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Immersion mode material pocket dynamic mechanical analysis (IMP-DMA): A novel tool to study gelatinisation of purified starches and starch-containing plant materials

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ABSTRACT

There is a clear need for improved methods for the study of the physical changes that occur in slurries and sol–gel systems that have significant water content. In this paper a novel immersion mode material pocket form of dynamic mechanical analysis (IMP-DMA) has been designed, combining material pocket technology to provide physical support to a powdered sample within an immersion bath. IMP-DMA allows the mechanical response of a powder during heating to be monitored in excess water. IMP-DMA was evaluated using a range of starch samples loaded as a slurry into a solid steel pocket, the mechanical responses of these samples were monitored as a function of temperature, and values for modulus and tan δ peaks were found to correspond well with events occurring at both the onset and peak gelatinisation temperatures as measured by differential scanning calorimetry (DSC) (e.g. wheat starch has an onset and peak DSC temperature of 49.3 °C and 57.2 °C, respectively, and shows a peak in tan δ at 52.8 °C and a modulus peak at 57.7 °C). Some limitations were found in the ability of DMA to detect transitions in starches with low or high amylose contents. IMP-DMA was shown to be an effective tool for monitoring the changes in starch structure that occur during gelatinisation, both in purified starches and in more complex starch-containing food materials. Thus, a new hyphenated form of DMA is now available that permits the thermally induced transitions of particle water dispersions to be characterised.

1. Introduction

Samples in the form of slurries (which for this paper is defined as a course suspension of starch granules in water) are used to explore a range of different problems in the analytical sciences. Physical changes in slurries/course suspensions may be used to investigate different polymorphs in pharmaceutics, specific sol–gel transitions and the physical properties of foods and food components, e.g. starch.

Starch is one of the primary sources of energy in the human diet (in the form of exogenous glucose supply) (Wang, Bogracheva, & Hedley, 1998), and is also used in a wide range of industrial processes (Jobling, 2004), including brewing (Bamforth, 2009), bioethanol production (Smith, 2008), paper manufacture (Blennow, Bay-Smidt, Leonhardt, Bandsholm, & Madsen, 2003) and in the production of biodegradable plastics (Zhao, Torley, & Halley 2008).

Starch exists in plants in a granular form, the granules being between 1 and 100 μm in diameter, and has a complex semi-crystalline structure (Buléon, Colonna, Planchot, & Ball, 1998). Starch consists of two polymeric components, *viz.*: amylose, which is an essentially linear α (1→4) linked glucose chain, and amylopectin, which is a branched polymer of α (1→4) linked glucose chains interspersed with α (1→6) branch points (Gallant, Bouchet, & Baldwin, 1997). The glucose chains of the amylopectin interact to form double helices, which are then built up into larger crystalline structures, in the form of lamellae, super helices and blocklets (Gallant et al., 1997; Oostergetel & van Bruggen, 1993; Parker, Kirby, & Morris, 2008; Waigh et al., 2000). These large crystalline structures are interspersed with regions of amorphous carbohydrate to build a complete starch granule (Pérez & Bertoft, 2010). The relative proportions of amorphous and crystalline material in the starch granule, and the arrangement of structure in the granule, have a significant bearing on the physico-chemical behaviour of the starch, interactions with hydrolytic enzymes and responses to hydrothermal treatments (Bogracheva, Meares, & Hedley, 2006; Colonna, Leloup, & Buléon, 1992; Tahir, Ellis, & Butterworth, 2010).

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One of the most important modifications of starch structure that occurs during processing of starch, for both food usage and industrial applications, is gelatinisation. When heated in excess water, starch goes through a thermal transition, termed gelatinisation, which occurs typically at temperatures between 50 and 70 °C (Donovan, 1979; Wang et al., 1998). Starch gelatinisation is an endothermic transition associated with rapid swelling of the granule and melting of crystalline regions. In the absence of water, the crystalline regions of starch granules go through a melting transition at a temperature of around 150–170 °C, although completely anhydrous crystallites may have a melting temperature in excess of 200 °C (Donovan, 1979; Roder et al., 2009; Steeneken & Woortman, 2009; Whittam, Noel, & Ring, 1990). The presence of excess water allows gelatinisation to occur at a much lower temperature (50–70 °C) (Perry & Donald, 2002; Roder et al., 2009). Water may be described as plasticising the gelatinisation of starch, as its presence, in excess, leads to swelling of the amorphous regions of the starch granule at the onset of gelatinisation. This is thought to destabilise crystalline regions of the starch granules, so that crystalline melting is possible at a temperature significantly below that at which melting would occur in the absence of water. Thus, during gelatinisation, it is believed that the transition is initiated by swelling of the amorphous background caused by water uptake. This introduces structural stress into the crystalline lamellae, leading to disruption of the crystal structure. Hence, there is a loss of crystalline order and breakdown in granule structure towards the end of gelatinisation. The presence of water may also stabilise the gelatinised state, thereby promoting gelatinisation (Jenkins & Donald, 1998; Perry & Donald, 2002).

A very common method used for monitoring transitions in sol–gel systems is differential scanning calorimetry (DSC), a technique that is commonly applied to starch gelatinisation (Donovan, 1979; Wang et al., 1998). From this it is possible to obtain the onset, peak and conclusion temperatures of the gelatinisation transition from the peak in the endotherm, which is obtained by heating starch in excess water, in addition to a value for the gelatinisation enthalpy, which is related to the proportion of ordered/disorder material in the starch (Bogracheva, Wang, Wang, & Hedley, 2002; Cooke & Gidley, 1992; Davydova, Leont'ev, Genin, Sasov, & Bogracheva, 1995; Donovan, 1979; Jenkins & Donald, 1998). DSC is limited, however, by the sample mass that can be loaded (especially important with more dilute slurries and material in the form of large (mm size) particulates), and in that DSC does not directly measure the physical properties of the slurry, so that DSC may not be sensitive to changes affecting the mechanical properties of the test material. Moreover, there are some limitations in controlling and modulating the environment (e.g. humidity) around test materials in DSC experiments. When DSC experiments are carried out with high water contents, there is a requirement that the DSC pan should be hermitically sealed, to prevent sample drying and endothermic heatflow as a result of water evaporation. Thus, water is unable to evaporate or condense, resulting in a high water vapour pressure in the headspace of the DSC pan. As all DSC measurements are made assuming a constant pressure, this may lead to errors. This problem is not encountered with the present IMP-DMA, because the sample is immersed in a water bath, and thus water is free to evaporate from the water/air interface at the surface of the bath without affecting the water activity encountered by the immersed sample (Fig. 1).

