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# Differences in amylose aggregation and starch gel formation with emulsifiers

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**Abstract**

The effects of different kinds of emulsifiers, polyglycerol ester and glycerol monostearate, and the surface-active lignosulfonate, on network formation and aggregation of amylose and starch in gels were studied. Pastes with emulsifier and 5% amylose or different starches were heated to between 90 and 150 °C, cooled and studied by means of transmission electron microscopy.

The fine-stranded amylose network aggregated into thicker strands when emulsifiers were added. At high emulsifier concentrations, spherical aggregates without internal structure formed, and the network disappeared. In wheat starch gels, a lower concentration of emulsifier was needed for amylose aggregation than in pure amylose gels. At high temperatures (>140 °C), aggregation was more ordered, and long, needle-like threads or brush-like aggregates were achieved. The amylose aggregated similarly with the complexing emulsifiers used in this work as with the non-complexing surfactant, which showed that amylose–lipid complex formation was not the primary explanation for aggregation.



*Keywords:* Amylose; Starch; Emulsifier; Gel; Aggregation; TEM

## 1. Introduction

Emulsifiers are common additives in industrially prepared food. Apart from their surface-active properties, they are known to affect the gelatinization of starch. For example, it has been shown in an earlier work that emulsifiers were able to delay the granule swelling and the amylose leakage in an 8% wheat starch paste up to very high temperatures (Richardson, Langton, Bark, & Hermansson, 2003). When the granules finally ruptured, they disintegrated rapidly due to the high temperature.

During cooling, the starch paste transforms into a gel, mainly as a result of amylose network formation. Amylose in dilute solutions generally forms quite an open network structure, with strands that are about 20 nm thick (Hermansson, Kidman, & Svegmark, 1995; Leloup, Colonna, Ring, Roberts, & Wells, 1992; Putaux, Buléon, & Chanzy, 2000). After using several techniques to study the amylose gel, for example acid hydrolysis, which degrades the amorphous parts of the molecules, Leloup et al. (1992) proposed that the amylose chains arranged themselves in helices obliquely to the length of the filament, linked by amorphous segments, thereby building up the network structure.

The network formation affects the final properties of the gel, and it can be changed by a number of factors. A change in relative concentrations of amylose and amylopectin due to emulsifiers hindering continuous amylose leakage can have a substantial effect, since the network formation in an amylose–amylopectin mixture can differ markedly from that in a pure amylose gel. The gel can also be influenced by other components than emulsifiers. For example, addition of ions such as $Na^+$ and $Ca^{2+}$ has been shown to change the network formation in a maize starch gel (Richardson, Sun, Langton, & Hermansson, 2004).

The microstructure of starch gels, and how it is changed by addition of emulsifiers or other additives, is still a poorly understood area. On a molecular level, the amylose conformation can be studied with, for example, DSC or X-ray diffraction. These techniques mainly give information on the amylose–lipid complex formation, which may be of considerable relevance in gels with complex-forming

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emulsifiers. It is generally believed that complexes between amylose and lipids are formed during starch gelatinization, since the V-type X-ray diffraction pattern develops during gelatinization of starch that contains lipids, and melt again at about 100–120 °C (Biliaderis, Page, & Maurice, 1986; Biliaderis & Seneviratne, 1990; Eliasson & Krog, 1985). On the other hand, pure amylose gels also have a DSC endotherm at about 120 °C (Leloup et al., 1992). This DSC endotherm may, therefore, indicate a melting of the crystalline parts of the amylose, as well as amylose–lipid complex melting.

