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Determination of the thermo-mechanical properties in starch and starch/gluten systems at low moisture content – A comparison of DSC and TMA

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ABSTRACT

The impact of heating rate on the glass transition (T_g) and melting transitions observed by differential scanning calorimetry (DSC) on starch and a starch/gluten blend (80:20 ratio) at low moisture content was examined. The results were compared to those determined by thermo-mechanical analysis (TMA). Comparison with dynamic mechanical thermal analysis (DMTA) and phase transition analysis (PTA) is also discussed. Higher heating rates increased the determined T_g as well as the melting peak temperatures in both starch and the starch/gluten blend. A heating rate of 5 °C/min gave the most precise value of T_g while still being clearly observed above the baseline. T_g values determined from the first and second DSC scans were found to differ significantly and retrogradation of starch biopolymers may be responsible. T_g values of starch determined by TMA showed good agreement with DSC results where the T_g was below 80 °C. However, moisture loss led to inaccurate T_g determination for TMA analyses at temperatures above 80 °C.

1. Introduction

Differential scanning calorimetry (DSC) and thermo-mechanical analysis (TMA) are two techniques that enable accurate determination of the temperature at which state changes occur in biopolymer systems. This can help to improve processing efficiencies and develop products with a longer shelf-life and enhanced textural attributes. While DSC and TMA both study changes in the state of materials as a function of temperature, the two techniques are fundamentally distinct from each other. DSC measures the energy required to undergo a transformation but TMA measures the mechanical properties of the material. How they relate to each other is still of some debate. Changes in material state can occur as either first and second order transitions. First order transitions are associated with either an endothermic or exothermic event (e.g. crystallisation and melting). In contrast, a glass transition (T_g) is a second order transition and involves a stepped change in the heat capacity of the material. DSC and TMA are used to study thermodynamic changes in materials but they both utilise non-equilibrium conditions and this can result in artefacts arising due to kinetic effects (e.g. sample relaxation and thermal conductivity). Variation in heating rate, instrumentation, sample presentation (e.g. sample holder, quantity and position in the holder) and a number of other factors can result in some degree of uncertainty in DSC measurements (Kousksou, Jamil, El Omari, Zeraouli, & Le Guer, 2011; Rudtsch, 2002; Simatos et al., 1996; Thomas, 2001; van Miltenburg & Cuevas-Diarte, 1989).

Biopolymers such as starch often exhibit very small changes in heat capacity when transitioning between the rubber and glassy state. This makes accurate determination of the T_g difficult by DSC although the T_g of both granular and pre-gelatinised starch has been well studied (Slade & Levine, 1993; Zeleznak & Hoseney, 1987). No doubt, work has already been conducted to investigate the effect of heating rate on starch transitions at low moisture content by conventional DSC, however there is little information in literature clearly stating the reason for choosing a preferred heating rate over any other possibilities. High heating rate DSC (>50 °C/min), has been used to investigate low moisture corn starch and the T_g was found to increase as heating rate was increased from 100 to 250 °C/min (Liu, Yu, Liu, Chen, & Li, 2009). The effect of heating rate on starch at high moisture content has been studied using conventional DSC (Andreev, Kalistratova, Wasserman, & Yuryev, 1999;

Abbreviations: DSC, differential scanning calorimetry; TMA, thermo-mechanical analysis; DMTA, dynamic mechanical thermal analysis; PTA, phase transition analysis; T_g, glass transition temperature; T_r, relaxation temperature.

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Whittam, Noel, & Ring, 1991; Yu & Christie, 2001). The findings from this work can provide clues as to what may be expected at low moisture content. Precise values for the temperature of a transition can be calculated by running a sample at a number of different heating rates and extrapolating back to 0 °C/min. However, this can prove time consuming and expensive. Hence, running experiments at the minimum heating rate that will give useful data is often sufficient to achieve very accurate data. Most conventional DSC on low moisture starch has been run at 10 °C/min. This has become an accepted experimental standard as it allows reasonably clear T_g determination on an acceptable experimental time scale and also means that results between researchers can be easily compared. However, it is likely that lower heating rates may be able to provide a more precise determination of T_g.

Plasticisation of a biopolymer T_g can be achieved by mixing with a suitable plasticiser. Water has been shown to be a universal plasticiser of biopolymers due to its very low glass transition temperature(s). Note, there is still some debate as to how many glassy states water may form (Yue & Angell, 2004). The commonly accepted T_g of water is often taken as 136 K (−137 °C) and the reduction in the biopolymer T_g is proportional to the quantity of water present in the sample. This can be estimated using the Gordon–Taylor equation (Gordon & Taylor, 1952):

$$T_g = \frac{w_1 T_{g_1} + k w_2 T_{g_2}}{w_1 + k w_2} \quad (1)$$

where T_{g_1} and T_{g_2} are the glass transition temperatures, and w_1 and w_2 are the mass based proportions of the pure components. k can be calculated using:

$$k = \frac{\rho_1 \Delta\alpha_2}{\rho_2 \Delta\alpha_1} \quad (2)$$

where ρ_1 and ρ_2 are the specific volumes and $\Delta\alpha_1$ and $\Delta\alpha_2$ refer to the changes in thermal expansion coefficients. Plasticisation can also be achieved by mixing with low molecular weight entities such as short chain biopolymers or sugars. Gluten is one such biopolymer that is often found in products containing starch. While the impact of gluten on starch at high moisture content has been studied (Eliasson, 1983), its effect at low moisture content is not as well published.