During gelatinisation the swelling of the granule and subsequent melting of starch crystallites leads to large changes in the physical structure of the starch granule and additionally large amounts of α-glucan material may be leached into solution. The starch granule rapidly swells, resulting in the loss of ordered structure and formation of a so called "granule ghost" (Debet & Gidley, 2007; Evans & Haisman, 1980; Jenkins & Donald, 1998). This large structural change may be used to characterise the gelatinisation transition of starch, but offers significant challenges to an analytical scientist, because following gelatinisation, the starch-water system is significantly phase-separated into solubilised glucan chains and granule ghosts. The gelatinisation of starch is unsuitable for study in conventional cone and plate or plate–plate configured rheometers, because the native starch granules will sink, creating a concentration gradient in the same direction as the direction of shear. This makes it impossible to obtain reliable estimates for the rheological properties of starch before and during gelatinisation accompanying hydrothermal treatment (Noosuk, Hill, Farhat, Mitchell, & Pradipasena, 2005; Rayment, Ross-Murphy, & Ellis, 1995; Rayment, Ross-Murphy, & Ellis 1998). The conventional approach to rheological characterisation of starch hydrothermal treatment is to introduce a stirring element in order to keep starch suspended during gelatinisation, as in the commonly used rapid visco-analyser (RVA) and visco-amylograph instruments. Although these instruments are useful, they are somewhat limited in application, because they measure torque on a rotor, which is difficult to relate to either the viscous or elastic parts of the modulus, limiting the amount of information that may be obtained about the gelatinisation process. Also the shear forces introduced by mixing may influence starch structural characteristics. The instruments give very broad ranges for the gelatinisation process and indicate initial temperatures that are much higher than those measured by DSC (Batey & Curtin, 2000; Deffenbaugh & Walker, 1989; Noosuk et al., 2005; Péret, Breene, & Bahnassey, 1998; Qian & Kuhn, 1999; Xie, Yu, Chen, & Li, 2008).

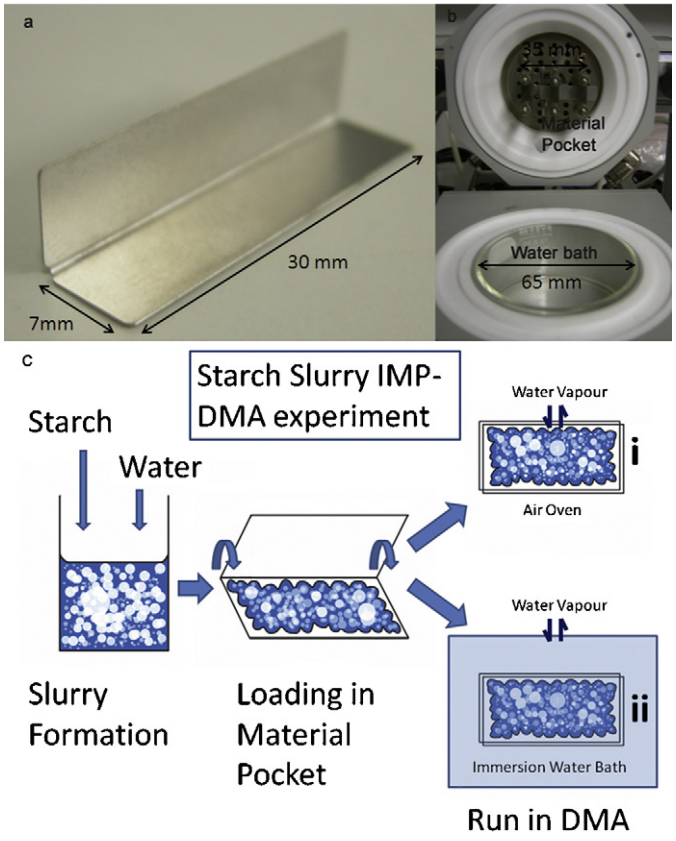

Fig. 1. The DMA equipment. (a) A close up of an open material pocket. (b) The material pocket loaded into the instrument showing the instrument head and water bath. (c) Schematic diagram showing the sample preparation and loading. The sample may be either (i) treated in a dry air oven, where water is free to evaporate directly from the sample, or (ii) immersed in a water bath, where the sample is completely submerged, and evaporation only occurs at the air–water interface at the surface of the water bath.

A method that has recently been introduced to the study of starch gelatinisation is dynamic mechanical analysis (DMA) (Madrigal, Sandoval, & Müller, 2011; Pereira & Oliveira, 2000; Xie et al., 2008), which has the potential to introduce water in its liquid and gaseous forms (i.e. changes in humidity) during testing. DMA is a physical characterisation method in which a sinusoidal force is applied to a test material and the stress and strain are measured as a function of temperature. Traditional approaches to DMA involve casting the test material into a solid bar that is capable of supporting its own weight (Madrigal et al., 2011; Pereira & Oliveira, 2000; Xie et al., 2008). This has obvious disadvantages for the study of a granular material such as starch, because compression of the sample to form a solid bar may alter the starches structure and properties, and limit the amount of water that may be incorporated into the sample. Starch gelatinisation is plasticised by water and the availability of water during gelatinisation is important in determining the nature of the structural changes of starch that occur (Bogracheva, Wang, & Hedley, 2001; Bogracheva et al., 2002; Perry & Donald, 2002; Roder et al., 2009).