Most of the research on emulsifier interactions has been done with amylose solutions, since amylose is known to form complexes. However, starch granules have other components than only amylose. Amylopectin gels can also be changed by the addition of emulsifiers. Nuessli, Handschin, Conde-Petit, and Escher (2000) have shown that a 2% potato amylopectin dispersion with different emulsifiers added had higher storage and loss moduli than the pure amylopectin dispersion, and also a lower iodine binding capacity, measured as the maximal wavelength during spectrophotometry. These differences were also explained by the complex-forming properties, since both ionic and non-ionic emulsifiers had the same effect, which excluded a purely electrostatic effect. Potato starch with emulsifiers added after swelling showed the same phenomenon; the storage and loss moduli increased with complex forming emulsifier, until the iodine binding capacity reached 0%, when there was no further change in rheological properties (Conde-Petit & Escher, 1992).

Emulsifiers comprise many different compounds. The main emulsifier in this study was a mixture of polyglycerol ester and monoglyceride (PGE/MG), produced for bakery applications where it has been shown to have a large impact not only on foam formation (Richardson, Langton, Fäldt, & Hermansson, 2002), but also on starch gelatinization (Richardson et al., 2003). In order to verify the results with this emulsifier, glycerol monostearate (GMS) was also used. Both PGE and GMS are able to form complexes with amylose. All emulsifying agents do not have this ability. As a comparison, the effects of lignosulfonate were also examined. The lignosulfonates are reaction products in the sulfite pulping process and forms large (20–100 nm), negatively charged spheres. It was shown in an earlier work (Richardson et al., 2004) that this surface-active product could affect the starch gelatinization. It was, therefore, considered interesting to examine whether it could also change the amylose aggregation.

The objective of this work was to determine how emulsifiers can affect the gel formation of starch and amylose from a microstructural point of view. Three different emulsifiers were chosen for the study, of which PGE/MG and GMS were able to form complexes and lignosulfonate was not. Thin sections of embedded starch gels were examined with transmission electron microscopy (TEM). In order to elucidate how amylose network formation was changed by the other starch components, the experiments were not only performed on native starch granule dispersions (wheat, potato and maize), but also on pure amylose gels.

## 2. Materials and methods

### 2.1. Materials

Wheat starch, C*Gel 20006 (starch >85.7%, protein 0.3%, moisture 12%), was obtained from Cerestar Benelux BV, Sas van Gent, Netherlands.

Native potato starch was from Lyckeby Culinar, Fjälkinge, Sweden.

Maize starch was from National Starch, Hamburg, Germany.

The amylose was potato amylose (heavy metals < 0.001%, water <5%) from Calbiochem, La Jolla, California.

The PGE/MG emulsifier, an α-gel with a mixture of polyglycerol ester and monoglyceride (extractable fat 31 ± 2%, iodine value <1), was a commercial ready-to-use α-gel (Colco) obtained from Aromatic AB, Stockholm, Sweden.

GMS, glycerol monostearate, was from Danisco Ingredients, Brabrand, Denmark.

Ca-lignosulfonate (DP751, 3.6% $Ca^{2+}$, 1.1% $Na^{+}$, 6.0% $S_0$, 2–3% reduced sugars) and Na-lignosulfonate (DP752, 0.4% $Ca^{2+}$, 6.1% $Na^{+}$, 5.3% $S_0$, 2–3% reduced sugars) were from LignoTech, Vargön, Sweden.

### 2.2. Starch paste preparation and microscopy

A starch suspension was prepared with 8% (w/w) wheat starch and 0, 0.5 or 4.0% PGE/MG in de-ionized water. The emulsifier:starch ratio was then 1:16 or 1:2. The suspension was heated in the Brabender Viscograph (Duisburg, Germany) at 75 rpm with a temperature increase of 1.5 °C/min. Viscograms were recorded with a 500 cmg measuring box. The suspension was heated to 90 or 97 °C and then poured into small glass cylinders with rubber stoppers. The cylinders were stored in a refrigerator (4 °C) for 1 h before small cubes were cut out for embedding. Eight-percent potato starch was heated with 0 or 2% GMS to 95 °C for 30 min, and treated in the same manner.

