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MICROSTRUCTURE AND RHEOLOGICAL PROPERTIES OF COMPOSITES OF POTATO STARCH GRANULES AND AMYLOSE: A COMPARISON OF OBSERVED AND PREDICTED STRUCTURES

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Abstract

Potato starch granules were gelatinised in amylose solution to study the effect of adding amylose to a high-swelling granular starch system. The effects of varying the amount of potato starch from 1-10%, added to a solution of 2% amylose, were studied by means of dynamic viscoelastic measurements and light microscopy.

The granules gelatinised in amylose solution had a lower degree of swelling than those gelatinised in water. The restricted swelling in amylose was reflected in a decrease in the complex shear modulus (G^*) at 75°C. Gelatinisation in 2% amylopectin also caused a decrease in G^* , but gelatinisation in 2% D-glucose did not affect the rheological behaviour. Microstructural analysis showed that the added amylose was present outside the granules after swelling, whereas the inherent amylose from the potato starch seemed to have diffused mainly to the inner aqueous centre of the granules.

The mixed potato starch/amylose systems showed a fast gelation comparable to that of cereal starch. The results were analysed by a model predicting the shear modulus of aqueous biphasic gels. When the system is regarded as a continuous network of added amylose with dispersed potato starch granules, the results from both microscopy and rheology are in excellent agreement with the model at potato starch concentrations below 6%. As the potato starch concentration was raised, the high swelling potential of the potato starch granules led to partial disruption of the continuous amylose network. The results imply that the inherent amylose from potato starch did not contribute to the gel strength caused by the added solubilised amylose.

Key Words: Potato starch, amylose, amylopectin, gels, light microscopy, rheology, viscoelastic measurements, incompatibility.

Introduction

Starches obtained from different botanical sources show various behaviour patterns and have unique areas of application. The main differences between the native structures of potato starch granules and cereal starches such as wheat and maize starch are that the former have a less dense crystalline structure, a lower lipid content and a lower ratio of amylose to amylopectin (Swinkels, 1985). An important feature of potato starch is the presence of phosphate groups attached to the amylopectin. Recent developments in plant breeding based on genetic engineering have enabled changes to be made in the ratio of amylose to amylopectin in e.g., potato starch. Interest has therefore been focused on the effects of varying the ratio of amylose to amylopectin.

Strong gels form considerably more slowly from potato starch than from cereal starches (Orford *et al.*, 1987; Svegmarm and Hermansson, 1991a). The structure of potato starch systems differs from those of cereal starch systems mainly in that the swelling ratio of the potato starch granules is higher, leading to close packing of the granules at low concentrations. At higher concentrations, the diffusion of amylose out of the swollen granules has been shown to be hindered owing to the almost complete lack of a continuous water phase outside the granules. The diffusion of amylose in potato starch was mainly directed towards the aqueous centre of the granules (Svegmarm and Hermansson, 1991b). In wheat starch, on the other hand, most of the amylose was leached out of the granule during heating to 95°C, though some amylose was solubilised in an aqueous phase inside the granules (Langton and Hermansson, 1989; Doublier, 1981). The volume ratio of the aqueous amylose phase outside the wheat granules was roughly estimated to be 0.25 in an 11% gel, judged from the micrographs published by Langton and Hermansson. The volume of the interstitial amylose phase of potato starch granules is practically negligible. The amylose content of potato starch is below that of cereal starch systems. Increasing the amylose content by raising the concentration of potato starch does not lead to significantly faster gelation (Svegmarm and Hermansson, 1990). The different locations of amylose in wheat and potato starch pastes, therefore, seem to be of importance for their

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functional properties.

Starch gels are often described as a continuous network of amylose in which starch granules are embedded. Swollen granules act as a filler when incorporated in amylose gels. Granules with the lowest degree of swelling were shown to give the highest reinforcement effect, but all granules reinforced the gel network (Ott and Hester, 1965; Ring, 1985). In those studies, pre-swollen granules of waxy maize and of pea starch were added to a 2% solution of amylose.

The present investigation was performed to evaluate the effect of amylose in a high-swelling granular potato starch system. The solvent partition in the potato starch paste is probably influenced by the fact that the amylopectin of the potato starch is more soluble than that of cereal starches. The effect of the added amylose on the swelling of the granules during gelatinisation has been taken into account. The effective concentration of amylose in the interstitial phase between the granules is increased by their gelatinisation. The competition between the amylose and the starch granules for available water is of particular interest in a high-swelling starch system such as potato starch. Clark (1987), has modified a model by Takayanagi *et al.* (1963) predict the upper and lower limits of the shear modulus in biphasic aqueous gels taking the water partition into account. Data predicted by this model were evaluated and compared with measured shear moduli and observed microstructures.

Materials and Methods

Materials

Potato starch was supplied by Lyckeby Stärkelsen (Kristianstad, Sweden). Amylose and amylopectin, prepared from potato starch, were purchased from Sigma Chemicals Company, St. Louis, USA. The molecular weight of the amylose sample has been determined to be 355,000 g/mol by light scattering (Roger, personal communication).

Solubilisation of amylose: Amylose was solubilised by heating in a pressure container in an oven at 155°C. The temperature was measured inside the pressure vessel during heating and the heating was stopped when the sample reached a temperature of 154°C. Increasing the temperature from 120°C to 154°C always took 120 minutes. Heating for a longer or for a shorter time decreased the final shear modulus of the gel formed after cooling. The maximum in shear modulus was obtained by heating between 90 to 140 minutes. The solubilisation of amylose was facilitated by degassing the amylose-water suspension prior to heating. Degassing serves various purposes, the amylose powder was moist-

ened, which increased the rate of solubilisation, and residuals of *n*-butanol and oxygen were removed.