In order to circumvent the production of a monolith or bar to allow DMA analysis, a DMA material pocket has been developed to allow particulate materials to be analysed in their native powdered form (Royall et al., 2005). Initial results using this technology to measure the thermally driven transitions of powdered semi-crystalline or semi-amorphous materials have proved successful (Mahlin, Wood, Hawkins, Mahey, & Royall, 2009). However, there is a need to conduct these powder DMA experiments in the presence of liquid water or more complex aqueous systems, since such situations model the processing end point for many powders that are heated in water and/or consumed by man. The aim of this paper, therefore, is to evaluate the novel combination of material pocket DMA and an immersion bath technology as a tool for the measurement of the physical changes that occur during the hydrothermal treatment of starch in its native powder form.

2. Materials and methods

Except where specified, all chemicals were obtained from Sigma–Aldrich Chemical Company (Poole, Dorset, UK) and were of the highest available grade.

2.1. Sources, preparation and characterisation of starches

Wheat starch (Cerestar, cv. GL04) and pea starches (WT, *r* and *lam* (Wang et al., 1998)) were gifts from Prof. T. Bogracheva and Prof. C. Hedley (formerly of the John Innes Centre, Norwich, UK). WT pea starch is a wild type pea starch comprising of ~30% amylose and 70% amylopectin (dry, w/w). The *r* mutant pea starch has a mutation at the *rugosa* gene locus, which results in a starch with a very high (~70%) amylose content, because of decreased activity of granule-bound starch synthase 1 (Lloyd, Hedley, Bull, & Ring, 1996). The *lam* mutant starch has a mutation at the *low amylose* gene locus and contains only ~10% amylose (Tahir, Ellis, Bogracheva, Meares-Taylor, & Butterworth, 2011;Tahir et al., 2010; Wang et al., 1998). Potato starch was obtained from National Starch and Chemicals (UK). Waxy rice starch (cv. Remyrise) was a gift from Dr. P. Rayment (Unilever, UK) and is essentially free of amylose. Normal maize starch (cv. Globzeta) was a gift from Prof. I. Rowland (University of Reading, UK). Starch-rich durum wheat semolina was a gift from Mr. Bruno Boggini (Millbo S.p.A., 28069 Trecate, Italy), and was used as 1 mm sized endosperm particles. The starches used in this study have been well characterised in our laboratory and contain very low levels of impurities (Tahir et al., 2010). The lipid and protein contents of the starch samples from different botanical sources were found to be ≤0.40% (w/w) and 0.31% (w/w), respectively, except for *r* mutant pea starch, which has the slightly higher level of 0.67% for protein (Tahir et al., 2010). Starch damage values for all samples, as determined by Congo red dye exclusion and microscopy, were found to be <0.5% of the total starch content (Slaughter, Ellis, & Butterworth, 2001; Warren, Royall, Gaisford, Butterworth, & Ellis, 2011).

2.2. Amylose/amylopectin ratio of starches

The amylose–amylopectin ratio of the starch was determined using the iodine dye binding method of Knutson (Knutson, 1986, 2000; Knutson & Grove, 1994), as modified by Warren (2011). Briefly, a 6 mM iodine solution was prepared by dissolving 0.229 g of re-sublimed iodine in 270 mL of dimethyl sulphoxide. To this solution, 30 mL of dH_2O was added. A standard curve was prepared from 1 to 5 mg of amylose (potato amylose, type III) in 15 mL Falcon tubes. Five milligrams of each starch sample were accurately weighed in triplicate and put into 15 mL Falcon tubes. To each of the samples and standards (and to a blank tube containing no starch) was added 10 mL of iodine solution. The tubes were then placed on a blood rotator providing end over end mixing and left overnight to allow the starch to dissolve.

After the starch had completely dissolved, 100 μL of solution was removed from each tube and added to 800 μL of dH_2O. This was then left for 30 min to allow the iodine–amylose complex to form a stable colour. The absorbance values of all samples and standards were then read spectrophotometrically at 600 nm (Cecil CE 2041 spectrophotometer) against the reagent blank. Apparent amylose concentrations were calculated from the amylose standards and these were converted to true values using the following equation:

$$\text{amylose concentration (\%)} = \text{apparent amylose concentration (\%)} - \frac{6.2}{93.8} \quad (1)$$

This equation corrects for the small amount of absorbance which occurs as a result of amylopectin binding iodine and producing some interfering colour (Knutson, 1986).

2.3. Immersion mode material pocket dynamic mechanical analysis (IMP-DMA)

Dynamic mechanical analysis is a method that measures the mechanical properties of a material as a function of temperature. A sample is clamped between a fixed cantilever and a drive shaft and a sinusoidal force is applied to the sample. The mechanical response of the sample to this force is measured as a function of the oscillatory frequency and the temperature.

When a viscoelastic material is analysed by DMA, an oscillatory stress is applied to the sample to deform it by a predetermined amount and the resultant strain is measured. As the majority of materials being investigated are viscoelastic in nature, a complex modulus results with an in phase (storage, G') modulus resulting from solid-like behaviour, and an out of phase (loss, G'') modulus resulting from liquid-like behaviour. The ratio G'/G'' is a dampening factor, describing the ratio of energy stored in the material to the energy dissipated and is termed $\tan\delta$ (Royall et al., 2005).

All DMA analyses were carried out using a Perkin Elmer DMA 8000 instrument fitted with a temperature-controlled immersion unit. The sample was loaded into a stainless steel material pocket (Perkin Elmer, Seer Green, UK, part no. N5330323) with dimensions of 30 mm by 14 mm. The pocket was scored lengthways to allow it to be folded in half and folded to an angle of approximately 60° to allow sample loading (Fig. 1a). Approximately 30 mg of starch was accurately weighed into the pocket, and either loaded dry, or mixed with 50 μL of water to make a slurry. The pocket was then folded in half, crimped closed to form a sandwich approximately

0.5 mm wide, reweighed, and clamped into the DMA (Fig. 1b). The pocket was loaded in a single cantilever bending mode, with one end of the pocket clamped to a fixed support and the other end clamped to the drive shaft. All the clamps were tightened using a torque wrench to a force of 5 N. This meant that one end of the pocket was held stationary, while the other end was subjected to an oscillating displacement by the driveshaft. This resulted in the pocket being deformed in an oscillating, bending motion in and out of plane, subjecting the starch powder (or slurry) in the pocket to a horizontal shear. Experiments were also carried out using a pocket manufactured from Dutch twilled woven steel (G. Bopp Ltd. MM10344 wire cloth).

