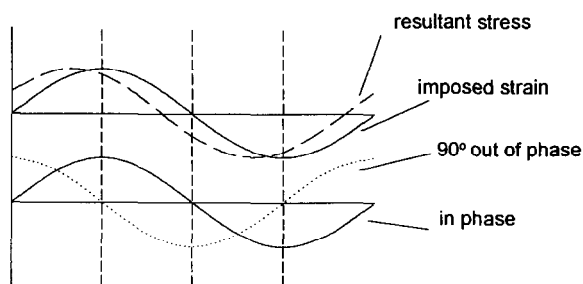


Fig. 2. Phase relationships between stress and strain. The storage modulus ( $G'$ ) is defined as the ratio of the in-phase stress and applied strain, the loss modulus ( $G''$ ) the ratio of out-of-phase stress and applied strain, and the loss tangent ( $\tan \delta = G''/G'$ ).

application is limited to a very small strain level when deviation from elasticity is relatively small. More detailed description of DMA can be found elsewhere [1]. The complex modulus ( $E^*$  or  $G^*$ ) is the stress/strain ratio which can be expressed as

$$E^* = E' + iE''$$

or

$$G^* = G' + iG''$$

where  $E'$  or  $G'$  is the in-phase component of the modulus,  $E''$  or  $G''$  is the out-of-phase component of the modulus, and  $i$  is the square root of  $-1$ .

Typically, the ratio between  $E''$  and  $E'$  is  $\tan \delta$ . Because  $E''$  represents the loss modulus and  $E'$  the storage modulus, their ratio ( $\tan \delta$ ) has been used to evaluate the loss process. This allows observation of the relaxation process as a function of time and temperature. The use of multiple frequency also allows further interpretations, such as dispersion frequency, correlation time, and calculation of the activation energy ( $E_a$ ). All of these expressions make this technique interesting in relating mechanical relaxation processes to microscopic or even molecular phenomena. However, it is extremely difficult to interpret unambiguously a macroscopic or rheological retardation or relaxation on a molecular level, i.e. bonding or conformational changes. Overlapping events result in an overlap of  $\tan \delta$  peaks, which can be deconvoluted mathematically by a peak analysis program [28–30]. Fig. 3 shows a deconvolution of fresh bread and a very staled bread of different moisture content. The  $\tan \delta$  peak in fresh bread has a left shoulder which may be a contribution from minor components in bread or a  $\beta$ -transition of the bread. The  $\tan \delta$  peaks in staled bread distinctively indicate two different phenomena; the one on the right is possibly related to the amylopectin melting peak.

The glass–rubber transition and melting observed by DMA is much clearer than when observed by DSC; but DSC is normally run in parallel with DMA so that endothermic or exothermic changes, as well as changes in heat capacity, can be identified and quantified.

TMA detects changes in thermal expansion while a stress is applied to the sample. Volumetric changes can be a result of melting, glass transition, and thermal expansion due to trapped gas or vapor inside the sample. Thus, interpretation of its data may require accompanying evidence from DSC or other methods.

Applications of DMA and TMA to cereal food systems have started recently. Shelf-stable bread (containing glycerol and a higher amount of lipids) at various moisture contents was studied by DMA and DSC [31]. Three transitions were found: one of glycerol (approx.  $-60^\circ\text{C}$  peak  $\tan \delta$ ), an ice melting (approx.  $-12^\circ\text{C}$  peak  $\tan \delta$ ) and a fat melting (approx.  $10^\circ\text{C}$ ). The transition responsible for the highest drop in storage modulus  $E'$  is that relating to the ice melting transition. In the case of white bread, this main transition was found to be in the approx.  $-2$  to  $-5^\circ\text{C}$  range [31]. It should be kept in mind that these transitions occurs over at least a  $10$ – $20^\circ\text{C}$  range and could cover as much as an  $80^\circ\text{C}$  or higher temperature range at lower moisture content. Using modified TMA (by applying a sinusoidal stress and observing the volumetric expansion upon heating), LeMeste et al. [32]

