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Analysis of the continuous phase of the modified waxy maize starch suspension

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ABSTRACT

The continuous phase of the suspension of swollen-in-water modified waxy maize starch was analysed. The composition, concentration and molecular weight of the substance released from modified starch granules were determined. Starch granules were swollen in excess water at 73 °C and held at this temperature for 1 min. Centrifugation was used to separate the granules from the supernatant; the latter was then submitted to physico-chemical analysis. Surface tension measurements showed that the supernatant was different from pure water indicating the presence of dissolved polymeric material(s). Differential Scanning Calorimetry and iodine staining results revealed the presence of amylopectin. Analytical Ultracentrifugation and Size Exclusion Chromatography coupled with a Multi-angle Laser Light Scattering were used to determine the sedimentation coefficient and weight–average molecular weight of the soluble amylopectin fraction as well as giving an indication of solution concentration. The molecular weight of dissolved amylopectin was around 1.5×10^6 g/mol and its concentration in the supernatant varied from 0.6 to 6.7 mg/mL for initial 10 mg/mL and 50 mg/mL starch suspensions, respectively. The sedimentation coefficient, weight–average molecular weight and amylopectin concentration in the supernatant all increased non-linearly with the initial starch concentration in the suspension.

1. Introduction

Starch is one of the main polysaccharides used in food applications due to its nutritional value. One of the most important starch properties is its swelling ability which makes it largely used as a thickening/gelling agent. The swelling of starch granules occurs when they are heated in excess water: the crystalline structure is lost allowing water to penetrate the granules. Further heating induces the bursting of the granule and thus a viscosity breakdown. To prevent granules from bursting and hence enhance the thickening properties, starches have been modified to inhibit their swelling (cross-linking and heat–moisture treatment).

When native starch is heated in water, amylose, if present, will leach out of the granule. What will happen when a physically modified waxy maize is heated? The conventional view is that nothing should come out of these starch granules as they are composed *only* of amylopectin and have been "physically" cross-linked. It has previously been reported that a small amount of material is solubilised in the continuous phase of suspension of swollen cross-linked waxy maize starch (Tecante & Doublier, 1999), however, the nature of the soluble fraction was not identified. To know the type and characteristics of the polymeric material that is present in suspension continuous phase is important as it may create a shear induced structure that might have an impact on the suspension rheology as well as influence mixing with other (bio)polymers. As far as the rheology of cross-linked waxy maize starch is concerned, Genovese & Rao (2003) showed that the exchange of the continuous phases of cross-linked waxy maize and tapioca (19.3% amylose content) starch pastes did not influence the overall suspension rheology. Mixing other polysaccharides with starch is performed to modify paste flow properties leading to desired specific textures for food applications (Eidam, Kulicke, Kuhn, & Stute, 1995; Savary, Handschin, Conde-Petit, Cayot, & Doublier, 2008; Tecante & Doublier, 1999) or to enhance film forming properties (Lafargue, Lourdin, & Doublier, 2007). If starch components are released into suspension continuous phase, interaction between this compound and other hydrocolloids present may become critical. Research on the rheology and compatibility of starch–polysaccharide mixtures is numerous (Abdulmola, Hember, Richardson, & Morris, 1996; Achayuthakan, Suphantharika, & Rao, 2006; Chaudemanche & Budtova, 2008; Kim & Yoo, 2006; Rodriguez-Hernandez et al., 2006; Savary et al., 2008; Tecante & Doublier, 1999). It has been reported that an enhancement of viscosity is due to the "excluded volume effect" of the starch granules and the apparent increase of concentration of the polysaccharide in the continuous

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phase. For example, it has been shown that interactions between amylose and galactomannans play an important role in the rheological responses of such mixtures (Alloncle & Doublier, 1991) because of amylose/hydrocolloid incompatibility.

In this study we used modified waxy maize starch with inhibited swelling through heat–moisture treatment. This starch is amylose free; thus theoretically no polymer should be present in the continuous phase of the suspension, as opposed to non-waxy starch. However, it will be shown that the continuous phase is not pure water. We detected the presence of a polymeric component and then identified and quantified it using a variety of analytical methods (Harding, 2005; Harding, Vårum, Stokke, & Smidsrød, 1991; Séne, Thévenot, & Prioul, 1997).