The samples were submerged in a water bath containing ~100 mL of deionised water (Fig. 1b), and subjected to heating from 20 °C to 90 °C at a heating rate of 1 °C/min (or placed in a dry air oven using the same heating conditions), while undergoing a dynamic displacement of 0.05 mm at 1, 10 and 30 Hz. The force was automatically controlled between 1 N and 10 N to achieve the target displacement. The modulus was calculated from the actual measured dynamic displacement amplitude (Royall et al., 2005).

All data were analysed using Origin® software to obtain the peak tan δ and modulus values.

2.4. Differential scanning calorimetry (DSC)

All DSC experiments were carried out using the method described by Warren et al. (2011).

2.5. Heated stage microscopy

Heated stage microscopy was carried out using a Leitz Dialux 22EB microscope fitted with crossed polarisers and a λ plate (red 1 compensator), with a Qi Imaging QiFastcam, Linkam HFS91 heated stage and Linkam TP92 controller attached to a computer running the Linksys32 software package (Linkam). Samples were heated from 25 °C to 90 °C at a rate of 1 °C/min, with an image being captured every 10 s. Microscopy was carried out following the method of Warren (2011).

3. Results

3.1. Method development and preliminary results

Preliminary experiments were carried out using wheat starch in a dry air oven. When a dry powdered wheat starch sample (~12% moisture (w/w) (Tahir et al., 2010)) was loaded into the material pocket and subjected to heating from 20 to 90 °C, there was no response observed in either the modulus or tan δ (data not shown), over the measured temperature range. This is unsurprising as the gelatinisation of starch is known to be plasticised by the presence of water, so no physical transition would be expected to occur at these temperatures in the absence of excess water (starch crystallite melting in the absence of water occurs at 150–170 °C) (Roder et al., 2009). Subsequently, an experiment was carried out where wheat starch was loaded into the material pocket as a slurry (containing 30 mg of starch and 50 μL of water), to provide water for gelatinisation. This pocket was sealed closed with nail polish to minimise water loss during heating and then subjected to heating in a dry air oven from 20 to 90 °C. A blank pocket was also run sealed with nail polish, but empty, and no transitions were observed (data not shown). No response was observed in the modulus for a starch sample under these conditions (Fig. 2a(ii)). A small response was observed in tan δ, in the form of a step change that occurred at around the gelatinisation temperature (Fig. 2b(ii)). This result indicated that in the presence of water, a change in the physical properties of the starch appeared to occur during gelatinisation.

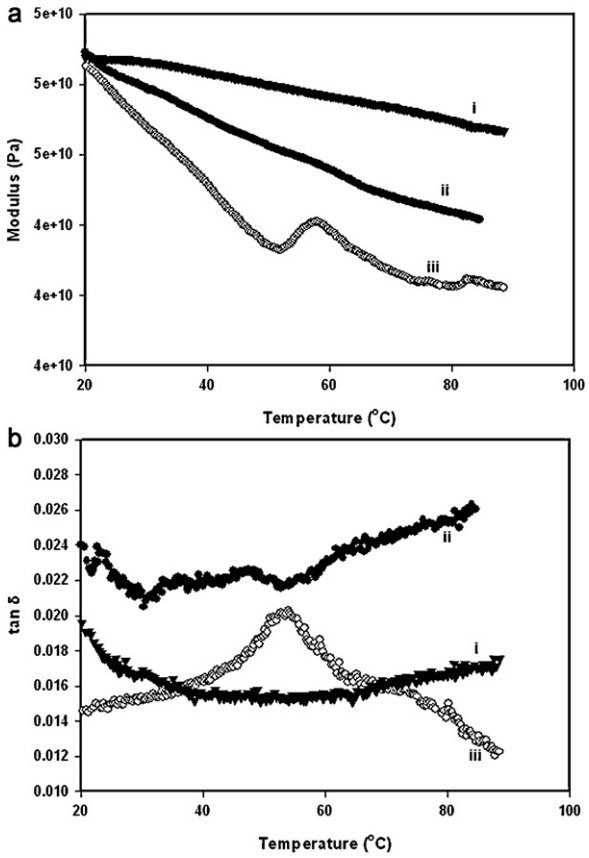

Fig. 2. (a) The modulus response for an empty material pocket (closed triangles, i), a wheat starch slurry heated in a material pocket in a dry air oven (closed circles, ii), and a wheat starch slurry heated in a material pocket in an immersion bath (open circles, iii). All data were recorded at 10 Hz (note that these data have not been baseline corrected). (b) The tan δ response for an empty material pocket (closed triangles, i), a wheat starch slurry heated in a material pocket in a dry air oven (closed circles, ii), and a wheat starch slurry heated in a material pocket in an immersion bath (open circles, iii). All data were recorded at 10 Hz.

The response was small and ill-defined, probably because insufficient water was present to fully gelatinise the starch, given that there was only a limited amount of water initially, which was further reduced by some drying during heating. A similar result was observed when wheat starch was loaded into a material pocket as a dry powder and then placed in the immersion heating unit and heated (data not shown). Upon examination of the samples in the pocket following heating, it was found that the sample had not fully mixed with water, leading to the formation of pockets of air that had stopped the starch from fully hydrating in the material pocket, leading to an inhomogeneous sample and inconsistent results. As a result of this, for subsequent experiments the starch samples were prepared as a slurry, prior to being submerged in the immersion unit. Examination of the samples both prior to and following heating showed that they were fully hydrated using this procedure, maximising the availability of water. Wheat starch heated in the DMA using this method was found to give very well defined, reproducible peaks in both the tan δ and modulus values (Fig. 2a(iii) and b(iii)).

