Rheomicroscopy of Starch Gelatinisation

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ABSTRACT

Rheomicroscopy offers simultaneous monitoring of microstructure through microscopy and rheology, in large deformation and small deformation oscillating shear as well as in compression. The method was used to visualise and explain starch gelatinisation and how it is affected by shear, granule architecture and botanical origin. Shear had a strong influence on paste viscosity and was explained by granule disruption following swelling. Gelatinisation occurred during narrower temperature range for potato starch than for maize starch, which in turn both had a different behaviour from wheat starch. A-type wheat starch was also shown to have different gelatinisation behaviour than B-type wheat starch.

INTRODUCTION

Rheometry as well as microscopy are commonly utilised to monitor transitions in fluid systems such as gelation, crystallisation, phase separation and melting. The two can be combined by mounting a microscope in a rheometer or vice versa, to simultaneously monitor rheological and microstructural changes.

Starch consists of the plant polysaccharides amylose and amylopectin which are arranged in predominantly crystalline granules in the plant cells. The ratio of amylopectin varies depending on botanical source and there are natural variants which almost exclusively consist of amylopectin ("waxy" variants). High amylose variants exist after plant breeding or genetic engineering. The granule structure remains during preparation into flour. Flours also contain protein, fat and fibres to a varying degree also depending on botanical source. E.g. potato starch consists of minimal amounts of protein and fat, whereas maize flour has about 10% protein and wheat up to 12-14%.

A further structural dimension is that the actual granule structure varies depending on botanical source[1]. The common feature is that the granules consist of alternating crystalline and amorphous shells. Granule size varies from 1-100 μm [2] and wheat even has a bimodal size distribution of large, disc-shaped so called A-type (10-35 μm) and small, spherical B-type (<10 μm) granules[3]. When a suspension of granules in water is heated the crystalline structure is gradually lost and the granules swell. The decreased crystallinity is observed under polarised light as loss of birefringence manifested as an extinction cross, often referred to as a "Maltese cross" of the starch granule. Amylose and amylopectin are dissolved in the continued phase and form an entangled polymer structure on further heating. When monitoring gelatinisation by

viscometry granule swelling induce an increase in viscosity, whereas further shearing and heating often disrupts the swollen structure resulting in a drop in viscosity[4]. The effects vary depending on botanical source and the initial increase in viscosity may occur at temperatures from 55 to 85°C. For wheat starch the granule type has been found to influence gelatinisation with the A-type granule gelatinisation starting at lower temperature than the B-type as measured with high shear, in excess of water (RVA)[5].

Rheomicroscopy setups are available from manufacturers (TA Instruments MMA, Anton Paar Rheo-Microscope). Several instruments designed in-house have been described in literature such as parallel sliding-plate, stress-controlled shear cells [6-8], rotational shear cells[9, 10] and rheometers mounted in confocal microscopes[11-18]. Rheomicroscopy offers a valuable tool to observe sample structure during shearing or compression. Some limitations to conventional rheometry are the comparatively low stiffness of a glass plate, positioning of the glass plate (parallel to the upper plate), one-sided heating and a limited range of measuring systems (parallel-plate and cone-plate). These limitations are worth considering when setting up an experiment, but do not significantly limit the outcome in reality.

Rheomicroscopy was used in the present study to simultaneously monitor rheological changes and microstructure during gelatinisation of potato, maize and wheat starch. A high concentration of starch was used to mimic behaviour of starch pastes rather than slurries.

MATERIALS AND METHODS

Materials
Commercial potato and maize starches were obtained in the local supermarket. Wheat A and B type starch was prepared in-house following a scaled up protocol of the preparation described by Park and co-workers[19]. Starch was mixed in in equal amounts by weight in deionized water to form a paste.

Methods
Rheomicroscopy was performed using a Modular Microscope Accessory (MMA) mounted in a HR 30 rheometer (both TA Instruments, New Castle, DE, USA). The MMA consists of a light microscope mounted under a transparent glass plate forming the bottom plate in a parallel-plate measuring system where the upper plate is temperature controlled (-20–100°C), see **Fig. 1**. The epi-illumination microscope (reflected light) operates in brightfield or polarisation mode using a blue laser (470 nm) and a 20x objective was used. The glass plate can counter-rotate relative to the upper plate to produce a stagnant plane inside the sample. The microscope is equipped with polarising filters and an optional fluorescence dichroic splitter.