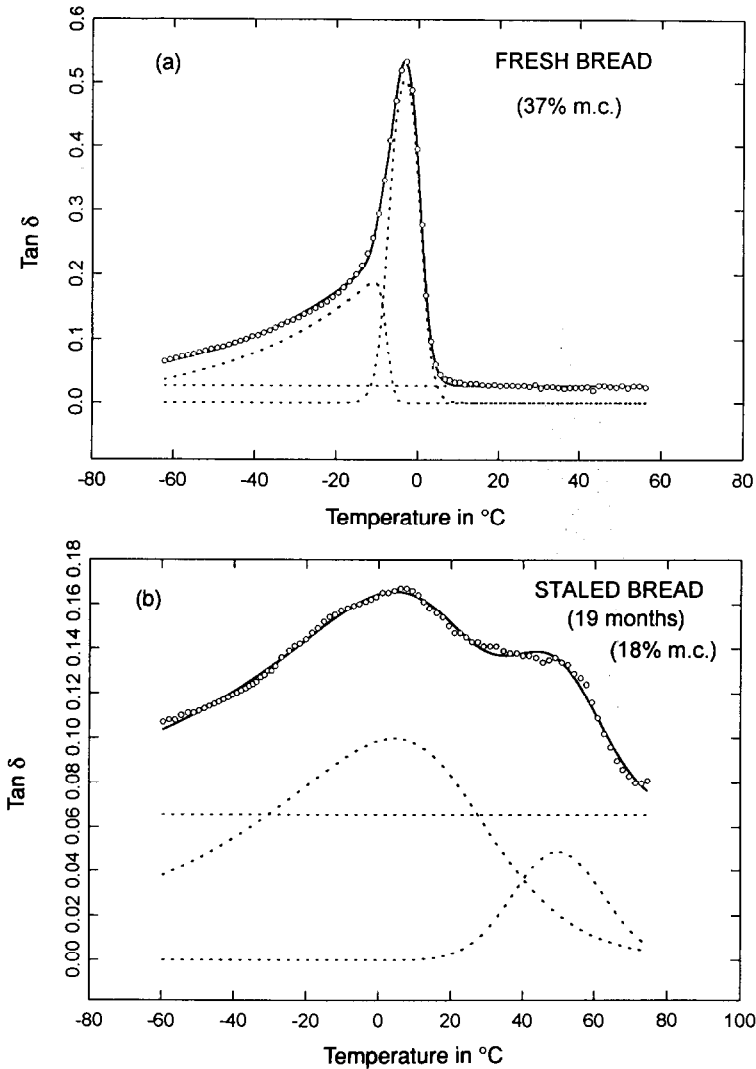


Fig. 3. Deconvoluted DMA plots for SWB bread: (a) fresh sample; (b) after 19 months storage. Note the decline in  $\tan \delta$  and the increased spacing of the two main peaks. The solid line is the fitted value, the dots the original observations.

reported that the onset of softening (37.4% moisture) occurred at  $-12^{\circ}\text{C}$ . This agreed with the DMA onset of  $E'$  (approx.  $-10^{\circ}\text{C}$ ) [31]. Transition temperatures by DMA and DSC have been reported to be in a good agreement [33]. Fig. 4 compares the results from white bread (37–38% moisture) using DMA, DSC [31] and TMA [32]. It can clearly be seen that the main transition resulted in a softening effect (a drop in  $E'$  by DMA and a broadening of the TMA curve) and this corresponds with the ice melting event. Thus, at this high moisture, ice melting



seems to be a key factor that results in the softening effect. It has been proposed by Levine and Slade [19] that the glass transition temperature of bread plays a key role in governing its textural properties and this corresponds to the onset of ice melting