The baseline of the modulus curves showed a slope (Fig. 2a). A control experiment was carried out (Fig. 2a(i)) where the modulus of the material pocket (without sample) over the temperature range investigated was measured. A reproducible baseline slope was observed in the modulus response of the pocket. This is likely to be due to the effects of thermal expansion in the steel pocket during the heating process. As a result, the average slope value

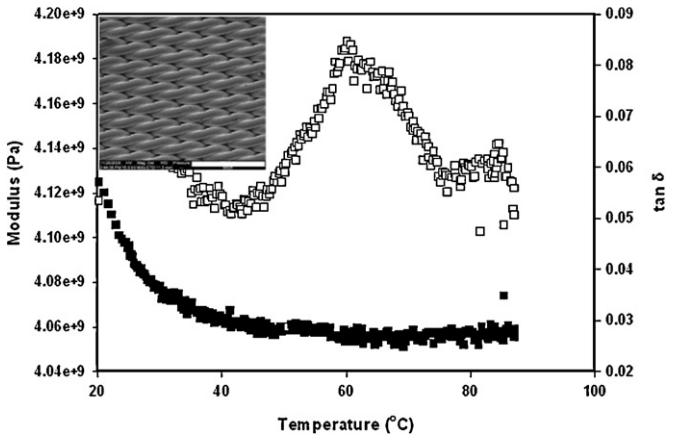

Fig. 3. The DMA response for a wheat starch slurry heated in a Dutch twilled pocket in an immersion bath. Modulus values (open squares), and tan δ values (closed squares) were collected at 10 Hz. Inset is an SEM image of Dutch twilled material. Scale bar 300 μm.

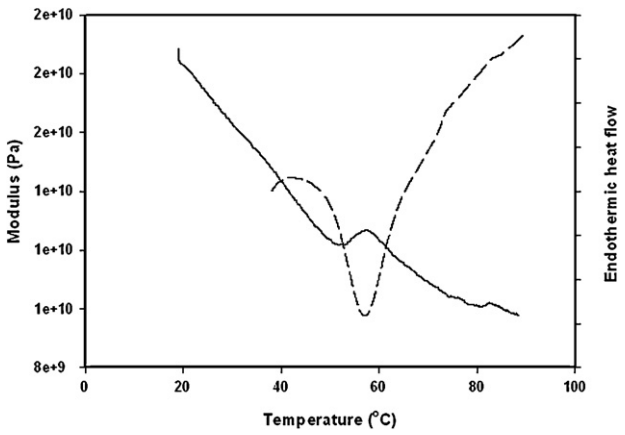

Fig. 4. An overlay graph of DSC (dashed line) and DMA modulus (solid line) data measured at 30 Hz in an immersion bath. DSC and DMA data are both obtained from wheat starch.

obtained (in triplicate) from an empty material pocket (i.e. blank) was subtracted from all subsequent analyses.

The DMA method was also tested with an alternative pocket material, Dutch twilled woven steel mesh. This material has small pores (~4 μm) (see inset scanning electron microscopy (SEM) image in Fig. 3 for detail), which would be expected to facilitate greater mobility of water into and out of the sample. Sensitivity is also increased due to the pocket material being less stiff, and thus making a smaller contribution to the total measured modulus.

Data for wheat starch DMA using the Dutch twilled pocket is shown in Fig. 3. The modulus measured was an order of magnitude smaller than that with a stiff pocket, as expected, due to the Dutch twilled steel pocket being less stiff than the solid steel pocket. The modulus peak observed was larger than that observed with a stiff pocket, but it was much broader and less well defined due to the greater noise generated by the Dutch twilled pocket, with greater variation in the position of the peak (±5 °C). The tan δ peak was almost completely lost in the noise, and could not be analysed. The position of the modulus peak with the Dutch twilled pocket was the same as with the solid steel pocket. The Dutch twilled allows free movement of water between the bulk and the sample. That the modulus peak position was the same for the solid steel and Dutch twilled pockets indicates that the water is able to enter the sample in the solid pocket as easily as in a Dutch twilled pocket.

3.2. The use of IMP-DMA to characterise the thermo-mechanical behaviour of starch and starch containing materials

As a result of these experiments, DMA runs for a range of starch samples were carried out using slurried samples, in a solid steel material pocket, using the immersion bath, because this method was found to produce the most reliable, reproducible peaks. The results of these experiments (tan δ and modulus peak temperatures) are shown in Table 1, along with onset and peak gelatinisation temperatures obtained by DSC. No frequency dependence was observed in peak position, when measurements were taken from 1 Hz to 30 Hz. This would suggest that the transition being observed in the tan δ is attributable to the swelling of the starch granules, rather than a glass transition, which would be expected to show a clear frequency dependence (Mahlin et al., 2009).

The DMA results appeared to be influenced by the amylose to amylopectin ratio of the starch samples. For starches with a normal amylose to amylopectin ratio (Table 1), peaks in both the modulus and tan δ could be reliably obtained for starch gelatinisation (Table 1). The *r* pea starch, which has a very high amylose content (65%) failed to show peaks in either tan δ or modulus. This was not a surprising result, as *r* pea starch differs significantly from other starches in its thermal characteristics (Bogracheva et al., 1999). *r* Pea starch does not have a defined gelatinisation peak, but instead goes through a long (>30 °C) transition, and even at high temperatures this starch still retains a granular structure, and therefore does not go through the physical changes that occur typically when more conventional starches are gelatinised. This makes gelatinisation of *r* pea starch difficult to study by IMP-DMA, RVA and DSC (Tahir et al., 2011; Tahir et al., 2010).

Low amylose varieties of starch (e.g. waxy rice, which is essentially amylose free, and *lam* pea starch, which is 7% amylose) did not produce reproducible peaks, and exhibited a large amount of variability between runs, particularly in the presence/absence of a modulus peak (data not presented). These findings may be explained by studies that show amylose to be an important determinant of starch mechanical properties post-gelatinisation, i.e. mediating the increase in viscosity during gelatinisation and maintaining the structural integrity of starch granule ghosts that remain after gelatinisation (Debet & Gidley, 2007). This makes the gelatinisation of low amylase varieties of starch difficult to study by thermo-mechanical techniques, where DSC is capable of detecting a defined gelatinisation peak (Jane et al., 1999). This may result, therefore, in low amylose varieties of starch showing less defined changes in structure during gelatinisation, which are more difficult to detect by DMA.