Five-percent potato amylose was mixed with emulsifier or lignosulfonate and de-ionized water in Reacti-Vial pressure vessels and dissolved in a Reacti-Therm heating/stirring module from Pierce (Rockford, Illinois) at 140 °C for 1 h. The vessels were left at room temperature for 10 min before the solution was poured into glass cylinders and stored in a refrigerator for 1 h. Since, the amylose concentration was lower than in the above-mentioned experiments, a lower concentration of PGE/MG emulsifier was used to ensure a similar emulsifier:amylose ratio (0, 0.31 or 2.5% PGE/MG). Eleven-percent potato starch,

11% maize starch and 8% wheat starch were heated in the same manner to 140 °C (potato) or 150 °C for 30 min.

After refrigeration, 1 $mm^3$ cubes were cut out of the gels and fixed with 2.5% glutaraldehyde and 0.2% ruthenium red in 0.1 M phosphate buffer of pH 7 overnight. The gels without emulsifier were fixed without ruthenium red. The gels that were too fluid for cutting were first embedded in agar capsules, to avoid dissolving. Dehydration steps with increasing ethanol concentration (50, 75, 95 and 99.5%) were followed by embedding in polybed (TAAB 812). Thin sections (50–100 nm) were cut with an ultramicrotome and collected on formvar-coated grids. The sections were stained according to the thiosemicarbazide-silver proteinate method described by Thiéry (1967). Images were taken with a LEO 906 E transmission electron microscope, TEM (Oberkochen, Germany) at an accelerating voltage of 80 kV.

## 3. Results and discussion

### *3.1. The microstructure of amylose gels with emulsifier*

When a 5% amylose dispersion was heated to 140 °C with different amounts of PGE/MG emulsifier, the network structure of the formed gel, seen with TEM, was drastically altered as shown in Fig. 1. The pure amylose gel had many short strands, and quite an open structure with large pores. Although, the structure was so open, the gel was firm enough to be cut without difficulty. The network was not completely homogeneous (local variations can be seen in, e.g. Fig. 1a), but it consisted mainly of strands. The strand thickness, calculated from a few manual measurements, was about 20 nm, which corresponds well to earlier measurements (Hermansson et al., 1995; Leloup et al., 1992). Addition of a low amount of emulsifier caused the strands to aggregate into somewhat thicker (about 25 nm), somewhat fewer, but definitely longer, strands. The maximum strand length seen in a section was about 2000 nm, compared to about 800 nm in a pure amylose gel. A few spherical aggregates, about 200–300 nm in diameter, were seen. This gel was also firm enough to be handled without difficulty. When a higher concentration of emulsifier was added, however, no gel was formed, only a thick, opaque, paste. The network disappeared completely and spherical aggregates with a diameter of about 400–800 nm were formed. The few strands that were visible were agglomerated together into very thick aggregates, about 1000–2000 nm long.

The kind of random spherical aggregation, without any discernible internal structure, that was seen in Fig. 1c and f has not been described before in amylose gels, except in a 6-week-old gel studied by means of cryo-TEM (Putaux et al., 2000). Such aggregates have been seen in starch gels as well, but they were described as hydrated amylopectin fragments (Hermansson et al., 1995). These results showed that amylose could aggregate randomly when some component including the aggregation is added. It was also very

