APPLICATION NOTE

Dynamic Mechanical Analysis

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Characterizing the Hydrothermal Behavior of Starch with Dynamic Mechanical Analysis

Introduction

Starch is one of the primary sources of energy in the human diet, and is also used in a wide range of industrial processes, including brewing, bioethanol production, paper manufacture and in the production of biodegradable plastics.[1]

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. Starch consists of two polymeric components: 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. 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 behavior of the starch and its response to hydrothermal treatments.[2]

One of the most important modifications of starch structure that occurs during processing of starch, for both food usage and industrial applications, is gelatinization. When heated in excess water, starch goes through a thermal transition, termed gelatinization, at temperatures between 50 and 70 °C. Starch gelatinization is an endothermic transition associated with rapid swelling of the granule and melting of crystalline regions. In the absence of water, starch crystallites go through a melting transition at much higher temperatures

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of around 150-170 ˚C. It is thought that the swelling of the amorphous regions of the starch granules, as they absorb water, introduces structural stress to the crystalline lamellae, allowing the crystallites to melt at a much lower temperature.

During gelatinization, "granule ghosts" (expanded, highly hydrated amorphous remnants of starch granules) are formed and large amounts of α-glucan material may be leached into solution. This large structural change may be used to characterize the gelatinization transition of a particular starch sample. However, the analysis is complicated by the significant degree of phase separation of the solubilized glucan chains and the granule ghosts. This phase separation renders starch gelatinization unsuitable for study in conventional cone-and-plate or plate-plate rheometers, because the native (un-gelatinized) 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 gelatinization accompanying hydrothermal treatment. The rheological changes that occur to starch during gelatinization are important to understand for a range of industrial applications, for example in starches used as a filler to provide texture in the food industry, and as a potential predictor of the starches behavior as an enzyme substrate.

Dynamic mechanical analysis (DMA) is a technique ideally suited to the investigation of relaxation events, and is often used for determination of glass transition temperatures in polymers and other amorphous materials, e.g. amorphous lactose and composite materials. DMA works by applying an oscillating force (stress) to the sample and measuring the resultant displacement (strain). From these measurements, the storage modulus, loss modulus and phase angle (tan δ; equal to the ratio of the loss and storage moduli) can be calculated. The phase angle gives information about the damping properties of the material: tan δ is plotted against temperature, and glass transitions are normally observed as a peak, since the material will absorb energy as it passes through the glass transition (a temporary increase in the loss modulus).

However, despite DMA's advantages, until recently it has not been widely used for powdered and granular materials due to the difficulty in handling them in mechanical tests. The Material Pocket for the DMA 8000 instrument from PerkinElmer was developed to allow straightforward, reproducible analysis of powders with DMA. The Material Pocket is capable of supporting 20-50 mg of granular or powdered material in a pocket formed by a piece of folded steel. The pocket is manufactured from stainless steel, which is mechanically inert over a wide range of temperatures, and hence any transitions that are observed during heating are due to changes in the material held in the pocket. The bulk of the mechanical response comes from the pocket, but any perturbations seen in the signal will result from transitions in the material held within the pocket.

An additional requirement for many applications, such as starch gelatinization and other transitions of food and pharmaceutical materials, is the ability to conduct 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. In this application note, we explore the use of immersion-mode material pocket DMA as a tool to investigate the structural changes associated with starch gelatinization.

Experimental

Wheat starch (Cerestar, cv. GL04) and pea starches (WT, *r* and *lam*) 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 a decreased activity of granule-bound starch synthase 1.[3] The *lam* mutant starch has a mutation at the *low amylose* gene locus and contains only ~10% amylose.[4] 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).

All experiments were carried out using a PerkinElmer DMA 8000 fitted with a water bath accessory. The sample was loaded into a stainless steel material pocket (PerkinElmer, 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. 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. 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.

The samples were submerged in a water bath containing ~100 mL of deionized water, 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.

Figure 1. The DMA 8000 dynamic mechanical analyzer with immersion bath accessory.