Viscoelastic measurements

The viscoelastic measurements were performed using oscillatory shear deformation in a Bohlin VOR Rheometer. A concentric cylinder cup and bob measuring system with an inner diameter of 25 mm (DIN 53019; part of Bohlin rheometer) with serrated walls was used in order to minimise slip. Measurements were performed at a frequency of 1 Hz, unless otherwise stated. The linear elastic region of warm starch pastes is quite narrow, and the maximum strain applied $2 \cdot 10^{-3}$, was within the linear region (Svegmak and Hermansson, 1990).

Sample preparation and measurement procedure

The mixed potato starch/amylose pastes were prepared in the Bohlin Rheometer. The potato starch was added to 2% aqueous amylose solution at 60°C. The dispersion was heated at a gradient of 1.5°C/minute from 60°C under gentle stirring.

The viscoelastic measurements were started when the viscosity was sufficiently high to avoid sedimentation of the unswollen starch granules. The temperature was raised to 75°C and kept at 75°C for ten minutes to equilibrate the samples. The frequency dependence was measured at 75°C. The samples were cooled to 30°C at a rate of 1.5°C/minute and kept at 30°C for 17 hours.

Samples for microscopical studies. After heating at 75°C the samples were frozen in liquid nitrogen and used for cryo-sectioning. Samples for plastic embedding were taken from the Bohlin Rheometer after the complete measurement cycle and then kept at 8°C for a week.

Light Microscopy

The microscopical techniques have previously been described in detail and discussed in Langton and Hermansson (1989) and in Svegmak and Hermansson (1991b).

The instrument used was a Nikon FXA microscope.

Cryo-sections. Droplets of the potato starch in amylose dispersions, at 75°C, were plunged rapidly into liquid nitrogen to freeze the starch pastes before the onset of amylose aggregation. The samples were then cut into 7 μ m thick sections in a Leitz cryostat at -12°C.

Plastic sections. The gels formed after cooling for 17 hours at 30°C in the Bohlin Rheometer were kept at 8°C for a week. The gel formation is the first step in the fixation of the starch molecules. The gels were then chemically fixed in 1% glutaraldehyde, dehydrated with ethanol and embedded in Histo-resin, as described by the manufacturer (LKB). The samples were cut into 3 μ m

thick sections in a Reichert-Jung Ultracut E microtome using glass knives.

Staining. The starch-amylose sections were stained with a droplet of Lugol's solution (1:1) and water (6.5 mM [I₂] and 20 mM [KI]) for one and a half minutes.

Results and Discussion

Effects of heating

Potato starch powder was added to a 2% amylose solution at a temperature of 60°C, and heating to 75°C was started immediately. Figure 1 shows the development of the complex shear modulus (G^*) as the starch granules gelatinise and swell during the heating of a 6% potato starch in a solution of either 2% amylose, 2% amylopectin or distilled water. The starch swollen in the amylose or amylopectin solution showed a lower complex modulus than the starch swollen in water. A particular feature is that the peak in G^* obtained for the starch-water systems disappeared on swelling in amylose or in amylopectin solution. A sharp increase in G^* was obtained by cooling the starch amylose systems (Figure 1). Such a fast increase in shear modulus is a characteristic of amylose gel formation. The effects that occur on cooling will be discussed later in detail.

The granules of potato starch seem to swell without restriction on heating until close-packing occurs. The swollen granules have been shown to fill up practically the entire volume of a potato starch paste already at a concentration of 5% (Svegmark and Hermansson, 1991b). Figures 2a and 2b (see color plate at page 188) show cryo-sections of a 6% potato starch paste swollen in 2% amylose. When the potato starch granules were swollen in amylose, the volume ratio of the phase outside the granular gel phase was increased. This interstitial phase contains solubilised amylose, which can be seen from the blue colour. Amylose is stained blue by iodine, whereas amylopectin is weakly stained brown or violet. A considerable amount of the amylose is located in the aqueous interior of the granules. This amylose phase will hereafter be referred to as the intragranular aqueous phase to distinguish it from the interstitial amylose phase.

The light violet staining of the walls of the granules shows that their content of amylose is low, which suggests that the added amylose did not enter the granules and that the original amylose of the granules was leaked out. There are inhomogeneities in the intensity of the blue-stained areas. The intragranular aqueous amylose phase is generally stained lighter blue than the aqueous amylose phase outside the granules. There are two possible explanations for this. Firstly, there might be differences in the staining of the native amylose and the

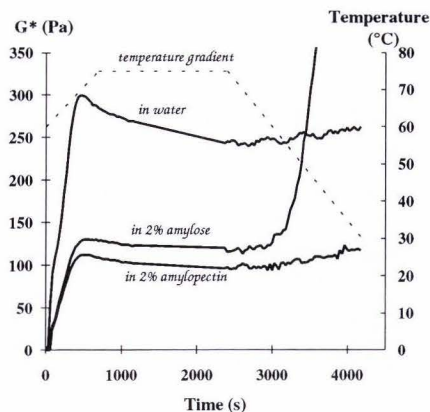


Figure 1. Changes in the complex shear modulus (G^*) during heating and cooling of 6% potato starch in different media. The temperature cycle is shown by the broken line.

added amylose. This suggests that the main part of the added amylose remains outside the granules in the interstitial phase, and that the native amylose diffuses to the aqueous phase in the middle of the granules. Secondly, the differences in staining might be due to differences in the amylose concentration caused by the granule swelling from some internal pressure, leading to an influx of water during gelatinisation.