The position of the peak in the modulus observed by DMA for starch gelatinisation showed a good correlation ($R^2 = 0.85$) with the position of the gelatinisation melting peak observed by DSC, as shown in Figs. 4 and 5a, where the position of the DSC peak can be seen to match up well with the modulus peak obtained by DMA, both in terms of peak position and peak width. Table 1 shows the relationship that was found between both modulus and tan δ with the DSC peak data (Fig. 5a). The relative peak positions observed by DMA for the different starch samples mirror the DSC peak positions. The modulus peak matches closely with the peak position found by DSC; a similar result to that obtained in recent experiments by Xie et al. (2008), using moulded blocks of starch with a low water content. Unlike the results of Xie and co-workers, however, who found that the tan δ peak also matches the DSC peak, the tan δ peak data presented in the current study was found to consistently occur 4–5 °C below the peak position obtained from the DMA modulus and DSC data (Table 1 and Fig. 5a). This can be explained if the tan δ peak is indicative of swelling of the granules as a result of water uptake. In an immersion bath the starch will

Table 1
Peak temperatures in the tan δ and modulus peaks for starch gelatinisation, measured at 30 Hz. Where data could be reliably obtained in triplicate, values are presented as means of triplicates (±S.E.M.). Otherwise values are from single readings. DSC onset and peak gelatinisation temperatures are presented as means of triplicates (±S.E.M.). Amylose contents of starch samples (%, w/w) determined by iodine binding are presented as means of triplicates (±S.E.M.).

Starch type	tan δ peak (°C)	Modulus peak (°C)	DSC onset temp. (°C)	DSC peak temp. (°C)	Amylose content (%, w/w)
Wheat	52.8 (±2.0)	57.7 (±0.6)	49.3 (±0.2)	57.2 (±0.2)	22.8 (±0.3)
Maize	59.9 (±2.3)	65.9 (±2.0)	64.3 (±0.2)	70.0 (±0.0)	22.8 (±2.2)
Waxy rice	58.5	66.3	57.3 (±0.1)	67.2 (±0.4)	1.2 (±0.1)
Potato	60.3 (±0.5)	64.4 (±2.0)	59.6 (±0.0)	65.0 (±0.0)	15.3 (±1.6)
r pea	N.D.	N.D.	43.9 (±1.1)	70.0 (±2.9)	65.1 (±1.3)
lam pea	60.9 (±2.9)	68.8	62.8 (±0.1)	67.0 (±0.2)	7.0 (±0.1)
Wild type pea	53.9 (±1.3)	57.7 (±0.4)	52.9 (±0.1)	59.0 (±0.0)	31.9 (±0.3)

N.D.: not determined.

have greater access to water than in a monolith, and will therefore be able to swell more fully. The tan δ peak position was in close agreement with the gelatinisation onset temperature measured by DSC (Table 1 and Fig. 5b), and can also be seen to correlate with the large changes in starch structure that are observed by light microscopy, although the IMP-DMA clearly provides a greater amount of detail than microscopy alone. Thus, microscopy can only be considered to be a semi-quantitative method due the limitations in the number of granules that can be analysed in a single field of view by image analysis. Nevertheless, the polarising microscopy method used in the current study is a useful complimentary technique for confirming the gelatinisation temperature and loss of order (Fig. 6). Micrographs of starch granules showed marked swelling and loss of birefringence (i.e. decrease in ordered structure) with increase in temperature, at temperatures similar to the peaks observed in IMP-DMA data.

In the current experiments presented in this paper, where water was available in excess for starch gelatinisation, the tan δ peak was found to occur at the onset of gelatinisation. It is well known that water availability plays an important role in starch gelatinisation (Perry & Donald, 2002; Roder et al., 2009). In the restricted water regime used by Xie and co-workers (40–60% (w/w) water) the position of the gelatinisation peak observed by DSC is unchanged relative to gelatinisation in excess water, but the peak is much smaller, and not associated with a pronounced glass transition. A significant fraction of the starch crystallites melt at a higher temperature, instead of going through gelatinisation plasticised by water (Roder et al., 2009). The position of the tan δ peak relative to the modulus peak has been shown to be highly dependent on water content in DMA experiments using moulded starch bars (Madrigal et al., 2011). In the excess water conditions used in the experiments presented here (with a material pocket and immersion bath), the gelatinisation of starch is not limited by water availability, and the tan δ peak occurs at the onset of gelatinisation, suggesting that this peak is an event which is water dependant, and occurs at the onset of gelatinisation.