Results and Conclusions

In the absence of water, starch does not go through a gelatinization transition. When starch was loaded as a dry powder into a material pocket and subjected to heating in a standard dry air oven, no transitions were observed between ambient temperature and 90 ˚C.

In order to induce a gelatinization transition, starch was loaded as a slurry with water in a material pocket, which was then closed with sealant. When this sample was heated from ambient to 90 ˚C, a small transition was observed at ~60 ˚C, near the gelatinization point of the starch. However, repeat runs showed poor reproducibility, with the temperature and intensity of the transition varying widely. This lack of reproducibility was attributed to variations in the degree of hydration of the sample.

Subsequently, a dry sample of starch was placed in the material pocket, and submerged in ~100 mL of water in the DMA 8000 immersion bath attachment. The starch sample was then heated from ambient to 90 ˚C. The gelatinization peak was again observed, but suffered from the same poor reproducibility. Inspection of the sample after analysis revealed that air bubbles had formed in the material pocket, leading to incomplete, uneven hydration of the starch. Finally, loading the material pocket with starch slurry prior to immersion in the water bath led to good reproducibility in both the measured modulus and phase angle. Typical results are shown in Figure 2.

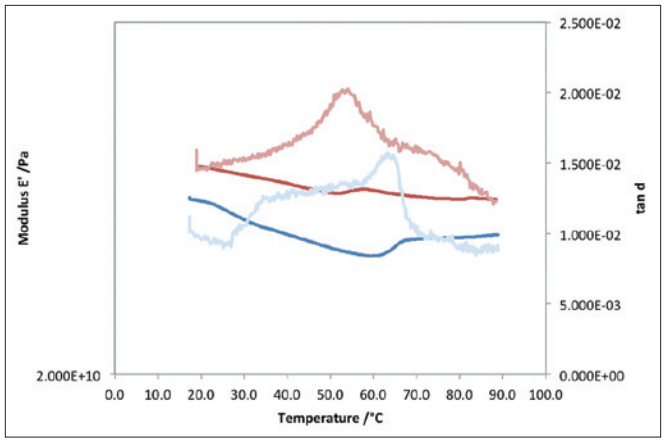

Figure 2. Tan δ (Maize – light blue, Wheat – light red) and modulus (Maize – dark blue, Wheat – dark red) values for starch slurries heated in the material pocket by immersion mode DMA.

The peaks seen in the modulus and tan δ for DMA analysis of starch in the material pocket clearly relate to structural changes in the starch that occur during gelatinization, which can also be observed by DSC and polarized light microscopy (Figure 3). The tan δ peak clearly correlates with the gelatinization onset temperature as measured by DSC and with the initial swelling of some granules (and loss of birefringence) observed with polarized light microscopy (Figure 3). No frequency dependence is observed for the tan δ, so it can be concluded that this is not a glass transition. A likely explanation for the tan δ peak is that it arises from the water absorption and swelling of the granules that occurs in the first stages of gelatinization.

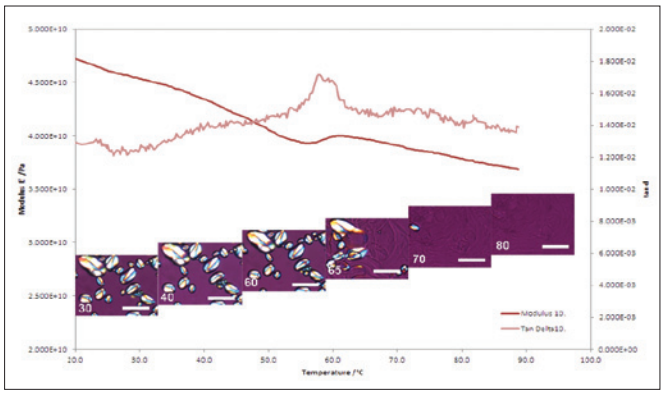

Figure 3. Tan δ (light red) and modulus (dark red) responses for potato starch heated in immersion mode DMA. Overlaid are polarizing microscopy images of potato starch suspended in water undergoing gelatinization. Temperatures are provided in ˚C. The scale bar represents 100 µm.