The reduction in G^* by swelling the potato starch in amylose solution, as shown in Figure 1, is believed to be due to the increase in the volume ratio of the interstitial phase. These results are in agreement with investigations in which the rheological behaviour of warm cereal starch pastes has been correlated to the degree of granular swelling (Bagley and Christiansson, 1982; Bagley *et al.*, 1983). Contrary to the cereal starch, potato starch granules swell practically without restriction and show close-packing at low concentrations (Evans and Haisman, 1979; Svegmark and Hermansson, 1991b). The rheological behaviour of close-packed systems has been suggested to be dependent mainly on the rigidity of the granules (Doublier *et al.*, 1987).

A special feature of potato starch is that leakage of amylose is directed into the aqueous phase in the middle of the granules, thus creating an intragranular amylose phase, whereas the amount of the interstitial phase is negligible (Svegmark and Hermansson, 1991b). The leakage of amylose in wheat starch has been shown to

Table 1. The complex modulus and phase angles of 6% potato starch gelatinised water, 2% amylose, 2% amylopectin or in 2% D-glucose.

6 % potato starch with	G* (peak value during heating)	G* (at 75°C)	Phase angle (at 75°C)
Distilled water	280 Pa	235 Pa	11°
2% Amylose	140 Pa	130 Pa	11°
2% Amylopectin	120 Pa	110 Pa	15°
2% D-Glucose	300 Pa	250 Pa	10°

occur in both directions; a substantial amount of the amylose diffuses out of the granules. The intragranular aqueous phase has not been included in any model describing starch paste rheology. Another reason for difficulties in modelling potato starch rheology in terms of the volume of swollen granules is the ease with which potato starch granules are broken down by shear and heat, causing solubilisation. In sheared potato starch pastes, the rheological behaviour is completely different from that of low sheared starch pastes, and the structure is dominated by a continuous phase of solubilised amylose and amylopectin (Svegmak and Hermansson, 1990, 1991b).

Apart from the decrease in the volume ratio of the swollen granules, the increase in the amount of amylose in the interstitial phase may also have an impact on the rheological behaviour.

The complex modulus was decreased to the same extent when potato starch granules were gelatinised in solubilised amylopectin instead of in solubilised amylose, as may be seen in Table 1 and Figure 1. Swelling in D-glucose did not significantly influence the rheological behaviour. This indicates that the action of amylose in restraining the swelling is partly due to the size of the molecules hindering the added amylose and amylopectin from entering the granules, causing an osmotic back-pressure.

Effects of potato starch concentrations

The influence of varying the concentration of the potato starch gelatinised in the 2% amylose solution has been evaluated. Figure 3 shows values of the complex modulus after heating at 75°C. The starches swollen in amylose showed considerably lower G* compared to the starches swollen in water up to the concentration of 8% potato starch. At 8% and at 10% potato starch concentrations, there were no distinguishable differences between the starch swelled in water and the starch swelled in 2% amylose. The mixed potato starch/amylose systems do not exhibit the typical linear concentration de-

G* at 75°C (Pa)

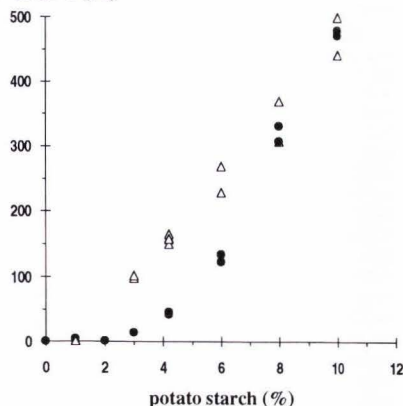


Figure 3. The complex shear modulus at 75°C against potato starch concentration. Starch gelatinised in water (Δ) and in 2% amylose (●).

pendence of native potato starches (Evans and Haisman, 1979; Svegmak and Hermansson, 1990). Within this concentration regime, the dependence is exponentially shaped. Wheat flour has previously been shown to exhibit such non-linear concentration dependencies (Evans and Haisman, 1979; Wong and Lelievre, 1981).

Addition of amylose increased the phase angle at starch concentrations below 8% and decreased the phase angle at starch concentrations above 8%, as shown in Figure 4. The phase angle of pure potato starch and water systems was practically independent of the concentration between 3 and 10%. In a previous study, we showed that the granular gel phase occupied practically the whole volume between 5 and 10% concentration (Svegmak and Hermansson, 1991b). These results indicate that the phase angle reflects the degree of granular close-packing in the systems. There is a sharp increase in the phase angle of the pure potato starch systems below the concentration of 3%, which may be interpreted as a loss of close-packing. The increase in phase angle by addition of amylose may reflect the decrease in the volume ratio of the granular phase, as shown in the micrographs in Figures 2a and b (see color plate, p. 188).