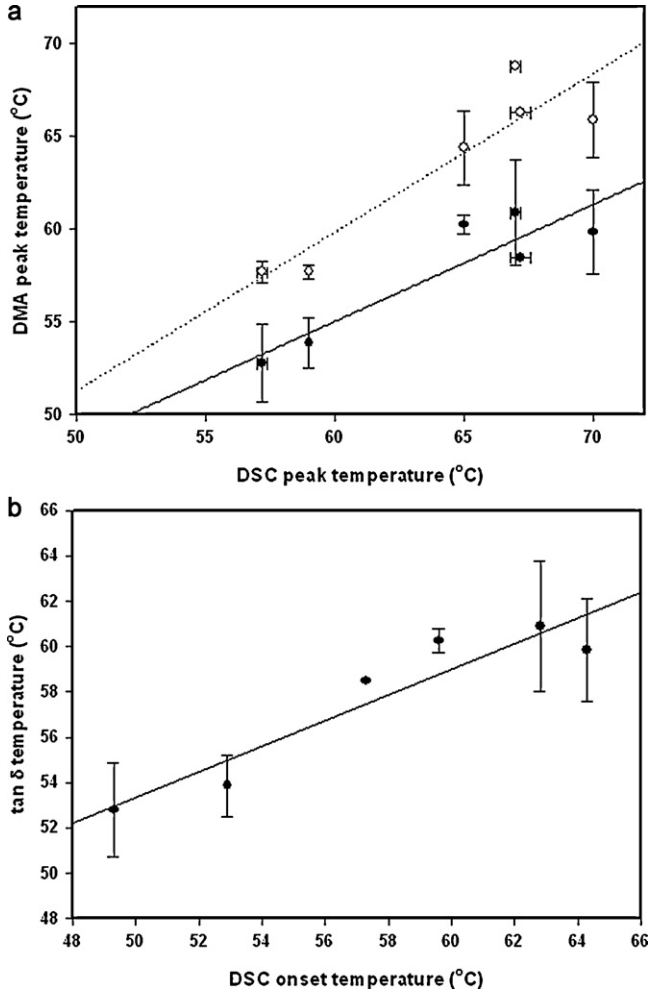

Fig. 5. (a) The linear relationship between DMA parameters modulus (open circles) and tan δ (closed circles) peak positions for starch gelatinisation and the position of the DSC peak observed for gelatinisation of the starch samples. Data points are means of triplicate determinations, and error bars represent S.E.M. Linear regression analysis has been included to demonstrate the strength of the relationships, so that the solid line has been fitted to the tan δ data (R^2 = 0.83) and the dotted line has been fitted to the modulus data (R^2 = 0.85). (b) Relationship between the tan δ peak and DSC onset for gelatinisation of starch samples. All data points represent means of triplicate determinations, and error bars represent S.E.M. A linear regression line has been fitted to the data (R^2 = 0.88).

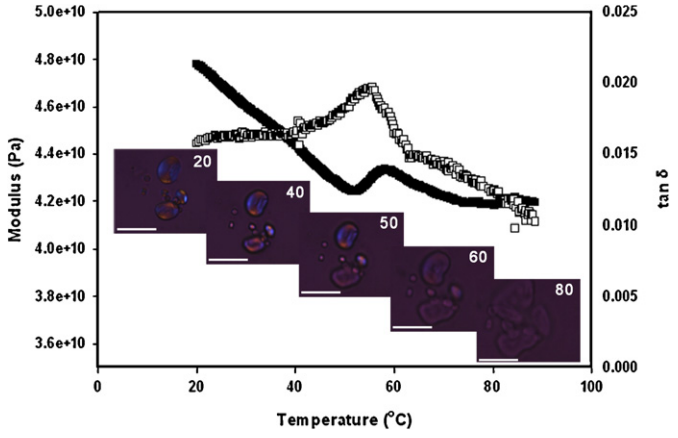

Fig. 6. tan δ (open squares) and modulus (closed squares) data for wheat starch gelatinisation. Data collected at 30 Hz. Heated stage microscopy images are included of wheat starch, heated in excess water, at 20, 40, 50, 60, and 80 °C. Micrographs of starch granules, examined by light microscopy under polarised light, exhibited swelling and loss of birefringence (i.e. decrease in ordered structure) with increase in temperature. The scale bar is 40 μm.

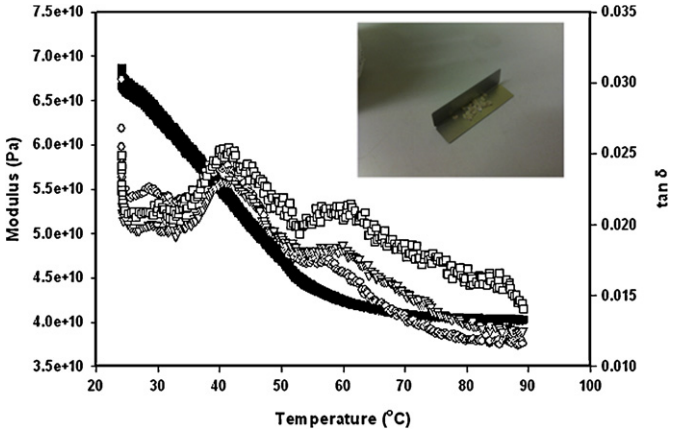

Fig. 7. The DMA response for durum wheat semolina (i.e. macroparticles of starch-containing wheat endosperm) heated in a material pocket in an immersion bath. Modulus values are closed data points, and tan δ values are open data points. Data collected at 30 Hz are squares, at 10 Hz triangles, and at 1 Hz circles. Inset is a sample of durum wheat semolina in a material pocket, prior to loading into the instrument.

In addition to studies with pure starches, the DMA with material pocket was also used to study gelatinisation of a more complex starch-containing material represented by durum wheat semolina. The semolina comprises small (~1 mm) chunks of intact wheat endosperm tissue that will fit into a material pocket (Fig. 7). The results of heating the semolina in water are shown in Fig. 7, where two peaks are observed. The first tan δ peak at ~42 °C is difficult to assign and may represent a change in endosperm cell wall structure, possibly a hydration step. The semolina was pre-hydrated at room temperature, in the same way as the starch samples, prior to submerging in the immersion bath, but this peak may represent a further temperature-dependant hydration stage. The second peak in tan δ is at 57.8 °C at 1 Hz, 59.9 °C at 10 Hz and 61.2 °C at 30 Hz, all of which are very close to the DSC peak of 57.2 °C obtained for starch extracted from the semolina (DSC data not shown), albeit slightly higher. It is interesting to note that the tan δ peaks associated with starch gelatinisation in semolina show a frequency dependence, that is unlike that of the purified starch, and which is above the DSC peak position. This may be due to limited water availability within the plant tissue and/or the influence of other cell components (e.g. cell walls) on the mechanical properties of the material. Obtaining the gelatinisation temperature of starch within a complex heterogeneous material, such as plant tissue, offers significant difficulties as the starch is at a relatively low (5–30%, w/w) concentration within the material, resulting in problems with sensitivity. Also plant materials may be too large, or awkwardly shaped to fit into a DSC or be analysed with microscopy techniques.