Aside from DSC, thermo-mechanical techniques can be used to determine T_g. These include thermo-mechanical analysis (TMA), dynamic mechanical thermal analysis (DMTA) and phase transition analysis (PTA). DMTA has been widely used to study biopolymer systems but moisture loss can lead to difficulties due to the open nature of most DMTA systems which limits its application to samples with T_g values below ~100 °C. PTA by comparison has been shown to be more reliable as it is better at controlling the moisture content in the sample compared to DMTA (Bengoechea, Arrachid, Guerrero, Hill, & Mitchell, 2007). While a number of studies have used DMTA and/or PTA to determine the T_g of starch based biopolymers, gluten or products containing them (Bengoechea et al., 2007; Kalichevsky, Jaroszkiewicz, Ablett, Blanshard, & Lillford, 1992; Kalichevsky, Jaroszkiewicz, & Blanshard, 1992; Le Meste, Huang, Panama, Anderson, & Lentz, 1992; Strahm, Plattner, Huber, & Rokey, 2000), TMA has not been well investigated. Being similar to PTA, TMA has the potential to give very clear transition data and also offers the advantage of using relatively small sample volumes. As it relies on displacement rather than heatflow (which can be difficult to measure by DSC), TMA may have the potential to be used to accurately determine T_g values for pure starch or starch/gluten blends.

This study aims to evaluate the effect of various heating rates on the T_g values of starch and a starch/gluten blend using DSC with particular focus on low moisture contents, i.e. below 20%. T_g values from the first and second heating scans will also be compared to investigate the effect of heating during the first scan. The T_g results derived from DSC will be compared with those determined by TMA. An evaluation as to how well the two techniques complement each other and the benefits and disadvantages of both methods will be discussed. The results should provide a greater understanding of the thermo-mechanical behaviour of starch and starch/gluten mixtures at low moisture content.

2. Materials and methods

2.1. Materials

Wheat starch (8.5% moisture, 0.2% protein) and vital wheat gluten (6.5% moisture, 71.8% protein) were kindly provided by Manildra Group (Nowra, Australia). In this work, all moisture contents are stated as a percentage of the total weight of the sample including the water therein.

2.2. Sample preparation

Samples were prepared at starch:gluten ratios of 100:0 and 80:20. The total weight of each sample was 150 g and the final moisture content was 35% for both samples. To prepare the blend, the wheat starch and gluten were mixed as supplied using a kitchen mixer (Chef Excel, Kenwood, Australia) fitted with a whisk attachment for 5 min to ensure homogeneity using a mid-range speed setting. Water was then added gradually to the starch or starch/gluten blend while being mixed on speed 10. The mixture was transferred to the bowl attachment of a stick blender (Stickmaster Plus, Sunbeam, Australia) and processed for approximately 1 min at the highest speed setting until no clumps were present. The starch and starch/gluten blends were left in sealed plastic bags overnight at 4 °C to allow full moisture equilibration.

These samples were then processed using a capillary rheometer (Bohlin Rosand RH2000, Malvern, UK) fitted with a 1 mm × 8 mm die at 110 °C using a plunger speed of 12.5 mm/min (shear rate ~150 s^{-1}). The resulting extrudates were dried in an oven at 50 °C for 48 h. The dried extrudates (7.5–8.5% moisture) were then ground in a blender (Grindomix GM200, Retsch, Germany) at 10,000 rpm for 5 min and sieved through a 250 μm sieve.

2.3. Moisture conditioning

Samples of the starch and starch/gluten blends (~3 g) were conditioned over saturated salt solutions (LiCl, $MgCl_2$, NaBr, NaCl, KCl) in sealed chambers. The moisture content was periodically monitored using a Karl Fischer coulometer (831, Metrohm, Australia) until it was relatively stable.

2.4. Differential scanning calorimetry (DSC)

Measurements were conducted on a differential scanning calorimeter (STAR System DSC I, Mettler Toledo, Australia). The instrument was calibrated using water, indium and zinc. Initial analyses were conducted to determine the approximate T_g endpoint for each sample at each moisture content. Analyses were then performed on the starch and starch/gluten blend (40.0 ± 1.0 mg) by heating from −20 °C to just past the T_g, cooling again to −20 °C and reheating to 230 °C. Heating was performed at 5, 10 and 20 °C/min while cooling was always conducted at 20 °C/min. Hermetically sealed Medium Pressure (stainless steel) pans were used with a Viton O-ring. An empty pan of the same type was used as a reference. Data collection and analysis was performed using the STARe software version 9.30. Samples were measured (ASTM

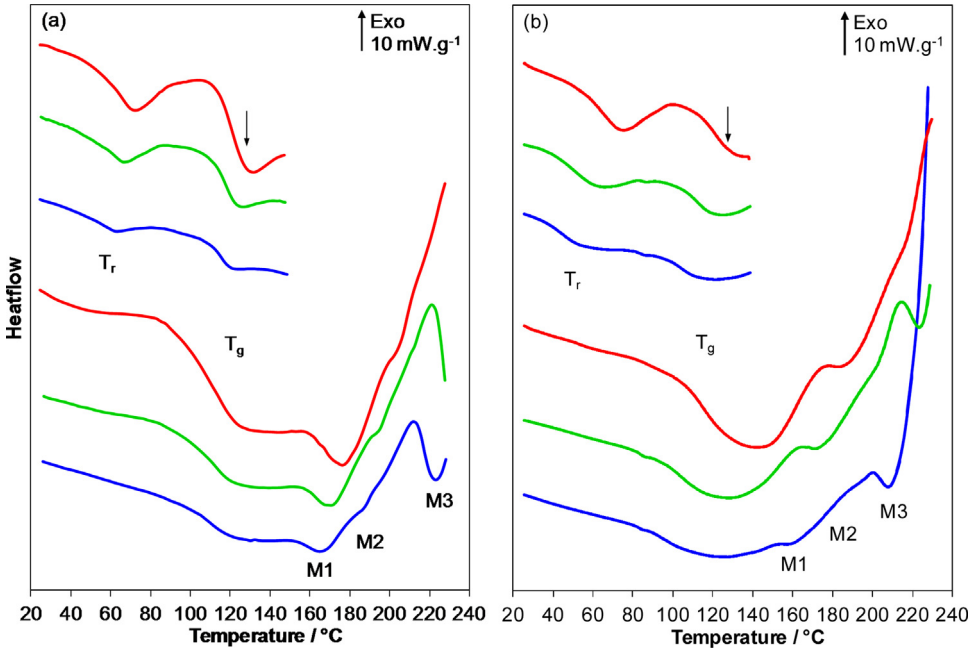

Fig. 1. 1st and 2nd DSC heating scans performed on the (a) starch sample at 10.5% and (b) starch/gluten blend at 5.5% moisture content at 5 (—), 10 (—) and 20 (—) °C/min.