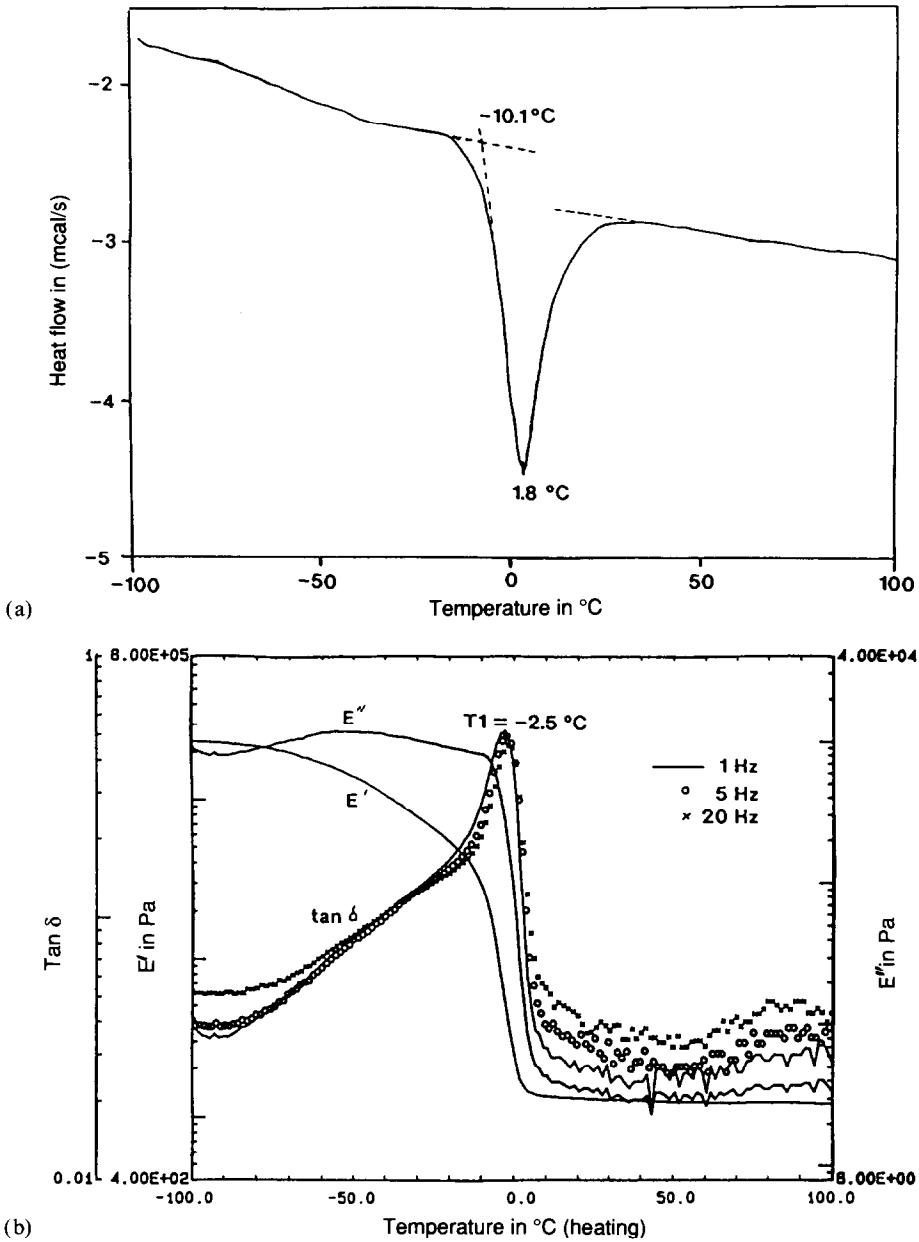
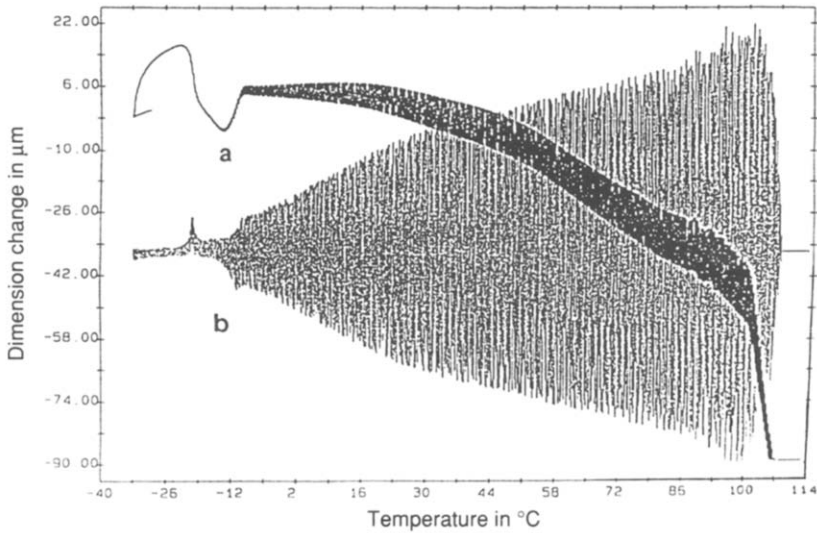


Fig. 4. (a), (b).