2. Materials and methods

2.1. Materials

Novation 2600 starch was generously provided by National Starch (Hamburg, Germany) and will be called "starch" in the following. This waxy maize starch has been physically modified through heat–moisture treatment to mimic the behaviour of a cross-linked waxy maize starch, i.e. demonstrating inhibited swelling.

Lysophosphatidylcholine (LPC), used for DSC measurements, was obtained from Sigma–Aldrich Chemei, (Gmbh, Seelze, Germany). It is used when biopolymer–lipid interactions are to be studied.

Amylose and amylopectin, both from potato, were purchased from MP Biomedicals Inc., France, and Sigma Chemicals Co., St. Louis, USA, respectively. Sodium hydroxide and acetic acid were purchased from Sigma–Aldrich (Dorset, UK). Sodium hydroxide was in the form of pellets (1 M NaOH aqueous solution was prepared in the lab); acetic acid was a 100% concentrated solution and was diluted to obtain a 1 M aqueous solution. Standard Lugol solution (0.2% iodine in 2% potassium iodide) and ethanol were obtained from Sigma–Aldrich, Dorset, UK.

Starch granules and all aqueous solutions were prepared with distilled water. For SEC-MALLS and AUC, deionised water was used as the buffer.

2.2. Methods

2.2.1. Preparation of starch suspension

The starch suspension is prepared by introducing dry granules in distilled water and heating with gentle stirring (~260 rpm). Three starch concentrations were prepared: 10, 30 and 50 mg/mL. The systems were heated up to 73 °C and held for 1 min. This gelatinisation temperature is determined separately using optical microscope equipped with a hot stage; it corresponds to the temperature at which the granules have lost their Maltese cross under polarized light. As a result, three suspensions with a dispersed phase of fully swollen starch granules and a continuous phase to be characterised were prepared. To separate the continuous phase from the granules, the suspension was centrifuged a first time. In order to study the influence of centrifugation parameters on the amount of substance released from the granules into the continuous phase, centrifugation time and speed were varied from 5 to 60 min and from 500 (340*g*) to 4000 rpm (2700*g*). After centrifugation the supernatant was collected and the sediment discarded. The supernatant was then centrifuged a second time for 20 min at 4000 rpm to ensure that there are no remaining starch granules. The supernatant was recovered for analysis.

The supernatant solution collected after centrifugation was used as it was or diluted with water in surface tension, Analytical Ultracentrifugation (AUC), Size Exclusion Chromatography (SEC) coupled with Multi Angle Laser Light Scattering (MALLS) methods as well as for viscosity and dry matter weight determination. For DSC and iodine staining methods the supernatant solutions were dried and dry matter re-dissolved (the exact procedure is explained in the corresponding section).

2.2.2. Statistical analysis

A statistical analysis was used to determine the influence of centrifugation speed and time on the concentration of released-from-starch dissolved component(s) using Design Expert software. The supernatant of the initial 30 mg/mL starch suspension was used and the studied "responses" were the viscosity and the concentration of dry matter in the supernatant. The design is of the factorial type, based on "extreme characteristics" in terms of centrifugation time and speed: very low rotation speed/short time, low rotation speed/long time, high rotation speed/short time and high rotation speed/long time. Four centre points (intermediate rotation speed and time) were added to complete statistical analysis. The Statistical significance of the results was assessed using factorial analysis of variance (ANOVA). All tests were conducted at a 5% significance.

2.2.3. Viscosity of the supernatant

The densities and viscosities of the supernatants of three suspensions and of the reference solvent (water) were analysed using an Automated Micro Viscometer and DMA 5000 Density Meter (both Anton Paar, Graz, Austria) under precise temperature control (20.00 ± 0.01 °C). Measurements were made in triplicate at a single concentration and intrinsic viscosities, $[\eta]$, were estimated using the Solomon-Ciutâ approximation (Solomon & Ciutâ, 1962):

$$[\eta] \approx \frac{(2\eta_{sp} - 2\ln(\eta_{rel}))^{1/2}}{c}$$

where $\eta_{rel} = \eta/\eta_0$ and $\eta_{sp} = \eta_{rel} - 1$, η is the dynamic viscosity (i.e. corrected for density) of a starch supernatant solution and η_0 is the dynamic viscosity of water (1.002 mPa s), c is the supernatant concentration determined from AUC and SEC-MALLS data.