D3418/IEC1006 standard) in duplicate and the mean value of the T_g and melting transitions was calculated for each sample.

2.5. Thermo-mechanical analysis (TMA)

The powder samples were tested in a TMA (Q800, TA Instruments, UK) using a cup and plunger test rig developed at CSIRO. The compression fixture for the TMA consists of an open ended cup made from aluminium and a plunger made from brass which fitted into the compression rig of the TMA instrument. The cup had an internal diameter of 4.5 mm and a height of 7.6 mm. The plunger had a diameter of 3.8 mm and a length of 5.6 mm.

The compression cup was filled with 0.3 ± 0.02 g of sample powder and a cap of silicone vacuum grease was applied to reduce moisture loss. The plunger was brought into contact with the sample. A constant force of 5 N was applied to the powder while the temperature was increased from −50 °C to 300 °C at 3 °C/min. The position of the plunger was monitored throughout the temperature sweep while the sample was under a constant compression load. Collection and analysis of the data was done through the Universal Analysis 2000, version 4.2E software. The temperature lag of the sample holder was determined using the melting points of acetonitrile, water and benzoic acid giving an average result of 14.5 ± 2.1 °C. The raw data for the starch and starch/gluten blend were adjusted to compensate for the temperature lag. Each sample was run at least twice. The onset, midpoint and endpoint temperatures of the T_g transitions were calculated using the tangents drawn on the displacement curve. Mean and average deviation from the mean was calculated for each value.

3. Results and discussion

3.1. Thermal transitions of starch and the starch/gluten blend by DSC

DSC has been widely used to investigate thermal transitions in materials. These thermal changes are associated with changes in material properties such as changes from a crystalline state to a liquid or from a glass to a rubber. A noticeable trend within literature is the use of standard experimental parameters, for example a 10 °C/min heating rate and use of the second scan data to determine T_g values. While this is useful in that it allows easier data comparison between experiments, it should be noted that methodology can significantly influence the results obtained.

The DSC curves of starch and the starch/gluten blend (at moisture contents of 10.5% and 5.5% respectively) obtained in this study using heating rates of 5, 10 and 20 °C/min are presented in Fig. 1. The relaxation endotherm (T_r), glass transition (T_g) and three distinct melting peaks (M1, M2 and M3) can be seen in these traces. Peak M1 corresponds to the melting of starch crystallites, M2 the melting of the amylose-lipid complexes and M3 the melting of amylose (Jang & Pyun, 1996). Furthermore, just after the M1 peak in the starch (Fig. 1a) and in starch/gluten sample (Fig. 1b), the baseline changed considerably to a positive gradient indicating the presence of an underlying exothermic process. In order to investigate this further, native starch at a moisture content of 7.0% was analysed using the same method. The change of slope was also present in the trace of the native starch (data not shown). In addition, the M1 peak was found to be much larger than in the pre-gelatinised samples due to the comparatively larger quantity of crystal structure in the native sample. Visual inspection of the native starch following the second scan in the DSC showed that the starch had changed from white to light brown in colour. This indicates that the starch had either begun to pyrolyse or oxidise during the second scan which may be attributable to the considerable headspace above the sample which is present with the size of the pans used in this work. This effect does not appear to have been discussed in literature.

The onset, midpoint and endpoint T_g values of the starch and starch/gluten blend as determined by DSC at various moisture contents (6.2–15.8%) are given in Table 1. It is worth noting that the T_g midpoint of both samples in Fig. 1 lies in the region of 100–120 °C even though the moisture content of the starch/gluten blend was considerably lower than that of the starch sample. In fact, the T_g values of the starch/gluten blend was comparatively lower than that of the starch sample at all comparable moisture contents. A comprehensive state diagram of the transitions for starch and gluten was produced by Cuq, Abecassis, & Guilbert, (2003). It showed that

Table 1
Onset, midpoint and endpoint T_g values from the 1st (left) and 2nd (right) heating scans and onset of the M3 peak as a function of moisture content for the: (a) starch and (b) starch/gluten blend.

Moisture content (wt%)	Heating rate (°C/min)	T_g (°C)			M3 (°C)
		Onset	Midpoint	Endpoint	Onset
(a) Starch					
6.2	20	163/140	171/158	179/176	>230
	10	154/137	161/149	169/161	>230
	5	146/135	151/143	157/151	220.2
10.5	20	112/93	119/107	127/121	>230
	10	109/93	115/105	121/117	222.5
	5	110/97	115/107	120/117	213.0
12.9	20	87/74	95/84	104/95	227.5
	10	86/74	93/84	100/95	218.2
	5	83/68	89/82	94/96	208.2
14.6	20	60/58	73/71	86/85	225.7
	10	66/54	72/67	79/80	215.5
	5	66/51	73/66	81/81	216.0
15.8	20	61/49	67/61	72/73	225.9
	10	61/41	64/54	66/66	214.4
	5	57/39	60/51	63/64	205.0
(b) Starch/gluten blend					
5.5	20	106/101	118/113	130/124	>230
	10	101/93	110/108	119/108	214.8
	5	99/88	106/96	113/104	198.1
9.7	20	72/68	83/80	93/91	218.5
	10	75/67	82/77	88/87	201.2
	5	76/71	81/78	86/85	188.0
12.0	20	60/55	82/80	105/105	220.7
	10	57/53	78/77	100/100	200.0
	5	53/37	74/71	96/104	183.3
14.2	20	65/41	74/63	83/85	211.7
	10	53/32	68/57	83/82	192.3
	5	51/27	66/55	80/83	178.4
15.8	20	57/35	67/53	78/71	207.2
	10	54/35	63/53	72/70	189.8
	5	51/26	58/50	66/71	174.0