Above 8%, the phase angles of the potato starch in amylose are lower than those of the potato starch in water, indicating that addition of amylose leads to an increase in elasticity, as shown in Figure 4. Partial aggregation of amylose may have been initiated at the higher

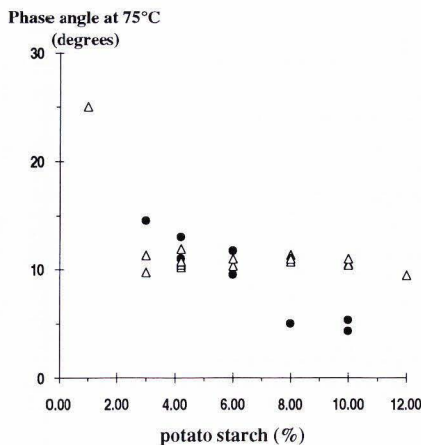


Figure 4. The phase angle at 75°C against potato starch concentration. Starch gelatinised in water (Δ) and in 2% amylose (●).

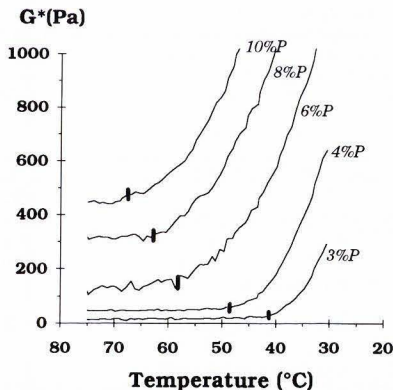


Figure 5. The changes in G^* on cooling mixed potato starch and amylose systems. The temperatures of the onset of amylose aggregation (T_g^*) are shown by marks.

concentrations of potato starch. The temperatures of the increase in complex modulus due to gel formation of amylose on cooling have been marked on the cooling curves in Figure 5. At the highest concentrations of starch, the increase in complex modulus takes place at temperatures close to 75°C.

Figure 6* shows a plastic section of an 8% potato starch paste. The clear blue phase of the amylose is located mainly in the middle of the granules, and the volume ratio of this phase is small. The walls of the granules consist mainly of amylopectin and are coloured brown-violet. The outer layer of the granular walls is considerably darker than the inner walls. This implies that the amylopectin in the outer layer differs from that in the interior of the granule, or that there is a denser packing at the surface of the granule. It has previously been shown that there was amylose present in the walls of the granules in a 10% potato starch gel; this can also be seen in Figure 7* (Svegmark and Hermansson, 1991b). At concentrations above 8%, there was not enough water available for completing the diffusion of amylose from the walls of the granules.

The 8% paste swollen in amylose differs from the one swollen in water mainly through the increase in volume of the interstitial phase, as shown by the micrographs in Figures 8a and b*. The intragranular and interstitial amylose show differences in structure, the interstitial amylose phase being smoother, as can be seen from the plastic section in Figure 8a. The microstructure of 10% potato starch in 2% amylose is shown in Figure 9a and b*. The diffusion of amylose from the granular gel phase seems to be more complete, as judged from the more even staining of the granules swollen in amylose, than that of the 10% potato starch swollen in water as shown in Figure 7. The restraint on swelling caused by the added amylose present in the interstitial phase may favour the diffusion of amylose from the granular gel phase.

Another effect of the addition of amylose can be seen in the characteristics of the frequency dependence of the storage modulus, G' at 75°C, as illustrated in Figure 10. For some systems, the slope of the frequency dependence in log-log scale varies in at least two frequency regions. The difference in the two slopes is increased with decreasing starch concentration. An even clearer split in the frequency spectra is given by the addition of the amylose. This type of heterogeneity in the frequency spectra has been interpreted in terms of a model with two types of regions, one with a high concentration of gel substance and a large number of cross-links and the other with free flow and no cross-links, as postulated by Weiss and Silverberg (1977). This model roughly fits the microstructure of a gelatinised starch paste. The gelatinised granules act as gel particles which are packed together forming a network. The gel particles are situated in a surrounding phase of water with solubilised amylose. As long as aggregation of amylose has not taken place, this interstitial phase fulfills

*Figures 2, 6-9 are on color plate at page 188.

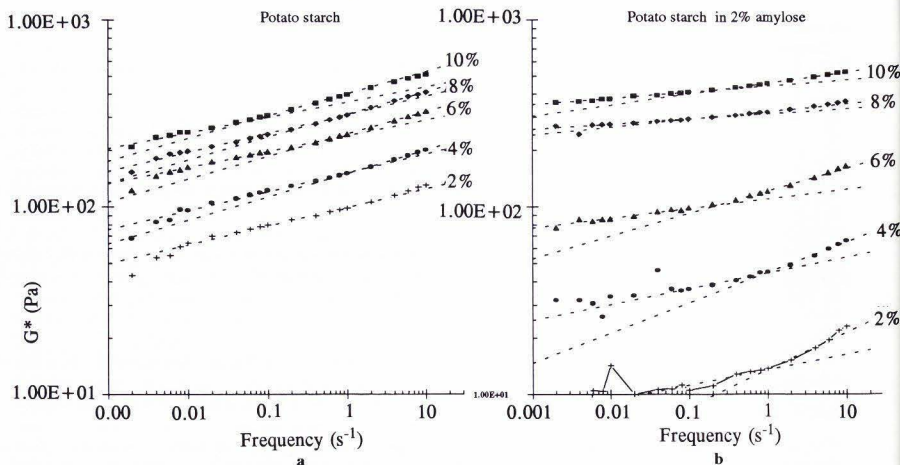


Figure 10. Frequency dependence of the storage modulus (G') at 75°C . The calculated slopes are shown by the broken lines. a. Pastes gelatinised in water. b. Pastes gelatinised in 2% amylose.

the criteria of the model by being freely draining and containing no cross-links. The effects of heterogeneity in gel structures on the frequency dependence and the relaxation spectrum have been discussed by Stading *et al.* (1992).