2.2.4. Dry matter concentration determination

The dry matter concentration in the starch supernatant of each of three suspensions was calculated after drying the sample for one night in a vacuum oven at 70 °C and measuring dry matter weight:

$$\text{Dry matter concentration}(\%) = [\text{dry matter weight}(g)/\text{supernatant}(g)] \times 100\%$$

2.2.5. Surface tension

The surface tension air/starch supernatant was determined using a static pendant drop method. The experiments were carried out on a PAT-1 (Sinterface, Germany). The results were compared to those obtained for the air/water interface. Values are the mean of 10 replicates.

2.2.6. Differential scanning calorimetry

A standard power compensated Perkin Elmer DSC-7 (Perkin-Elmer, Ltd., Beaconsfield, UK) was used to investigate the presence of polysaccharides in the suspension supernatant. Portion of the dried, milled supernatant was weighed in a high pressure, stainless steel pan and water was added to make up a ratio of 1:3 dry sample to water. LPC was added at 5% concentration of the dry sample weight and the pan was hermetically sealed and left overnight on rotating rollers for moisture to equilibrate. For comparison purposes, the initial starch was prepared in the same manner. The occurrence of any thermal event was studied by heating the previ-

ously mentioned sample in the DSC at scanning rate of 40 °C/min from 5 to 140 °C. For each sample, three heating/cooling cycles were performed (cooling at 10 °C/min) and the temperature was held for 5 min after each ramp. Only the heating scans are reported in the results section. All measurements were performed in duplicate.

2.2.7. Iodine staining

This method was used to determine the type of polysaccharide present in the supernatant. The procedure was based on the ISO method (ISO, 1987) and reported by Tongdang (2001). First, several reference systems were prepared: pure amylopectin and its mixtures with amylose. Pure amylose (1 mg) and pure amylopectin (1 mg) were dispersed separately in the mixture of 0.1 mL of ethanol and 0.9 mL of 1 M NaOH aqueous solution. Once the solutions prepared, they were mixed in the volume proportions ranging from 0% to 40% of amylose. The mixtures were heated for 30 min at 95 °C and then left to cool. The samples were then diluted to 10 mL with distilled water. A 1 mL aliquot was pipetted and mixed with 5 mL of distilled water, 0.1 mL of a 1 M acetic acid solution and 0.2 mL of Lugol solution. This volume was then diluted to 10 mL with distilled water and the solution well mixed. Finally, the flasks were left for 20 min in a dark room for the colour to develop.

The supernatant to analyse was prepared in the same manner: 10 mg of dry matter (dried supernatant) was dissolved in 1 mL of a mixture of 0.1 mL of ethanol and 0.9 mL of 1 M NaOH solution, followed by heating and dissolution with water, acetic acid and Lugol solution, as described above. The optical absorbance was recorded for wavelengths from 400 to 800 nm in spectrophotometer (DU-640, Beckman Instrument, Palo Alto, USA). A blank sample (distilled water) was taken as a reference.

2.2.8. Size exclusion chromatography coupled to multi-angle laser light scattering

Analytical fractionation was carried out on supernatant solutions from three starch concentrations using a series of SEC columns TSK G6000PW, TSK G5000PW and TSK G4000PW protected by a similarly packed guard column (Tosoh Bioscience, Tokyo, Japan) with on-line MALLS (Dawn DSP, Wyatt Technology, Santa Barbara, USA) and refractive index (Optilab rEX, Wyatt Technology, Santa Barbara, USA) detectors. The eluent (deionised distilled water at 25 °C) was pumped at 0.80 mL/min (PU-1580, Jasco Corporation, Great Dunmow, UK) and the injected volume was 100 μL for each sample (in duplicate). Absolute molar masses, radii of gyration and concentrations were calculated for each sample using a Debye 1st order model (where the refractive index increment, dn/dc = 0.146 mL/g) incorporated into the ASTRA® (Version 5.1.9.1) software (Wyatt Technology, Santa Barbara, USA). The concentrations of soluble material were estimated from the areas under curves for each starch supernatant sample and used as a guide to prepare the samples of an appropriate concentration for sedimentation velocity experiments.