at any given moisture content, the T_g of gluten was considerably lower than that of starch. This implies that the interaction between gluten and starch in the blend results in a T_g that is lower than that of starch but higher than that of gluten. The Gordon-Taylor equation (Eq. (1)) can be used to approximate the T_g of a blend using the T_g values from the individual components and assumes that the polymers are compatible.

3.1.1. Heating rate impacts the T_g and other thermal transitions in starch

One of the most common questions when conducting calorimetric measurements using DSC is "which heating rate is most suitable?" Use of slow heating rates reduces the heatflow and can make distinguishing transitions from the baseline very difficult. Use of fast heating rates tends to increase the measured temperature of the transition. While onset temperatures are often little affected, midpoints and more so endpoints can be shifted considerably. In samples with very small transitions, even the onset temperature can appear at higher temperatures due to the manner in which it is determined from the DSC trace. Furthermore, if the heating rate is too high and the transition is small, the stretching effect can also lead to difficulties in differentiating it from the baseline.

The trend in T_g values determined from the second heating scan for the starch and starch/gluten blend at heating rates of 5, 10 and 20 °C is shown in Fig. 2. The results show that higher heating rates generally shifted the glass transitions to higher temperatures. The same was seen with the M3 peak (Table 1) as well as the M1 and M2 peaks (data not shown). This effect became more prominent with decreasing moisture content and was most noticeable between data collected at 10 and 20 °C/min and was less pronounced between 5 and 10 °C/min. The reason for this shift is primarily the result of thermal lag between the thermocouples of the DSC and the sample which is more significant at higher heating rates. This effect is well known and has been discussed in detail elsewhere (Kousksou et al., 2011; Thomas, 2001; van Miltenburg & Cuevas-Diarte, 1989).

While the effect of heating rate on conventional DSC of starch at low moisture content does not appear to be reported in literature, the effect at higher moisture content has been investigated. Andreev et al. (1999), found no difference between the measured gelatinisation temperature of starch when determined at heating rates of 3 and 0.25 °C/min by DSC. However, Whittam, Noel and Ring (1991) did show that a decrease in heating rate from 20 to 2.5 °C/min results in a decrease in the measured gelatinisation temperature for B-type amylose crystals. Yu and Christie (2001) also observed that higher heating rates can shift the observed transition to higher temperatures in starch samples at high moisture content. Similarly, at low moisture content, Liu et al. (2009) have shown that higher heating rates using high speed DSC can lead to a stretching effect on the transition such that it occurs over a much wider temperature range. These findings suggest that significant differences are only evident at higher heating rates. Our results show that higher heating rates can also affect the T_g values at low moisture and the differences arise in part from the kinetics of the transition. This may be the reason for the large differences between the

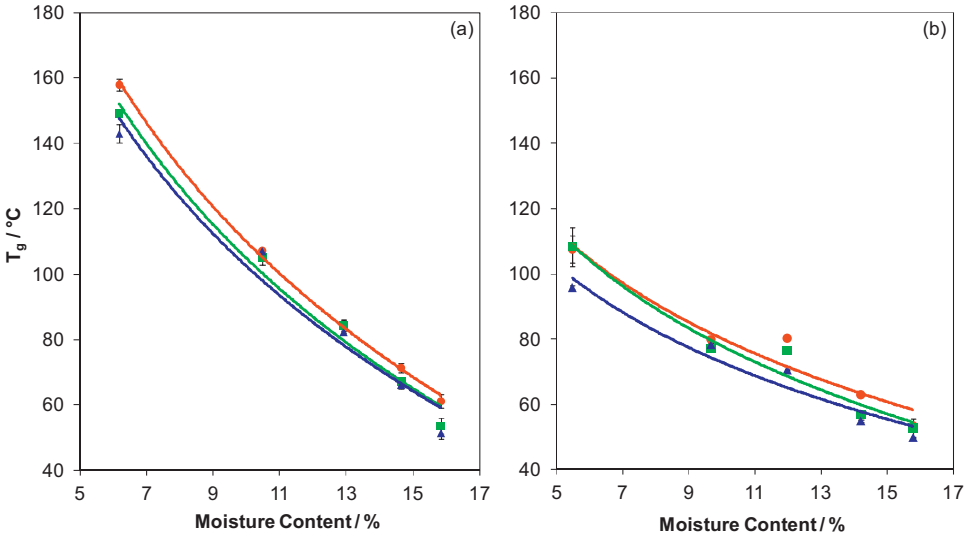

Fig. 2. Midpoint T_g values obtained from the 2nd heating scan determined at heating rates of 5 (—), 10 (—) and 20 (—) °C/min as a function of moisture content for the (a) starch and (b) starch/gluten blend. Error bars represent the average deviation from the mean.

M3 onset values while differences in T_g values are by comparison smaller (Table 1).