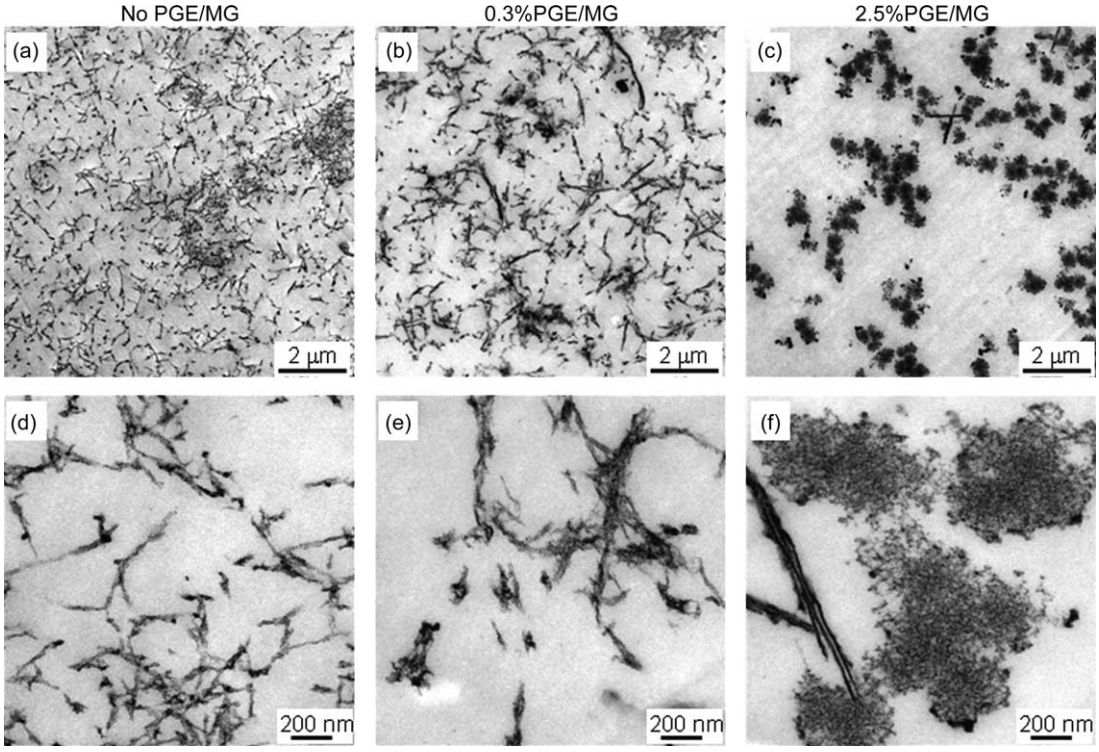


Fig. 1. Five percent amylose gels with PGE/MG emulsifier under the TEM at two magnifications. They were embedded and sectioned after heating to 140 °C for 1 h and cooling in refrigerator. (a and d) Only amylose; (b and e) emulsifier:amylose 1:16; and (c and f) emulsifier:amylose 1:2.

interesting that different aggregation forms (thick strands or random aggregation) could be achieved by changing the emulsifier concentration.

An interesting question was, of course, whether these effects on the amylose aggregation were due to amylose–emulsifier complex formation or not. The complex formation was not measured in these gels, but it should be kept in mind that complexes are formed on the molecular scale, while this aggregation involves hundreds or thousands of molecules. It has also been shown that, for example, salt, such as NaCl, $CaCl_2$ and $AlCl_3$, was able to increase the amylose aggregation at 155 °C, producing a more bush-like appearance (Hermansson et al., 1995). Aggregation can thus be induced by other components than lipids. A possibility is that it could be induced by a change in electrostatic properties or surface-activity of the surrounding solution.

### 3.2. The microstructure of starch gels

Studies on pure amylose gels are very informative, but when whole starch granules are used they contain many other components than amylose. It is, therefore, also necessary to study the aggregation phenomena in starch gels. Fig. 2 shows the appearance of an 8% wheat starch gel, after heating to 97 °C and cooling in a refrigerator. It had the same emulsifier:starch ratios as in Fig. 1. The thick sections examined under the light microscope (Fig. 2a–c) corresponded well to the smears of the same samples that had been studied earlier (Richardson et al., 2003). Without emulsifier, the granules were deformed and the polysaccharides (amylose and/or amylopectin) that had leaked out formed a continuous bulk phase, as revealed by light microscopy (Fig. 2a). With emulsifier (PGE/MG),