The starch paste was applied on the glass plate and the upper plate was lowered to a gap of 0.3-0.4 mm. The sample perimeter was covered with paraffin oil to avoid drying. Temperature was then increased from 30°C to 95°C at 5°C/min. In shear experiments three shear rates were used, 3 s^{-1}, 30 s^{-1} and 150 s^{-1} which were applied during the heating gradient. In small amplitude oscillatory shear (SAOS) a sinusoidal strain of 0.5% was instead applied during the heating gradient. This strain was in the linear region of the samples. Videos of the complete experiments were recorded as well as time lapse videos with one frame per 0.4 s which corresponds to 10x the real video speed for 25 fps.

FIGURE 1: Experimental setup of the Modular Microscope Accessory mounted in the rheometer.

RESULTS

A general note on the results is that videos of the sample changes give much more information than the snapshots presented in the paper. Rheomicroscopy videos are therefore available on-line[1], and specific video files are referenced in the text.
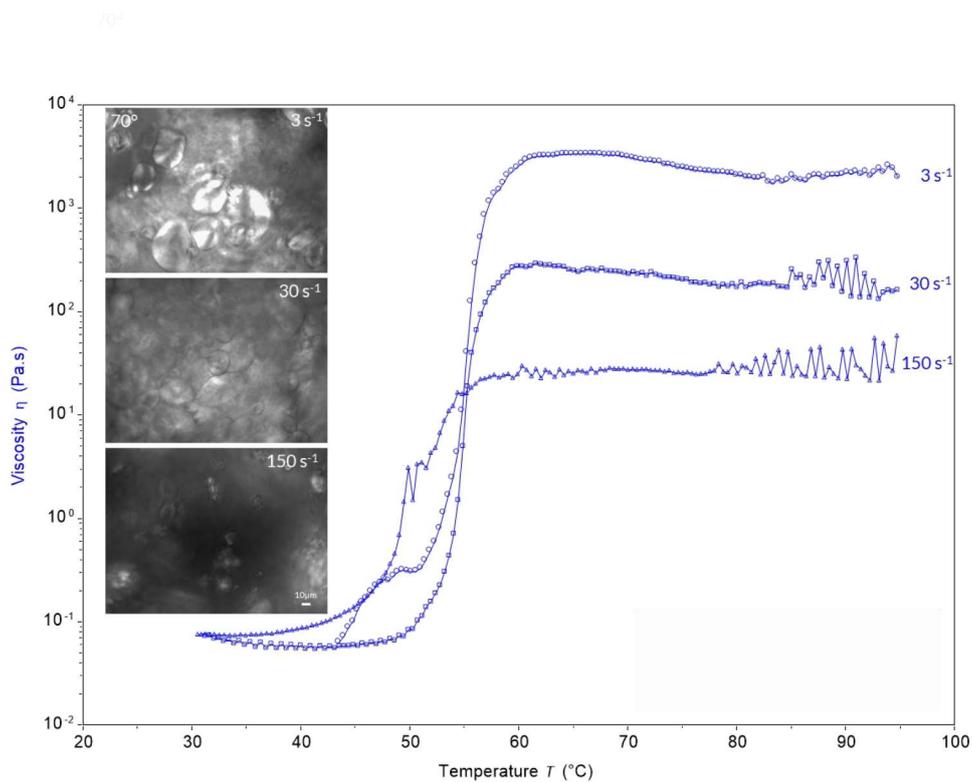
Fig. 2. visualises gelatinisation of potato starch through the viscosity of the paste and the microstructure at specific temperatures. As heat disrupts hydrogen bonds holding the starch granule structure together the granules swell and lose their crystalline structure. As the temperature rises, water molecules penetrate the granule structure. The interaction between water molecules and starch macromolecules breaks hydrogen bonds and weakens the crystalline structure. The amorphous regions of the starch granules start to absorb water, causing the granules to swell and increase in size. The swelling induces a dramatic increase in viscosity between 50° and 60°C. Some crystallinity remains at 65 °C as can be seen as bright structures (Maltese cross) in some of the remining granules. The viscosity peaks at 60°C at this shear rate of 30 s^{-1} after which shear disrupts the swollen structure. Birefringence is lost except a few remaining granules or granule fragments at the higher temperatures.