Aggregation of amylose in potato starch systems. A comparison between observed and predicted data

Aggregation of amylose is initiated by cooling. The mixed potato starch and amylose systems were cooled and kept at 30°C for 17 hours in order to reach a steady state value. Amylose gels normally reach a plateau value in shear modulus within a few hours. The shear modulus of the amylose gels has been shown to be strongly dependent on both the concentration and the chain length (Clark *et al.*, 1989; Ellis and Ring, 1985). The concentration dependence of amylose gels in this study gave the best fit at $c^{3.35}$ in the range between 2 and 9% amylose (Svegmarm *et al.*, 1993).

In order to understand effects of the granules as fillers, a model proposed by Takayanagi *et al.* (1963), which predicts the mechanical properties of binary composites was used. The overall shear modulus (G_{tot}) of a weak material (x) dispersed as particles in a continuous phase of a stronger material (y) has been suggested to be related to the moduli of the components by:

$$G_{tot} = \phi_x G_x + \phi_y G_y \quad (1)$$

where ϕ_x and ϕ_y are the volume fraction of each

component ($\phi_x + \phi_y = 1$).

The reverse situation with a strong filler (y) in a weaker medium (x) is expressed by:

$$\frac{1}{G_{tot}} = \frac{\phi_x}{G_x} + \frac{\phi_y}{G_y} \quad (2)$$

In order to be able to use this model for aqueous systems with two gelling biopolymers, x and y, the water participation had to be included. Clark (1987) introduced the use of a p-factor describing the relative potential of water-binding for the biopolymers. A p-value less than one means that polymer y binds more water than polymer x.

$$\alpha = \frac{pc_x}{pc_x + c_y} \quad (3)$$

where α is the fraction of the water associated with x; c_x and c_y are the overall weight % concentrations.

The phase volumes and the local concentrations of the biopolymers in each phase can be calculated from the p-factor (Clark, 1987), as shown by the equations in Table 2. Equations 1 and 2 have been used to predict the upper and lower limits of the complex modulus for mixtures of incompatible gelling biopolymers (Clark, 1987; Kasapis *et al.*, 1992; Morris, 1992).

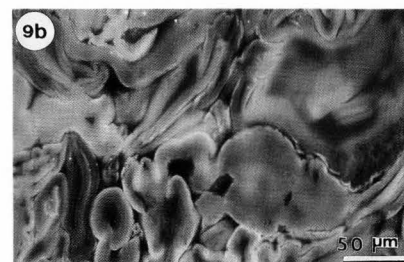
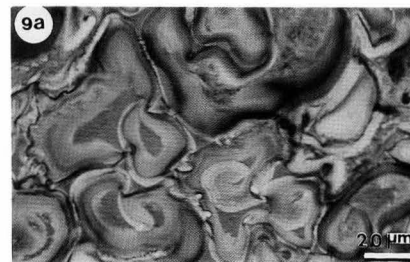
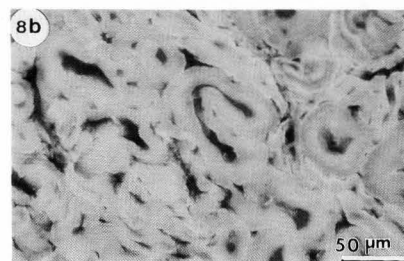
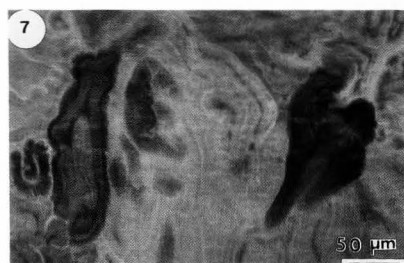
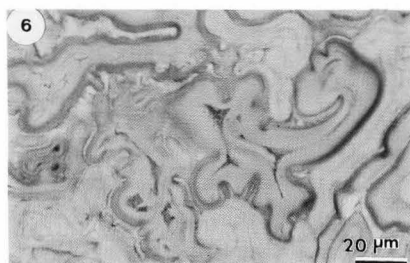
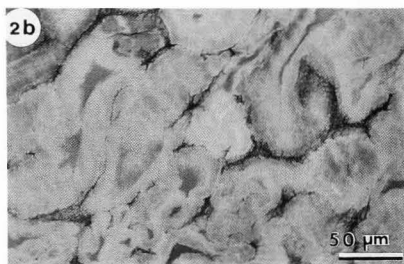
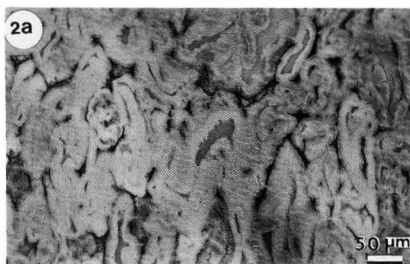
In this study, we have had access to a number of variables used in this model, such as:

Composites of Potato Starch Granules and Amylose

Table 2. Parameters and formulas used in the calculation of the upper and lower bounds of the shear modulus according to Clark (1987). The p-value was set to 0.85.