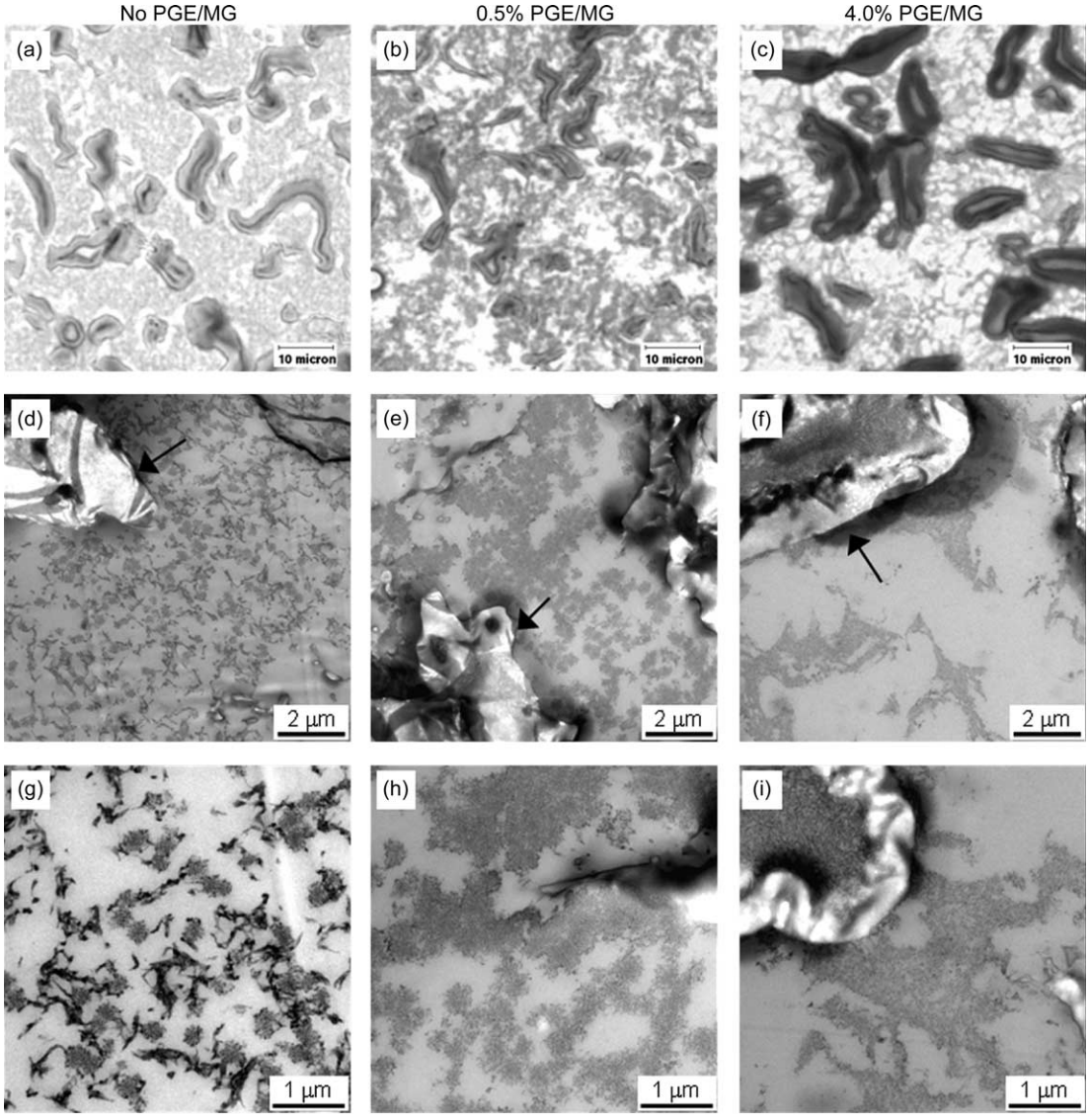


Fig. 2. Eight-percent wheat starch gels with PGE/MG emulsifier. They were embedded and sectioned after heating to 97 °C and cooling in a refrigerator. (a–c) 1 µm sections, stained with iodine for LM; and (d–i) 100 nm sections, TEM, at two magnifications. The emulsifier:amylose ratio was the same as in Fig. 1. Arrows indicate non-dissolved granules.

the granules remained larger and less deformed, the amount of leaked amylose decreased, and the aggregation in the bulk phase increased (Fig. 2b and c).