3.1.2. Determination of T_g from first and second scan data

The temperature at which the starch relaxation peak (T_r) occurs depends on the temperature at which it was stored (Appelqvist, Cooke, Gidley, & Lane, 1993). In these samples T_r occurred at 60–80 °C and in the samples with lowest moisture content, it was well separated from T_g in the first heating scan (Fig. 1). However, at higher moisture contents, T_g and T_r can overlap causing complications in determining an accurate value for T_g from first scan data. For this reason, the second heating scan is often used to determine T_g values and this is currently standard practise for biopolymer systems (Steele, 2004). However, this relies on the fact that T_r is relatively slow to recover and assumes that T_g is fully reversible and remains unaltered by the first heating scan. Hence, in the timeframe of the experiment, the first scan effectively eliminates the relaxation peak, leaving the T_g to be clearly identified in the second heating scan.

When this was attempted with the processed starch or starch/gluten blend samples, the T_g in the second heating scan was found to be significantly lower than that in the first heating scan (Fig. 1). This was most easily observed in the data from the starch sample (Fig. 1a) but was also present in the starch/gluten blend (Fig. 1b) and the existence of this effect was independent of heating rate (Table 1). Close comparison of the T_g values revealed that it was in fact the T_g onset temperature that differed most between the first and second heating scans (Table 1). The midpoint data was affected to a lesser extent while the endpoint values were generally least affected (Table 1). The observed differences between first and second scan data appeared less pronounced in samples with higher moisture contents than those at lower moisture contents. The reason for this is probably due to the fact that the *relative* change in water content is smaller as moisture content increases. Also of note, was that T_g always appeared far more defined in the first heating scan than in the second. This can also be seen in data presented by Kalichevsky, Jaroszkiewicz, Ablett, et al. (1992).

A possible explanation for the difference seen between the first and second scan curves near the T_g transition point is that the degree of crystallinity and therefore the amount of amorphous starch in the sample may have changed following the first heating scan. Zeleznak and Hoseney (1987) did indeed show that the T_g of pre-gelatinised starch is lower than that of native starch which lends support to this idea. During heating, starch polymers that exist as crystallites in the native granule become hydrated so long as sufficient water is present. If insufficient water is present, higher temperatures are required to melt partially hydrated starch polymer chains. When stored at room temperature for extended periods of time, hydrated starch polymers can re-crystallise through the retrogradation process.

Hence, it is possible that starch crystallite melting was incomplete in these samples following extrusion at 110 °C and 35% moisture content but it is also possible that some retrogradation may have taken place. In fact, both un-melted and retrograded crystallites could have been present in the samples. Microscopic examination showed no evidence of intact granules of starch in either sample (results not shown) but this does not prove that small crystallites were not present. There is in fact evidence that incomplete crystallite melting was present in the sample which is shown by the existence of the small M1 peak in the second heating scan (Fig. 1). The sample of native starch analysed by DSC showed the M1 peak to be much larger (data not shown) than in the extruded sample which confirms the association of the M1 peak with starch crystallite melting. Support to the idea of retrograded crystallites comes from the existence of a small endothermic peak near the endpoint of the T_g in the first heating scan (labelled with an arrow in Fig. 1) which is absent in the second heating scan. Retrograded starch tends to melt at a lower temperature than the native crystallites and hence, we believe the peak indicated by the arrow in Fig. 1 corresponds to retrograded starch. Thus, small amounts of un-melted starch crystallites and retrograded starch may well have been present in the samples prior to analysis on the DSC and could account for the difference in the shape and width of the T_g transition between the first and second heating scans.

The fact that T_g could be altered by the first heating scan appears to be ignored in literature and the common practice of using the second heating scan to determine T_g may not be as pertinent as it seems. As this is the case, it may be of value to consider use of the T_g from the first heating scan over that from the second heating scan as a closer approximation to real food systems during storage. However, this also has its difficulties as T_g and T_r begin to overlap at moisture contents above ~12% in pure starch systems and at even lower moisture contents in blends or real food systems.

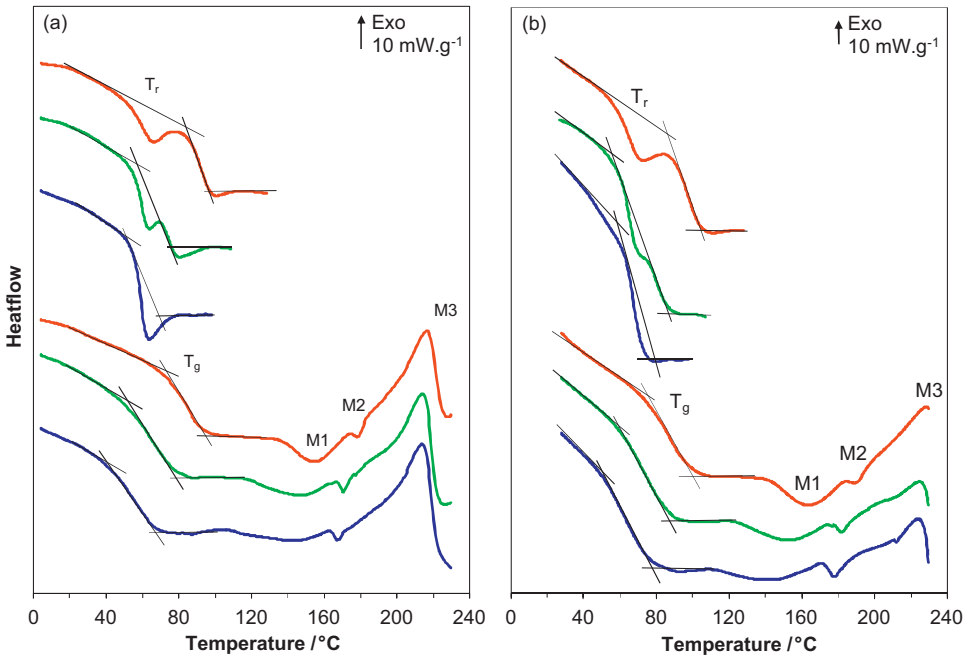

Fig. 3. 1st and 2nd DSC heating scans at (a) 10 °C/min and (b) 20 °C/min on the starch sample at 12.9 (—), 14.6 (—) and 15.8 (—) % moisture content.