c_{po}	0.0	1.0	2.0	3.0	4.2	6.0	8.0	10.0
c_{am}^*	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
$c_{am}^{eff} = \frac{100(p c_{po} + c_{am})}{100 - (1-p)c_{po}}$	1.8	2.7	3.5	4.4	5.4	7.0	8.7	10.5
$c_{po}^{eff} = \frac{100(p c_{po} + c_{am})}{100p + (1-p)c_{am}}$	-	3.1	4.1	5.1	6.3	8.1	10.1	12.1
$\phi_{am} = \frac{c_{am}(100 - (1-p)c_{po})}{100(p c_{po} + c_{am})}$	1.0	0.68	0.51	0.41	0.33	0.26	0.21	0.17
$\phi_{po} = \frac{c_{po}(100p + (1-p)c_{am})}{100(p c_{po} + c_{am})}$	0.0	0.32	0.49	0.59	0.67	0.74	0.79	0.83
$G_{am}^* = 50(c_{am}^{eff} - 0.7)^{3.3}$	68	456	1513	3650	8282	21295	47859	9198
$G_{po}^* = 50(c_{po}^{eff} - 1)$	53	102	151	200	259	347	444	542
$G_{tot} = \phi_{po} G_{po} + \phi_{am} G_{am}$	68	342	850	1621	2931	5762	10249	1628
$\frac{1}{G_{tot}} = \frac{\phi_{po}}{G_{po}} + \frac{\phi_{am}}{G_{am}}$	68	216	281	327	382	465	556	654
$G^*_{measured}$	62	370	849	1660	2100	2300	2800	2830

c_{am}^* Variation in nominal concentration due to addition of potato starch has not been accounted for. The concentration is based on dry weight.



Legends for Figures on color plate at page 188.

Figure 2. Cryo-sections (Figure 2b at a higher magnification) of a 6% potato starch heated to 75°C in a solution of 2% amylose.

Figure 6. Plastic section of a 8% potato starch gel. The sample was heated to 75°C, cooled and kept at 8°C for a week.

Figure 7. Cryo-section of a 10% potato starch paste in water. The sample was heated to 75°C.

Figure 8a. Plastic section of a mixed gel of 8% potato starch in 2% amylose. The sample was heated to 75°C, cooled and kept at 8°C for a week.

Figure 8b. Cryo-section of a mixed system of 8% potato starch in 2% amylose. The sample was heated to 75°C.

Figure 9a. Plastic section of a mixed gel of 10% potato starch in 2% amylose. The sample was heated to 75°C, cooled and kept at 8°C for a week.

Figure 9b. Cryo-section of 10% potato starch in 2% amylose. The sample was heated to 75°C.

G_{tot} The shear modulus of the composite system has been measured at potato starch concentrations between 1 and 10% in 2% amylose.

G_y The concentration dependence of G^* in amylose gels (Svegmærk *et al.*, 1993)

$$G_{am}^* = 50(c_{am} - 0.7)^{3.3} \quad (4)$$

G_x The concentration dependence of the granular gel particle phase, G_x , is unknown but has been estimated to equal the shear modulus of hot potato starch at 75°C. This assumption is based on the facts that the potato starch granules are closely packed at concentrations above 4% and that amylose aggregation takes place at lower temperatures. The rheological behaviour of potato starch pastes was thus assumed to depend on the rigidity of the granules which are built by an amylopectin gel phase. Cooling potato starch pastes has an almost negligible effect on the shear modulus, which again implies that the amylose in potato starch has little influence on the rheological behaviour. In the range between 2-17%, the complex modulus was modelled by the linear equation:

$$G_{po}^* = 50(c_{po} - 1) \quad (5)$$

ϕ_x, ϕ_y The volume fractions of amylose and amylopectin can be roughly estimated from the micrographs.

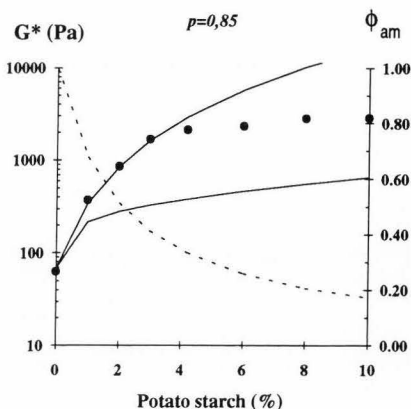


Figure 11. The G^* of mixed gels of potato starch in 2% amylose against potato starch concentration. The predicted upper and lower bounds of the shear modulus from the Clark model at a p -value of 0.85 are shown by the continuous curves. The phase volume of the added amylose is shown by the broken line.

The mixed gels of amylose and potato starch can be expected to follow the upper limit of the shear modulus as described by eq. 1, the amylose being the stronger gelling agent and the granules being the dispersed weaker component. In the first step of modelling, the p -factor was estimated by iteration to give a good fit between the measured G_{tot}^* and the G_{tot}^* from eq. 1. The best fit was anticipated from the lowest concentrations of potato starch at which the granules could be expected to be dispersed.

Two different cases were studied: In the first case, the amylose content was calculated from the sum of the inherent amylose from the potato starch and the added 2% amylose. The amylose content of potato starch was set at 18%, which is the lower limit of amylose content of potato starch reported in the literature (Shannon and Garwood, 1984). No satisfactory fit could be found in this case; the high concentration dependence G_{am}^* cannot be counterbalanced by decreasing the phase volume of amylose. Decreasing the phase volume increases the effective amylose concentration above the regime tested. Using a dependence of c^2 , which can be expected to model the concentration dependence at high amylose concentrations (Clark *et al.*, 1989), did not give a good fit either.