The gelatinisation is influenced by shear rate which is demonstrated in **Fig. 3**. High shear rate yields a lower viscosity as it breaks more of the entangled polymer structure formed by the swollen granules. The micrographs in **Fig. 3.** shows the microstructure at 70°C where considerably more crystalline structures are left at the low shear rate of 3 s^{-1} than at the higher shear rates.

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[1] Rheomicroscopy videos are available at
https://www.youtube.com/playlist?list=PLcF2837zn0ZbdDIexKrbbgkh9Thaxssw8
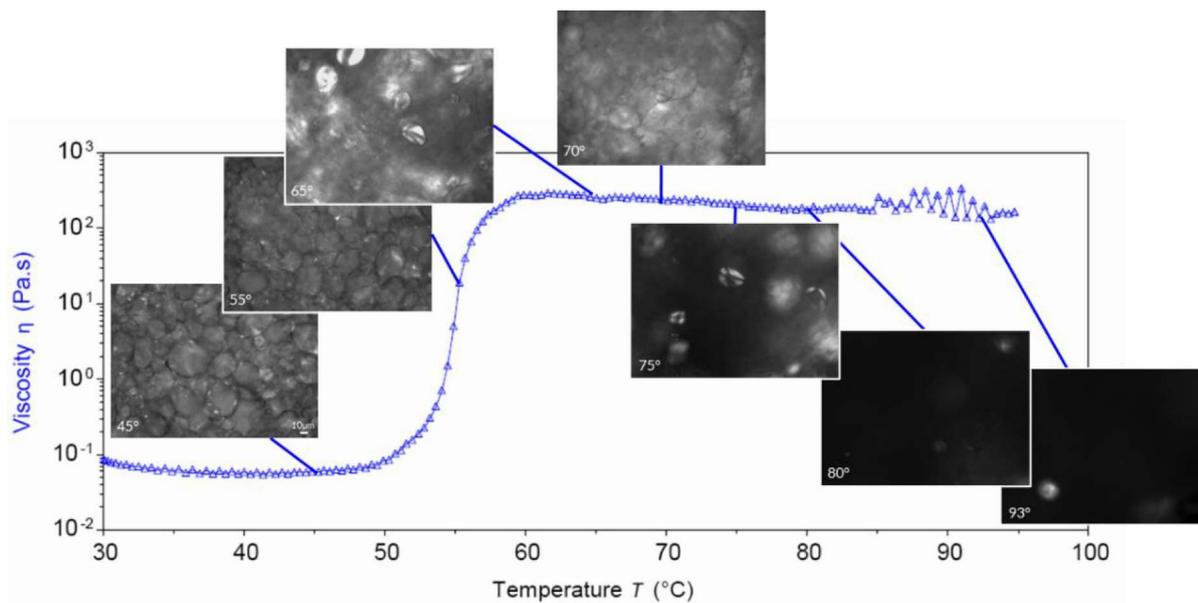
FIGURE 2: Gelatinisation of potato starch during shearing at 30 s⁻¹. The micrographs show the structure using cross-polarised light at specific temperatures. Video available on-line.

FIGURE 3: Effect of different shear rates on viscosity during gelatinisation of potato starch. The micrographs show the microstructure at 70°C. Video available on-line.

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Botanical origin also influences the gelatinisation process as can be seen in **Fig. 4**. Maize starch has a considerably broader viscosity increase than potato starch which can be associated with the differing granule architecture[1]. However, shear has the same effect on viscosity for both starches.
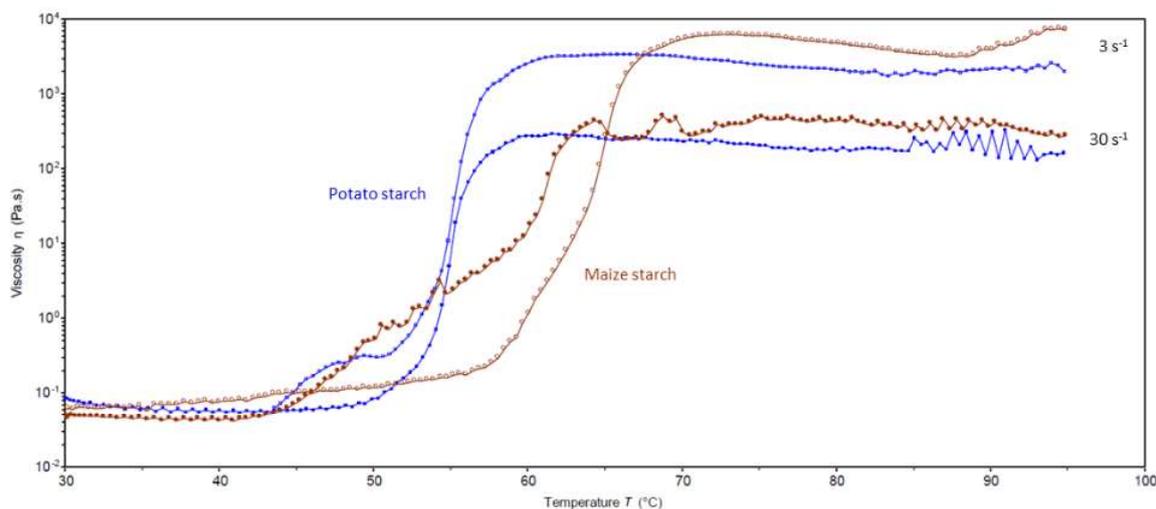