3.1.3. Measuring T_g close to the relaxation temperature

As discussed above, T_g and T_r in the first DSC heating scan began to overlap as moisture content increased. Fig. 3 presents the first and second DSC heating scans of starch at various moisture contents analysed using a heating rate of (a) 10 and (b) 20 °C/min. The tangents and bisecting lines have been included to demonstrate how the T_g values were determined for both the first and second heating scans. Although T_g and T_r have some overlap in these samples, only for the sample at the highest moisture content (15.8%) do they completely overlap. Analysis of the full array of data on each sample showed that the bisector line always had a very similar slope in the first heating scan regardless of the moisture content of the sample. This slope was by comparison significantly shallower in the second heating step. By using slopes from first heating scans conducted at the same heating rate on samples at lower moisture content, as well as parts of the T_g that were not overlapping with the T_r peak, a good estimation of the T_g from the first heating scan was obtainable for most starch and starch/gluten blend samples (Table 1). Comparison of the results at the different heating rates showed that lower heating rates tended to reduce the chance of T_g and T_r overlapping due to both transitions occurring over smaller temperature ranges. While some of the T_g values were difficult to accurately determine from first scan data, the relatively large difference between T_g values calculated from the first compared to the second heating scan implies that this technique is still worthy of consideration when attempting to analyse aged starch or starch/gluten samples.

3.2. Determination of thermal transitions by TMA

While DSC measures heat flow of a sample, TMA measures the compressibility. Below the T_g, the material behaves as a solid and displacement of the plunger is minimal. As the sample heats up it begins to flow and fills the voids between the particles stacked in the compression cup. Theory dictates that volume change is closely related to T_g and as the cup walls are of a fixed circumference, it is important to note that the displacement measured by the TMA instrument is directly proportional to the volume change in the sample. Thus, in this instance, T_g is theoretically related to displacement of the plunger as well as the volume change in the sample. This reduces the volume of the sample which is detected by the downward movement of the plunger on top of the sample. The temperature and degree of compression indicates the degree of plasticisation of the sample.

TMA has been used to investigate starch, its constituent biopolymers and gluten but none of these have been under low moisture conditions, where T_g is of importance. Strahm et al. (2000) used a phase transition analyser (PTA) with a continuous heating ramp to examine the T_g of one specific cereal formulation at various moisture contents. The formulation was composed of corn, oat and wheat flours, sugar and salt but the results were not compared with DSC measurements.

TMA was used to investigate the mechanical properties of rice starch granules using a 50% starch/water mixture (Biliaderis, Page, & Maurice, 1986; Biliaderis, Page, Maurice, & Juliano, 1986). However, at this moisture content, expansion occurs and T_g appears below 0 °C. Le Meste et al. (1992) applied TMA but used an oscillatory load rather than a direct compression method to determine the T_g of bread at moisture levels from ~7 to 40%. No information on the protein and starch levels or other additives (other than fat content) was specified and these would have undoubtedly impacted the value of T_g. The same method was used to study gluten and starch in a later publication (Huang, Haynes, Levine, & Slade, 1996).

3.2.1. T_g determination of starch and the starch/gluten blend using TMA

A typical TMA displacement versus temperature curve for starch is shown in Fig. 4. Two transitions were observed in all samples during heating up to 300 °C, the first transition is usually associated with the T_g and the second with the starch melting point (M3). As M1 and M2 are very minor transitions having little or no impact on the rate of probe displacement, they were not detected during measurement by TMA.

Fig. 5 shows the displacement curves for starch and starch/gluten blends at a range of moisture contents. As may be expected, the magnitude of overall displacement was mostly dependent on the moisture content of the sample. The onset, midpoint and endpoint of the first transition obtained at each moisture content using TMA was determined using tangents and a bisector

Table 2
Onset, midpoint and endpoint temperatures for the first transition and the onset temperature of the M3 peak as a function of moisture content in the TMA curve for the: (a) starch and (b) starch/gluten blend. Errors are quoted as the average deviation from the mean.

Moisture content (wt%)	1st transition (°C)				M3
	Onset	Midpoint	Endpoint	Derivative	Onset
(a) Starch					
6.2	69.5 ± 9.0	126.0 ± 0.5	150.1 ± 0.7	125.3 ± 0.8	249.5 ± 0.3
9.3	75.2 ± 12.1	100.5 ± 4.4	110.7 ± 2.2	100.3 ± 4.3	251.9 ± 1.0
12.6	74.6 ± 0.2	85.0 ± 0.0	97.3 ± 2.6	85.5 ± 0.2	253.8 ± 1.2
14.5	55.3 ± 2.1	66.4 ± 2.6	81.1 ± 1.9	66.4 ± 2.6	253.3 ± 1.9
16.7	38.6 ± 0.5	47.5 ± 0.3	63.5 ± 1.9	47.5 ± 0.1	253.5 ± 2.0
(b) Starch/gluten blend					
5.8	90.8 ± 2.2	115.7 ± 1.4	127.8 ± 0.8	116.1 ± 1.3	245.9 ± 6.1
8.8	87.4 ± 3.6	109.3 ± 0.6	116.1 ± 1.3	109.9 ± 0.9	253.1 ± 1.1
12.2	73.8 ± 1.9	86.4 ± 3.0	97.6 ± 1.1	86.2 ± 2.9	255.5 ± 0.4
13.8	57.1 ± 0.7	67.8 ± 0.9	79.4 ± 0.4	67.8 ± 1.0	253.4 ± 5.0
15.7	42.2 ± 0.9	52.2 ± 1.7	71.0 ± 3.2	52.6 ± 1.5	258.7 ± 0.8