In the second case, the mixed system was regarded

as a mixture of 2% amylose (1.8% DS) and potato starch of different concentrations. The effect of the inherent amylose from the potato starch was thus not accounted for. The upper (eq. 1) and lower limits of the shear modulus (eq. 2), at a p -value of 0.85, are shown as continuous lines in Figure 11; the measured values of G_{tot} are shown as dots. The upper limit gives a good fit to the measured values up to 4% potato starch at $p = 0.85$. All the calculated values and the equations used are shown in Table 2 together with the measured G_{tot} . The volume fraction of the added amylose can be calculated from the p -value as shown in Table 2, and it has been depicted by the broken curve in Figure 11. The estimate of the phase volume of the amylose phase by the Clark model was in fairly good agreement with the phase volume of the interstitial amylose as shown in the micrographs in Figures 2, 8 and 9.

The model can be verified from another angle by the viscoelastic measurements. The p -value predicts the effective concentration of amylose in the continuous phase. This effective concentration of amylose can also be estimated from the viscoelastic measurements by the procedure described below; the gelation of amylose during cooling was reflected by a sharp increase in G^* , as shown in Figure 5, and by a fall in phase angle to values close to zero. The temperature of the onset of amylose aggregation (T_g^0) during the cooling at $1.5^\circ\text{C}/\text{min}$ can be seen in Figure 5 by marks on each concentration curve. The T_g^0 of the sol-gel transition of pure amylose solutions has been measured at the same cooling gradient ($1.5^\circ\text{C}/\text{min}$) as shown in Figure 12. The amylose concentration corresponding to the T_g^0 from the mixed systems was determined from Figure 12. An indirect measurement of the local concentration in the continuous phase (C_{am}^{eff}) was derived by this procedure. A comparison of the effective local amylose concentrations predicted by the p -factor and those obtained from the T_g^0 of the rheological measurements is shown in Table 3 and Figure 13. The high correlation between the estimated c_{am}^{eff} and the predicted at a p -value of 0.85 implies that the model is applicable to the modelling of potato starch and amylose mixed systems.

The measured values of the G_{tot} deviate from the upper limits of the rheological model at starch concentrations above 4%; at concentrations above 8% there was little change when the starch concentration was raised, as shown in Figure 11. The model predicts that a phase inversion takes place when the shear modulus shifts from following the upper to the lower limits. A complete phase inversion cannot be expected in our systems due to the granular state of the potato starch, but on increasing the concentration of granules some domains of the interstitial amylose can be entrapped within a continuous network of granules.

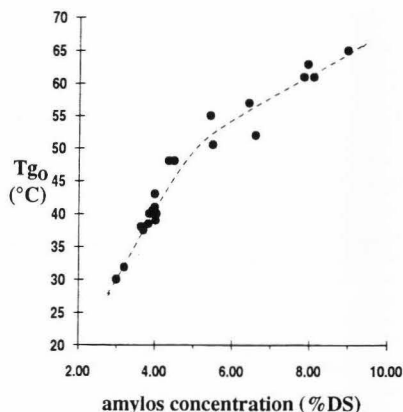


Figure 12. The temperature of the onset of gelation (T_g^0) on cooling pure amylose dispersions at a rate of $1.5^\circ\text{C}/\text{min}$ determined from viscoelastic measurements.

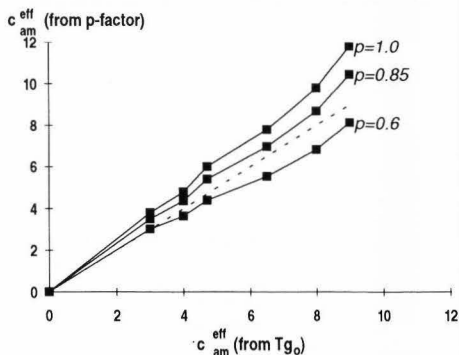


Figure 13. The predicted effective local concentration of amylose calculated at different p -values ($p = 1.0, 0.85, \text{ and } 0.6$) against the amylose concentration corresponding to the T_g^0 derived from viscoelastic measurements of the mixed systems.

The lack of response from the inherent amylose of the potato starch may be due to its location in dispersed domains inside the granules. Another possibility is that the inherent amylose has a considerably slower gelation pattern. Even though the added amylose was prepared

Table 3. Comparison between the effective local concentration of amylose (c_{am}^{eff}) calculated from Clark's model, 1987, and the concentration of amylose corresponding to the gel point from the viscoelastic measurements (T_g°).

Potato starch (%)	0	1	2	3	4	6	8	10
C_{am}	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
$C_{am}^{eff}(p=0,85)$	1.80	2.65	3.51	4.37	5.40	6.96	8.70	10.46
T_g°			30	41	49	58	63	67
$C_{am}^{eff}(\text{from } T_g^\circ)$			3	3.8	4.5	7	8	8.8

from potato starch, the molecular properties may differ from that of the inherent potato starch amylose.

If amylose aggregation was initiated before the granules were swollen, e.g., by slightly lowered temperatures, the gels formed were considerably stronger. The swelling of the granules can be expected to be restricted if the amylose forms a gel network prior to the gelatinisation, and this would increase the phase volume of the interstitial amylose phase.

Conclusions

The gelatinisation of potato starch in a medium of solubilised amylose restricted the swelling of the granules. The inherent potato starch amylose diffused to the aqueous phase in the middle of the granules, whereas the amylose added outside the granules did not enter the granules. The outer layer of the potato starch granule seemed to act as a restraint on the diffusion of amylose both into and out of the granules.