At a higher magnification with TEM, the granules were seen as densely packed objects with a crystalline, poorly stained, outer core. Due to the dense packing, no effects of emulsifier on the internal granule structure were seen, and it was not possible to see whether the emulsifier interacted with the granule surfaces. The amylose network did not seem to be specifically attached to the granule surfaces. The evaluation was, therefore, restricted to the bulk phase. It was seen that the bulk phase resembled that of the amylose gels, but the spherical aggregates had increased at the expense of the network. The gel without emulsifier had some network interwoven with aggregates (Fig. 2d and g), but even at the low emulsifier concentration (Fig. 2e and h) the ordinary network had disappeared completely. At the high concentration, the aggregates were no longer spherical. They covered large, irregularly formed areas, with large empty spaces between them (Fig. 2f and i). This gel was too soft to be cut. The aggregates did not seem to be attached to the granule surfaces.

A similar aggregation was seen with another emulsifier, glycerol monostearate (GMS). Hermansson et al. (1995) observed that when GMS was added to potato starch, amylose aggregated randomly around spherical droplets, which were thought to consist of emulsifier. The 8% potato starch gels with or without 2% GMS are shown in Fig. 3. With emulsifier (Fig. 3b), a dense, non-structured aggregate was formed instead of a network. The behavior was similar to that of wheat starch with PGE/MG in Fig. 2, although the amylose:amylopectin ratio, as well as the composition of other components, in wheat starch and potato starch differs markedly.

It seemed that the effects of PGE/MG on the network formation were much more pronounced in the wheat starch gel (Fig. 2) than in the amylose gel (Fig. 1), since less emulsifier was needed in the starch gel for the same effect. To some extent, the increased aggregation was possibly an effect of the effective emulsifier:starch ratio. The emulsifier:starch ratio in the whole dispersion was similar, but a large portion of the wheat starch amylose was still trapped inside the granules, while the pure amylose was dissolved.

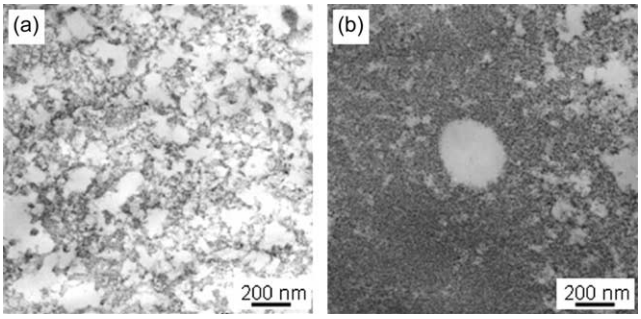


Fig. 3. Eight percent potato starch gel after heating to 95 °C for 30 min. (a) Without GMS; and (b) with 2% GMS.

This led to a lower relative concentration of amylose outside the granules in the wheat starch gel. However, the amylose was more aggregated in the wheat starch even if no emulsifier was added at all, which indicated that the other components in the starch were also capable of inducing aggregation. Apart from amylose, wheat starch contains amylopectin, lipids, proteins and several other components. These may have an effect although they do not form amylose–lipid complexes. For example, it has been shown that the network formation and the amount of spherical aggregates can be changed in about the same way with ions, such as $Na^+$, or with lignosulfonate, which is a bulky, surface-active molecule that does not form complexes with amylose (Richardson et al., 2004). However, the emulsifier had an extremely powerful effect on the amylose network.