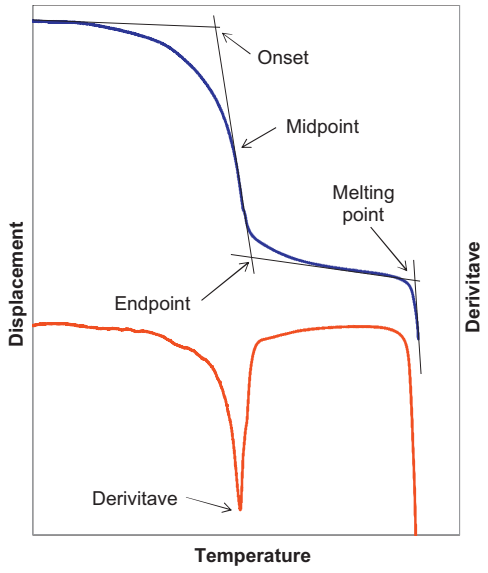

Fig. 4. Example compression test data showing the displacement (—) and its 1st derivative (—) for a powder analysed using TMA.

line. The midpoint was determined from the point of inflection in the TMA displacement curve as it gave more reproducible data than the mean of the onset and endpoint temperatures. Additionally, the derivative of the displacement curve resulted in a peak which was found to be very close to the temperature obtained from the midpoint/point of inflection in the curve. These results are detailed in Table 2.

3.2.2. Comparison of DSC and TMA method for the determination of T_g

Fig. 6 compares the midpoint of the first transition as determined by TMA with the T_g values determined by DSC for both the starch and starch/gluten blend as a function of moisture content. The values of the first transition determined by TMA and values for T_g as determined by DSC decreased with increasing moisture content for both starch and the starch/gluten blend. Both DSC and TMA data showed that this transition tended to occur at lower temperatures in the starch/gluten blend compared to the starch at equivalent moisture contents (due to the plasticisation of starch by the gluten polymers). However, the differences between starch and starch/gluten blend transition temperatures were not as pronounced in the TMA data compared to the DSC data. Good agreement was found between DSC and TMA data for samples at the higher moisture contents but poor agreement was found at lower moisture contents (<13%). It is suggested that loss of moisture from the samples during measurement was responsible. Examination of

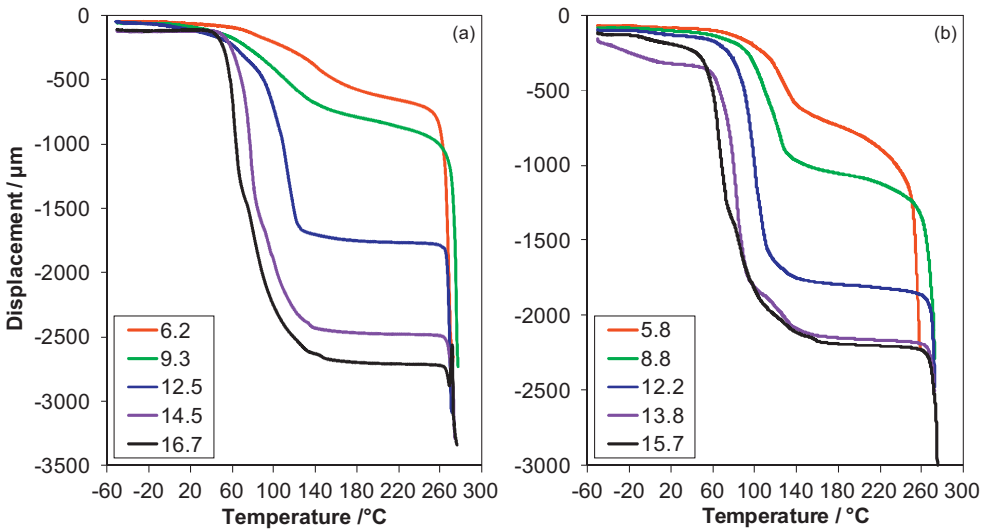

Fig. 5. Compression TMA results for the (a) starch and (b) starch/gluten blend at various moisture contents using a constant force of 5 N and heating rate of 3 °C/min.

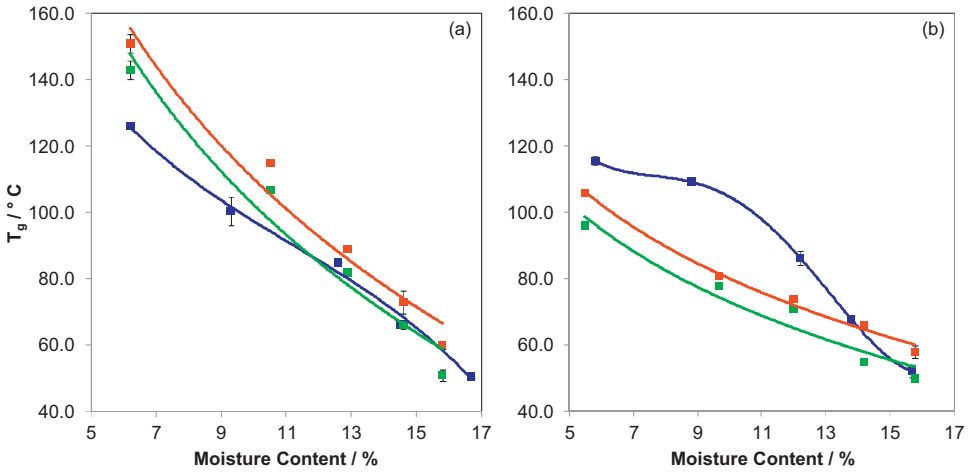

Fig. 6. Midpoint T_g values from 1st (—) and 2nd (—) heating scans on the DSC conducted at a heating rate of 5 °C/min and compared to the midpoint values of the 1st transition obtained by TMA (—) for the (a) starch and (b) starch/gluten blend as a function of moisture content. Error bars represent the average deviation from the mean.