(c)

Fig. 4. (a) DSC, (b) DMA, and (c) TMA thermograms for fresh white bread. (DSC and DMA data from Ref. [31]; TMA data from Ref. [32].)

(at  $T_g$ ) in maximally freeze-concentrated samples. The fact that data in Fig. 4 show a two log cycle reduction in  $E'$ , a shift in DSC baseline, and a volumetric expansion, could suggest that there is a glass–rubber transition involved. However, this clearly could not be the only event. Ice melting would generally result in: (1) a decrease in  $E'$ ; (2) a shift in the baseline due to a change in specific heat; and (3) an increase in specific volume. Thus, the changes observed in  $E'$ , the DSC baseline, and the specific volume are largely influenced by ice melting. A softening effect is expected because an immediate plasticization of the polymers by this liquid water is expected. As moisture content is decreased, the  $\tan \delta$  peak becomes broader and moves to a higher temperature range [31]. This could be due to the inhomogeneity of the matrix which is plasticized at various temperatures. The distribution of temperatures over which the transitions occur leads to a broad  $\tan \delta$  peak (broad range for the decrease in  $E'$ ).

Attenburrow et al. [34] studied the fracturability of extrudate in a glassy state using DMA and acoustic emission techniques. They emphasized that with a very low moisture content (when  $T_g < 20^\circ\text{C}$ ), the acoustic emission events (related to crispiness) may be related to the glass transition phenomenon. The tendency for brittle fracture decreases upon hydration due to an increased energy absorption on strain cycling [35]. Perhaps the distribution of many small events at low moisture might play a key role in monitoring the crispiness. Unfortunately, resolving such a broad transition would be difficult.

With regard to bread staling, Hallberg and Chinachoti [31] reported the DMA thermograms of shelf-stable bread during storage (no moisture loss) showing that the  $\tan \delta$  peak did decrease slightly in amplitude but not in general temperature

range. Thus, at least in this bread, there was no increase in network formation in the amorphous region that led to an increase in  $T_g$  during storage, as previously suggested by Levine and Slade [19]. However, in white bread, when a significant amount of moisture loss during storage was observed (due to a different storage packaging material), there was a significant increase in the  $\tan \delta$  peak temperature range, but not in the  $T_g$  from the midpoint temperature [35].  $T_g$  was found to be insensitive to staling; only when moisture loss was severe below the unfreezable water region ( $W'_g$ ) was  $T_g$  increased. During staling of white bread, it was found that the  $\tan \delta$  peak shape (width and amplitude) was very sensitive to staling and it was concluded that the changes were not simply due to moisture loss.

There are many factors involved in thermal analyses that must be considered before any conclusions can be drawn. For DMA, because the geometry and porosity of samples are important, slight deviations in these parameters can result in a significant change in the absolute  $E'$  values. Because samples are normally pressed into a bar and because DMA is restricted to a low strain level, it is not clear how meaningful the rheological DMA parameters are with respect to the texture of real bread. The use of the  $\tan \delta$  midpoint temperatures alone does not seem to be appropriate. One should pay attention to the temperature range, the  $E'$  values, and the magnitude and breadth of the transition. In addition, variations among samples (in composition, geometry, compressibility) can be so great that ten or more replications may be needed in some situations, especially with samples that are porous, brittle, and sensitive to even slight moisture loss.

### 3. Summary

Combinations of thermal analyses and molecular spectroscopy have provided scientists with new avenues to better understand polysaccharides in cereal systems. In the case of starch, much is yet to be understood on the molecular level concerning the importance of glass transition (including the  $T_g$  values for various starches of varying moisture content, degree of crystallinity and history of the samples). Though the role of starch retrogradation has been emphasized, amorphous components are yet to be better understood, in terms of molecular interaction, glass–rubber transition, and phase separations. The use of DMA, TMA, DSC and other techniques would provide an interesting approach to characterize amorphous and crystalline components in cereal systems. And finally, it would be very useful to establish a relationship of these parameters to the actual texture quality as well as to the molecular structure.

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