The amylose aggregation due to emulsifier addition was already visible at an earlier stage of the heating. Fig. 4 shows the wheat starch gels after heating to only 90 °C. The gel of wheat starch without emulsifier, formed after heating to 90 °C (Fig. 4a), had a network quite similar to that of pure amylose (Fig. 1a). The absence of spherical aggregates in the pure wheat starch at 90 °C (Fig. 4a) indicated that the components that induced aggregation had not yet been released from the granules. With emulsifier (Fig. 4b), only a very small amount of amylose had leaked out of the granules at this early stage, due to the restricted gelatinization that has been described before (Richardson et al., 2003), but in this case the released amylose was packed tightly in separated areas without any network connection, just as at the higher temperature.

### 3.3. Aggregation at high temperatures

When different kinds of starch are heated, the amylose often forms the kind of network shown in Fig. 1a, i.e. quite a homogeneous, well-distributed network. However, the network changed when gels containing more than only pure amylose were heated to at least 140 °C. Some examples are given in Fig. 5. At, or above, this temperature, the network strands gathered into thicker bundles, which were either very long and rigid or more brush-like. This was already seen in Fig. 1b–c, when emulsifier was added to amylose.

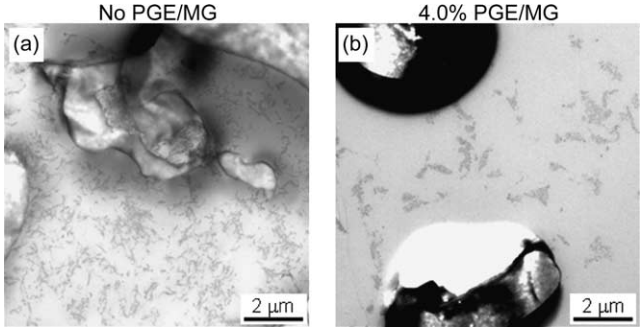


Fig. 4. Eight percent wheat starch gels with PGE/MG emulsifier. They were embedded and sectioned after heating to 90 °C and cooling in a refrigerator. (a) Without PGE/MG; and (b) with 4.0% PGE/MG.