4. Discussion

Historically, the only samples that could be analysed by DMA were those that could support their own weight when clamped between two (or more) cantilevers. The recent introduction of the powder or material "pocket" has allowed the analysis of loose powders and slurries, by holding the sample in a solid steel support (Royall et al., 2005). The use of suitable pockets, combined with the addition of a temperature controlled water bath to the Perkin Elmer DMA 8000 instrument used in this study, produces a highly versatile system for the study of the physical changes in starch structure associated with hydrothermal treatment and gelatinisation of granular starch.

Previous work (Xie et al., 2008) has found that DMA may be applied to the study of starch gelatinisation using blocks moulded from wetted starch. This technique has the disadvantage that it limits the water content that can be included with the starch to a very narrow range of between 40 and 60% (w/w). Water plays a key role as a plasticising agent in starch gelatinisation (Bogracheva et al., 2001; Perry & Donald, 2002; Roder et al., 2009), so investigating starch gelatinisation under the water limited conditions necessitated by forming the starch into blocks leads to results that cannot be directly compared with those obtained under conditions of excess water, with the starch in a free flowing powder form. The novel material pocket method for DMA analysis of powders (Royall et al., 2005) has shown promise for the detection of water-dependant glass transitions in carbohydrate polymers (Raschip et al., 2008). When the material pocket is used with an immersion bath it makes a useful tool for studying starch gelatinisation in excess water, producing peaks in both the modulus and tan δ that correspond to changes in the physical properties of starch during gelatinisation as measured by DSC (Figs. 2 and 6). Mutant starches with different amylose/amylopectin ratios showed unusual results. The *r* pea starch, with a very high amylose content, gelatinises over a very broad temperature range (~35 °C as measured by DSC), maintaining its granule structure throughout gelatinisation (Tahir et al., 2010), and subsequently does not show a detectable transition in either modulus or tan δ DMA data. This would qualitatively agree with results obtained by RVA that show high amylose starch varieties to have a negligible peak pasting viscosity (Song & Jane, 2000). The *lam* pea starch has a lower than average amylose content of 6% (w/w), and produces a reproducible tan δ peak, but does not produce a modulus peak on every run. Waxy rice starch, which is essentially free of amylose, did not reproducibly yield a peak in either modulus or tan δ values (although the peak positions were consistent when peaks were obtained). This would suggest that the starch structural changes that occur during gelatinisation of low amylose varieties of starch are more difficult to detect by DMA. Interestingly, RVA data has indicated a higher peak viscosity, and earlier pasting temperature for waxy varieties of starch, compared with those with normal amylose/amylopectin ratios (Chakraborty et al., 2004; Song & Jane, 2000; Yoo & Jane, 2002). This has been attributed to the role of amylose in maintaining starch granule integrity during gelatinisation (Debet & Gidley, 2007). In the absence of amylose the granules completely disintegrate during gelatinisation, resulting in a rapid increase in viscosity. It may be that as the waxy granules disintegrate during gelatinisation, there is very little starch granule structure remaining to shear in the material pocket, thus making the detection of peaks associated with gelatinisation extremely difficult.

As well as being able to determine correctly the onset and peak temperatures for the gelatinisation of pure starches from the physical changes that occur to the granule during gelatinisation, hydrothermal mechanical analysis is also sensitive enough to detect the physical changes that occur to the structure of a complex starch-containing plant tissue, such as durum wheat semolina, when the starch component of the material gelatinises. The method is therefore capable of accurately determining the gelatinisation temperature of starch when it is part of a more complex system. This can prove challenging with more conventional techniques, such as DSC, due to the low proportion of starch in many food materials.

There are aspects of the methodology that require further development work, most notably when studying starches with very high, or very low, amylose contents. As discussed above, this is likely to be due to intrinsic properties of these highly unusual starches, in that they undergo different (smaller) thermo-mechanical transitions upon gelatinisation, which are difficult to detect, compared with starches with normal amylose/amylopectin ratios (Chakraborty et al., 2004; Song & Jane, 2000; Yoo & Jane, 2002). This poses an interesting challenge for research workers analysing starch by IMP-DMA. A possible methodological improvement would be to mix the

starch sample with an inert powder filler, such as aluminium oxide (Mahlin et al., 2009). This would have the effect of isolating the individual starch granules within the matrix formed by the inert filler, thus allowing single starch granules to swell and leach fully without interference from other starch granules, thereby increasing the sensitivity of the method. Such an improved sensitivity has been reported for hydroxypropyl methyl cellulose when analysed by DMA in conjunction with an inert filler (Mahlin et al., 2009).

A range of instrumental set ups were tested to determine which gave the most accurate and reproducible results. It was found that using a steel material pocket in an immersion bath provided the most reproducible data, when compared to the data obtained from DSC experiments. In excess water conditions it was found that the tan δ peak was positioned at the onset of gelatinisation, as measured by DSC. The tan δ peak did not show any frequency dependency, and may represent the swelling of the starch that occurs at the onset of gelatinisation. The modulus peak corresponded with the position of the DSC peak gelatinisation temperature, indicating the temperature at which crystalline melting occurs. Different starch samples showed different DMA responses, depending on their composition and structure, in particular their amylose/amylopectin ratio, which mirrors results obtained by other techniques such as RVA. The IMP-DMA was found to have the sufficient sensitivity to reliably detect starch gelatinisation in more complex heterogeneous food samples such as durum wheat semolina.

5. Conclusions

In conclusion, IMP-DMA allows the changes in physical structure that occur during the gelatinisation of starch to be correctly measured using starch in the form of a free flowing powder. Supporting the starch in a material pocket provides the flexibility to allow the sample to be fully immersed, either in pure water for the accurate determination of gelatinisation properties, or potentially in other liquids of interest. Alternatively, as the sample is physically supported by the material pocket it may potentially be heated in a dry air oven, or at different water activities using a humidity unit. The data presented in this study have revealed that some limitations exist with the technique, particularly in detecting transitions in low and high amylose varieties of starch. The data, however, show that IMP-DMA is a highly flexible tool that may be used to study starch gelatinisation of both purified starch powders and starches in complex food matrices, under a wide range of conditions. Thus, IMP-DMA makes a useful addition to the range of tools available to study starch gelatinisation behaviour.

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