FIGURE 4: Gelatinisation of potato starch (blue) and maize starch (brown) at low shear rate (3 s^{-1}) and medium shear rate (30 s^{-1}).

Starch gelatinisation can also be monitored by SAOS which does not disrupt any structures. Gelatinisation can in one sense be regarded similar to gelation, even though it does not fulfil the conventional rheological criteria for gelation[20, 21] as the phase angle is clearly below 45° throughout the whole process (see **Fig. 5**). The swelling and transformation from a suspension of granules to a concentrated polymer solution still induces a peak in the phase angle indicative of a second order transition.

Fig. 5. and **Fig. 6.** show gelatinisation of wheat starch type as compared to wheat flour. The wheat starch was fractionated into the larger A-type granules and the smaller B-type granules. The micrographs show the microstructure on either side of the phase angle peak and clearly displays the effect of swelling and loss of crystallinity. B-type starch, however, has too small granules to be observed under the current magnification and resolution, and therefore appear homogeneous, but when observing the video of the transition a structure difference can be detected.

A-type and B-type starch have previously been observed to have different gelatinisation kinetics under shear, and SAOS rheomicroscopy also detects a small difference in peak temperature. A-type starch peaks at about 1.5 °C lower temperature than the B-type starch. The wheat flour has even higher peak temperature and it may depend on delayed swelling in the wheat flour influenced by the water transport between gluten and starch. The composition of flour is, however, more complex with considerable amounts of protein (wheat gluten) and fat. As there is no shear present (no kneading) the paste properties can be assumed to be dominated by the starch.
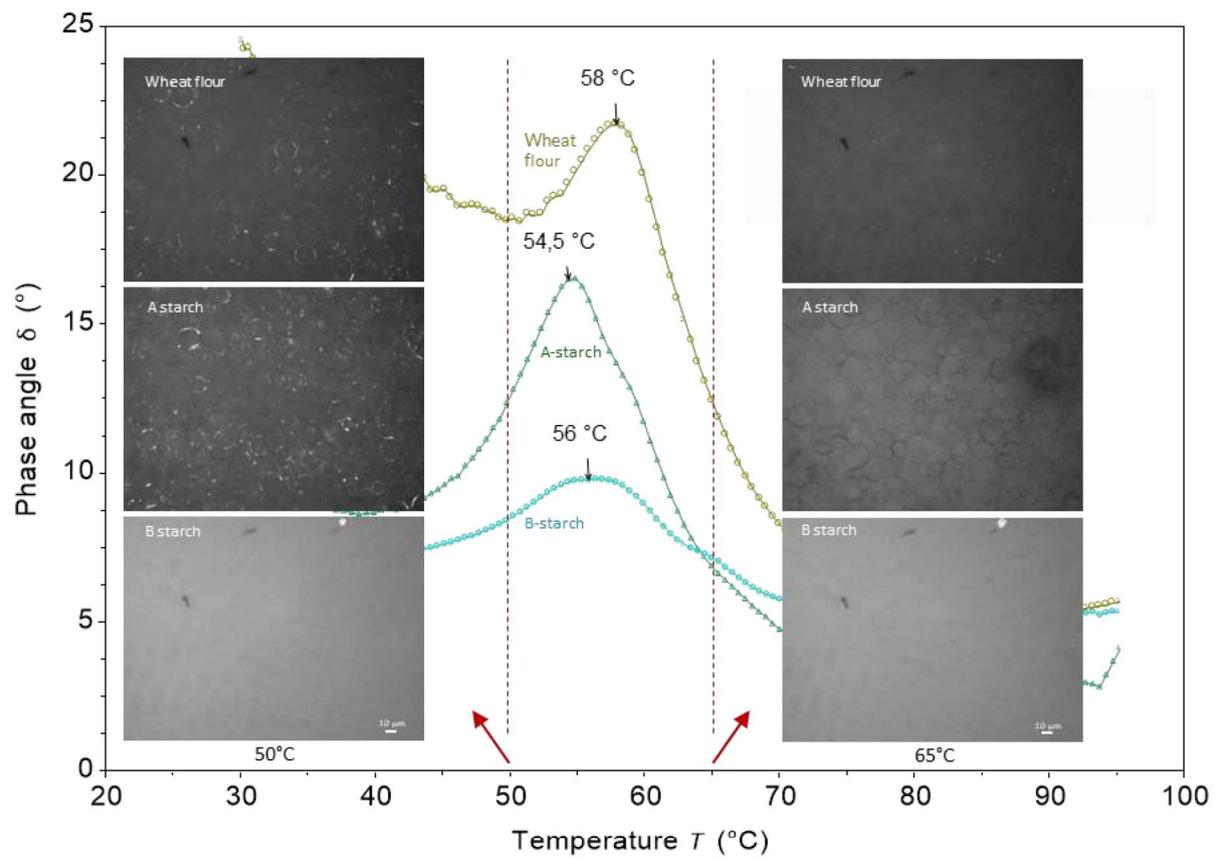
FIGURE 5: Phase angle during gelatinisation of A-type and B-type starch from wheat and wheat flour. The micrographs show the microstructure at 50 °C (left) and 65 °C (right). Video available on-line.
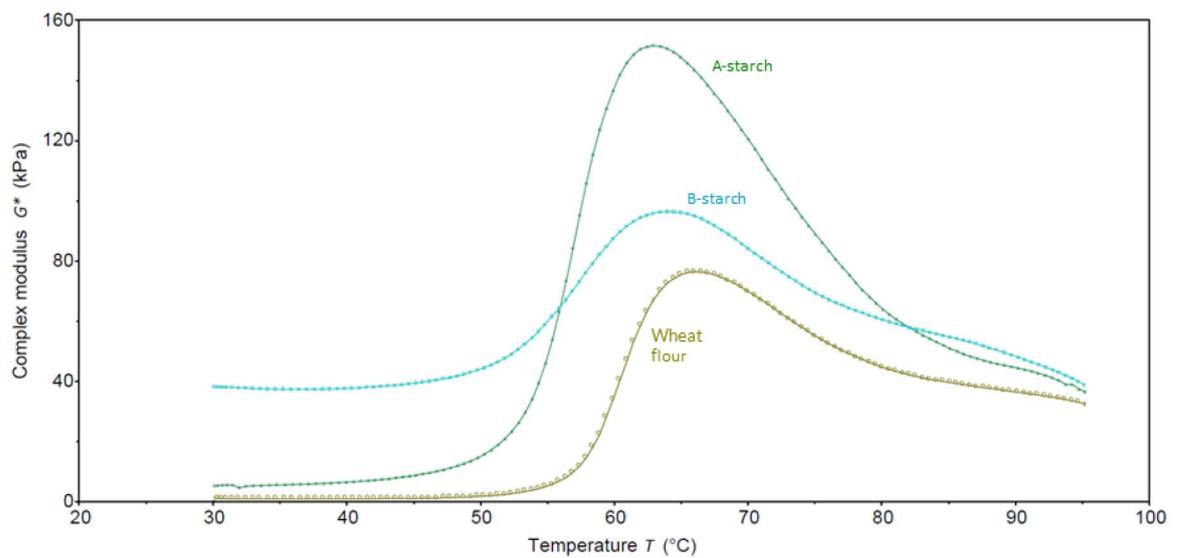
FIGURE 6: Complex shear modulus during gelatinisation of A-type and B-type starch from wheat and wheat flour. Video available on-line.

CONCLUSIONS

Rheomicroscopy offers a unique insight into the microstructural influence on rheological properties. For the investigated starch pastes the effect of shear, granule size and botanical origin on starch gelatinisation was detected by rheology and was largely explained by microscopy observation of the starch microstructure. By simultaneous monitoring by both techniques the events can be connected with high precision.

ACKNOWLEDGEMENTS

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