The granules fill up almost the whole volume in the pure potato starch water systems. The restriction in the swelling of the granules caused by the added amylose leads to a loss of close-packing. The lowered volume fraction of the granules resulted in a substantial decrease in the complex shear modulus and an increase in the phase angle of the hot dispersions.

The shear modulus of the amylose/potato starch gels was modelled by regarding the system as a binary composite of starch granules dispersed in amylose, thus ignoring the effects of the inherent potato starch amylose. The Clark model taking the water partition into account was in good agreement with the results both from viscoelastic measurements and from observation of the microstructure by light microscopy.

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Discussion with Reviewers

A.-C. Eliasson: In Fig. 1 you have included measurements on a potato starch/amylopectin composite, but in the discussion the role of amylopectin is almost completely neglected. Would you say that it is to expect that this composite behaves very much in the same way as the potato-starch/amylose composite, or have you described an effect that is related to amylose, and not to the presence of a polysaccharide?

Authors: The amylopectin was added in order to evaluate if the effects were specific to amylose or if they were general. The restriction in swelling seems to be general, and we would expect to inhibit the swelling by addition of other water-soluble polymers as well. Addition of another hydrocolloid could be a way of restricting the swelling of potato starch granules. This could be a means of decreasing the shear sensitivity of potato starch.

A.-C. Eliasson: Is it really possible to use the microscope to measure phase volume? I would have expected that there are several difficulties related to an approach where you extrapolate from two dimensions to three.

Authors: We believe that an approximate estimate can be made regarding the thickness of the thin sections from the plastic embedding (3 μm) compared with the diameters of the swollen granules (100 μm) and the relative large number of granules in the sections. By image analysis and by mathematical treatment, it is possible to calculate the phase volume using a micrograph that is representative and that includes a large number of granules. The area fraction of the phase on the plane is equal to the volume fraction.

M.M.G. Koning: It was stated that the calculated phase volumes correspond with those observed in the micrographs. Is this only the trend or are real phase volume data determined from the micrographs? If so, how is this done?

Authors: The correspondence is based on visual comparison of sections of plastic embeddings at varying starch concentrations and 2% amylose. Plastic sections are more suitable for such determinations, since they are thinner than cryo-sections. An investigation of the phase volume could be performed by an image analysing system. Such a study would be of great interest, though this would be rather time consuming.

A.-C. Eliasson: From a nutritional point of view, it is beneficial with starch that is not completely gelatinised; it will then function as a "slow" carbohydrate. Do you think that the composites you describe would be a way to change "rapid" starch into "slow"?

Authors: We do not believe that the degree of gelatinisation is affected at such a high water content as that in this study. At higher concentrations the presence of a hydrocolloid, competing for water, could possibly hinder the gelatinisation.

J.-L. Doublier: Estimations of G^* variations for granular gel particles are based on the shear modulus of hot starch pastes (at 75°C) instead of cold starch systems (at 30°C). I do not understand, why? The rigidity of granular gel particles is assimilated to that of the starch gel. Is this assimilation plausible? Please comment.

Authors: The values were calculated from the hot pastes in order to avoid contribution from the amylose, which may aggregate on cooling. At temperatures above 70°C amylose is in solution, and its effects on the rheological behaviour can be disregarded. Close-packed sediments of gel particles have been shown to be mainly dependant on the rigidity of the particles (Orford *et al.*, 1987). Since the concentration dependence of the granules is not one of the critical parameters in the calculations, we think such an estimate is sufficient for the system studied.

J.-L. Doublier: An important conclusion clearly illustrated by Figure 10 is that potato starch pastes can be regarded as suspensions of closely-packed swollen particles. In contrast, these particles cannot be packed so tightly when amylose is present, the result being either a suspension in a macromolecular medium of low viscosity or a composite gel, depending upon amylose concentration. I feel these systems, in each case, would be better described on the basis of the G' and G'' variations as a function of frequency. Can the authors comment on these patterns? On the other hand, what about the same variations of G' and G'' in case of mixtures with amylopectin?

Authors: The range of sensitivity of the instrument did not allow for proper studies of the frequency dependence of G'' before gel formation when the final gel strength of the systems is studied in sequence. After cooling and gelation all samples studied were independent of frequency.

M.M.G. Koning: An increase in phase angle was observed when solubilised amylopectin was added to a swelling potato starch solution compared to addition of solubilised amylose. How do you explain this observed difference?

Authors: The effect of amylopectin has to be studied at different concentrations of added potato starch to draw any conclusions. There is a dependence between the concentration of potato starch and the phase angle for the mixtures with amylose.

As also pointed out by Dr. Doublier, it would be of interest to compare frequency dependence of G' and G'' for mixtures of granules and amylose or amylopectin. However, the Sigma amylopectin has a notably low viscosity, and it can be questioned if this sample is the best choice for such a study.

M.M.G. Koning: In the comparison in Table 2 between the measured and calculated data, a value of $p = 0.85$ is assumed. Does this value indeed give the best agreement between model and experiment?

Authors: This value gives the best fit to the upper limit using the Takayanagi's equations as shown in Figure 11. We also estimated the p -value from the point of onset of amylose gelation by the rapid increase in G^* and by the decrease in phase angles, as shown by Figure 12. With this approach 0.7 gave the best fit.