published literature suggests that samples analysed by DMTA and oscillatory TMA also suffer from moisture loss at elevated temperatures. Bengoechea et al. (2007) compared the T_g values obtained by PTA, DSC and DMTA for casein, soya and gluten proteins. Their results showed that while PTA and DSC gave fairly similar values of T_g for all 3 proteins, DMTA generally gave a higher value (particularly at the lowest moisture contents). It is also worth noting that many publications only appear to examine samples with transition temperatures below ~100 °C, most likely to avoid this issue (Bengoechea et al., 2007; Kalichevsky, Jaroszkiewicz, Ablett, et al., 1992; Kalichevsky, Jaroszkiewicz, & Blanshard, 1992; Le Meste et al., 1992; Nicholls, Appelqvist, Davies, Ingman, & Lillford, 1995).

There are several key aspects to the TMA results which suggest that moisture loss is indeed a significant artefact – particularly with the lowest moisture samples. For instance, moisture loss would tend to increase T_g values and this is exactly what is seen in the TMA data for a number of the samples of the starch/gluten blend when compared to the DSC values (Fig. 6b). The onset of the first transition in the samples with the two lowest moisture contents occurred at around 80–100 °C according to the TMA results which is the temperature range where significant evaporation would be expected. These curves also have a fairly shallow onset as might be expected from moisture loss. The endpoint on the other hand is fairly sharp as might be expected from a glass transition. Hence, this data suggests that the first transition in the TMA curves of the two samples of the starch/gluten blend at the lowest moisture contents is actually a composite of two overlapping effects, one being sample compaction due to moisture loss and the other being an elevated T_g from what may have been expected.

The displacement curves for the starch samples at the two lowest moisture contents also show a shallow onset of the first transition (Fig. 5a), but in this instance the endpoints also had a shallow gradient change. While TMA showed an increased midpoint value for the first transition compared to the DSC data for the starch/gluten blend at the lowest moisture contents, the opposite was true for the starch samples. This however, can also be explained by the moisture loss hypothesis. As the starch sample does not contain any gluten, the true T_g increases drastically at the lowest moisture contents as is shown by the DSC data (Fig. 6a). However, the loss of moisture is likely to occur at a similar rate in the starch sample as in the starch/gluten blend and hence it is suggested that the first transition in the TMA curve of the starch sample is only attributable to the volume reduction caused by the moisture loss and that the sample remains in the glassy state above this transition. The absence of the contribution attributable to the T_g in the displacement of the plunger explains why the magnitude of displacement is smaller for the two lowest moisture starch samples than that of the starch/gluten blend. As the starch would have been very dry at these temperatures, it is believed that the measured T_g by TMA actually occurred just prior to the starch melting peak (M3).

Further evidence of moisture loss can also be found in the value of the onset temperature for the M3 peak. DSC data shows that the onset temperature of this transition is dependent on the moisture content and ranges from 174 °C to over 230 °C. However, all of the TMA curves show the midpoint of the M3 transition to occur at ~255 °C – i.e. they were independent of the initial moisture content and far higher than suggested by DSC. In fact, the value of 255 °C is in good agreement with the 248 °C value estimated by extrapolation for starch with a 0% moisture content from DSC findings by Donovan, Lorenz, and Kulp (1983). This suggests that the samples had almost completely dried out by the time the starch crystallite melting peak (M3) was measured by TMA.

4. Conclusions

There are a number of factors to consider when evaluating the glass transition temperature of starch and starch/gluten materials. As others have pointed out, sample size, pan type and instrument type can all have a significant impact on DSC transitions (Kousksou et al., 2011; Rudtsch, 2002; Simatos et al., 1996; Thomas, 2001; van Miltenburg & Cuevas-Diarte, 1989). The results of this work show that higher DSC heating rates artificially increase the T_g as well as the temperatures of M1, M2 and M3 peaks. The lowest heating rate investigated (5 °C/min) was found to yield a clearly defined T_g in the Mettler Toledo DSC1 instrument when a sample size of ~40 mg was used with stainless steel crucibles. The T_g values determined from the first heating scan were found to differ significantly from those of the second heating scan. Melting of remaining intact starch crystallites or retrograded crystallites during the first heating scan may be responsible for this difference. In our opinion, the first heating scan gives a more representative T_g value of a real food systems although, it is acknowledged that it can be difficult to obtain accurate T_g values where T_r and T_g overlap. We have shown that a slower heating rate e.g. 5 rather than 10 °C/min reduced the likelihood of T_g and T_r overlapping which made data analysis easier and more accurate. In order to overcome such issues and to generate data from a sample without having to heat it prior to analysis, modulated DSC may prove a more useful tool.

The advantages of TMA over DSC are that the transition from a glassy to rubbery state is very clear so long as moisture loss is negligible and only a single heating step is required. Small sample volumes may also be an advantage over techniques such as PTA. However, moisture loss significantly limits the use of TMA in a similar manner to DMTA for T_g determination in biopolymers such as starch where the T_g is relatively high. While silicon grease may slow moisture loss during TMA measurements, it is not enough to produce reliable data at temperatures above 80 °C.

On a final note, it is often very convenient to utilise midpoints in T_g determinations due to their relatively good reproducibility. However, from a practical point of view it is often more worthwhile to consider the onset and endpoints and to use the one that is most appropriate. For example, the endpoint of the T_g may be of more practical importance to processability while the onset may well be more useful for evaluating shelf-life stability.

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