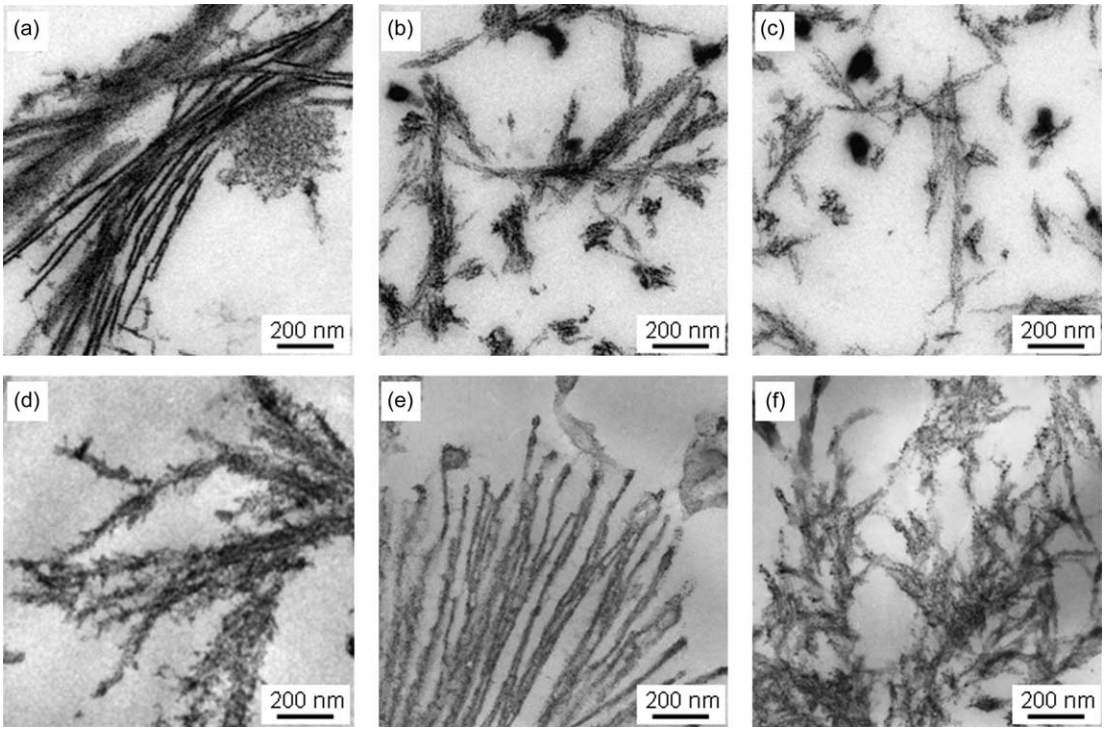


Fig. 5. Gels with different starches and additives under the TEM. They were heated to 140 °C unless indicated otherwise. (a) 5% amylose with 2.5% PGE/MG; (b) 5% amylose with 1% Ca-LS; (c) 5% amylose with 1% Na-LS; (d) 11% potato starch; (e) 11% maize starch, 150 °C; and (f) 8% wheat starch, 150 °C.

A very large amylose aggregate in the gel with 2.5% PGE/MG, consisting of several needle-like threads packed together, is shown in Fig. 5a. This kind of ordered aggregation could, therefore, be induced by the presence of PGE/MG.

A similar effect was seen when lignosulfonate, the charged, non-complexing emulsifier, was added. Both Ca- or Na-lignosulfonate induced a brush-like appearance in amylose gels (Fig. 5b and c). Lignosulfonate seemed to attach to the network, since there were darkly stained balls of about the estimated lignosulfonate molecule size. Aggregates were also formed in starch gels without additives, such as in potato, maize and wheat starch (Fig. 5d–f). Potato and wheat starch showed the brush-like aggregates, while maize starch had long needles bundled together. Aggregates of this kind were not seen in potato, wheat or maize starch heated to less than 140 °C.

Since these aggregates were formed by starch or by amylose with additives, but not by pure amylose, it was concluded once again that there are components present in starch that can induce amylose aggregation. These components seemed to be present in both cereal and root starches. The effects could not be explained by complex formation, since lignosulfonate is unable to form complexes with amylose, and root starches contain a very low amount of lipids (in potato they only contribute 0.05% of the dry weight, according to Swinkels (1985)). However, PGE/MG and lignosulfonate are surface-active components and might affect the interaction between the amylose molecules to induce phase separation.

The reason why these aggregates were seen only at such high temperatures could not be determined in this work. Since crystalline amylose has been reported to melt at quite high temperatures (about 95–135 °C), it is possible that these molecules do not interact properly with other components before they melt. The melting temperature depends on both chain length and degree of branching, and the molecules that are most difficult to melt could also have the most perfect structure for building up these aggregates, and they might also be more apt to interact with components that change the properties of the surroundings. The effects could also be enhanced by the high temperature, which may increase the phase separation or the ability to rearrange. These are only some suggestions, since the theories could not be verified in this project.

## 4. Conclusions

In this study it was shown that emulsifiers exerted a drastic effect on the amylose aggregation. The normal amylose network consists of thin strands. At moderate emulsifier concentrations, the amylose aggregated into thicker and more rigid strands than normal. At high emulsifier concentrations, the amylose gathered into unstructured, spherical aggregates. The aggregate formation was enhanced in starch gels, and the prevalence of aggregates has been shown to be increased also by other components than emulsifiers. Therefore, the aggregation was not considered to be primarily due to

amylose–emulsifier complex formation but rather to phase separation, which may be induced by several components.

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