The modulus peak is observed at a temperature 5-6 ˚C higher than the tan δ peak, at a temperature corresponding to the peak in the DSC gelatinization endotherm (Table 1 and Figure 4). The modulus peak also shows no frequency dependence. This peak may be interpreted as resulting from the near simultaneous relaxation in the amorphous regions of the starch granule and melting of crystalline regions that occurs during gelatinization.

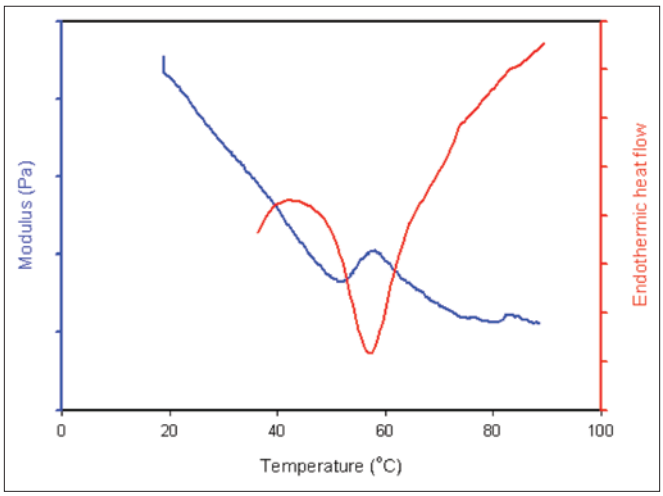

Figure 4. Modulus values for wheat starch heated in immersion mode DMA (blue) overlaid with endothermic heat flow for wheat starch in excess water measured by DSC (red).

The natures of the peaks that are observed for starch gelatinization by DMA are dependent on the type of starch that is used. Starches with very low (<10% w/w) amylose content have smaller tan δ and modulus peaks, reflecting the smaller change in viscosity on gelatinization. This is at least in part because amylose leaching into solution is one factor in increasing the viscosity of the aqueous phase. Starches with very high amylose contents (>60% w/w) fail to show peaks, as these starches do not go through major structural changes during gelatinization.

For more a more detailed discussion see Warren, et. al. (2012).[5]

In conclusion, DMA is a tool that may be used to measure structural changes in starch due to hydrothermal treatment. DMA is capable of accurately measuring changes in starch structure associated with both the onset (tan δ) of gelatinization, and the main gelatinization transition (modulus). The DMA offers more flexibility than alternative methods (e.g. DSC) as more awkward samples may be loaded into the material pocket, and the environment around the sample may be far better controlled. Using the water bath and humidity unit available with the PerkinElmer DMA 8000, sample behavior may be investigated under a wide range of humidity values, and immersed in a range of aqueous or non-aqueous liquids. This great flexibility and control make the DMA 8000 a powerful tool for characterisation of starch under a range of conditions not available with other techniques.

Table 1. **Peak temperatures in the tan δ and modulus peaks for starch gelatinization, measured at 30Hz. 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 gelatinization 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

References

1. Jobling, S. (2004). Improving starch for food and industrial applications. *Current Opinion in Plant Biology,* 7, 210-218.

2. Buléon, A., Colonna, P., Planchot, V., and Ball, S. (1998). Starch granules: structure and biosynthesis. *International Journal of Biological Macromolecules,* 23, 85-112.

3. Lloyd, J.R., Hedley, C.L., Bull, V.J., and Ring, S.G. (1996). Determination of the effect of *r* and *rb* mutations on the structure of amylose and amylopectin in pea (*Pisum sativum* L.). *Carbohydrate Polymers,* 29, 45-49.

4. Wang, T.L., Bogracheva, T.Y., and Hedley, C.L. (1998). Starch: As simple as A, B, C? *Journal of Experimental Botany,* 49, 481-502.

5. Warren, F.J., Royall, P.G., Butterworth, P.J., and Ellis, P.R. (2012). Immersion mode material pocket dynamic mechanical analysis (IMP-DMA): a novel tool to study gelatinization of purified starches and starch-containing plant materials. *Carbohydrate Polymers under review.*

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