2.2.9. Analytical ultracentrifugation

Sedimentation velocity experiments were performed using a Beckman Instruments (Palo Alto, USA) Optima XLI Analytical Ultracentrifuge. Supernatant solutions (500 μL) from three starch concentration and distilled water (510 μL) were injected into the solution and reference channels, respectively, of a double sector 20 mm optical path length cell. Samples were centrifuged at 20,000 rpm at a temperature of 20.0 °C. Concentration profiles and the movement of the sedimenting boundary in the analytical ultracentrifuge cell were recorded using the Rayleigh interference optical system and converted to concentration (in units of fringe displacement relative to the meniscus, j) versus radial position, r (Harding, 2005). The data was then analysed using the *ls*-$g^*(s)$ model incorporated into the SEDFIT program (Schuck, 1998). This software based on the numerical solutions of the Lamm equation follows the changes in the concentration profiles with radial position and time and generates an apparent distribution of sedimentation coefficients in the form of $g^*(s)$ versus $s_{20,w}$, where the $*$ indicates that the distribution of sedimentation coefficients has not been corrected for diffusion effects. Usually sedimentation coefficients $s_{20,w}$ are measured at a series of concentrations and extrapolated to infinite dilution ($s^0_{20,w}$) to account for non-ideality, however in this case as concentrations were very low the weight average sedimentation coefficient was taken equivalent to that at infinite dilution (supernatant solutions from 10, 30 and 50 mg/mL starch suspension were diluted by 2, 6 and 20, respectively). The concentrations of each sample after dilution were estimated from the areas (A) under the $g^*(s)$ curves using the following relationship: c (mg/mL) = 0.388A (fringes). The "true" concentrations in the supernatant were then calculated according to the dilution factor.

3. Results

The Results section is divided into several parts to progressively demonstrate how the substance dissolved in the supernatant was identified and characterised. First, using surface tension measurements we show that there is some dissolved material present in suspension continuous phase. Viscosity and dry matter concentration results coupled with statistical analysis approach discuss the influence of centrifugation time and speed on supernatant concentration; the concentration of the supernatant is estimated for 30 mg/mL starch suspension. Using DSC and iodine staining the type of the polysaccharide present in the supernatant is determined. Finally, using AUC and SEC-MALLS methods, a precise polymer concentration in each of three suspensions continuous phases is obtained and the molecular weight is determined. In the Discussion section we shall consider all results together to hypothesise about the reason of the presence of the polysaccharide in the continuous phase of swollen-in-water modified waxy maize starch suspension.

3.1. Surface tension

The results for the (starch suspension supernatant)/air surface tension are obtained for a 50 mg/mL initial starch suspension. The supernatant/air surface tension is 67.9 ± 1.5 mN/m; it is slightly lower than water/air, 72.1 ± 1.3, the latter being in good agreement with the literature (Bergeron et al., 1997). Although the modified starch studied mimics cross-linked starches, it seems that a slightly surface active component is leaching out from the granule.

3.2. Statistical analysis

To rule out the effect of centrifugation on the release of matter into water from starch granules, a statistical analysis was performed as described in Section 2. This will answer the question as to whether the release is due to swelling and gelatinisation of the granules or just due to high spinning of the sample. Supernatant solution viscosity varied from 1.053 to 1.081 mPa s and dry matter concentration in the supernatant from 2.35 to 2.85 mg/mL, not depending on centrifugation time and speed. The viscosity mean value was around 1.07 ± 0.009 mPa s and it is slightly higher than water (1.002 mPa s). The mean value of the concentration of the dissolved matter in the supernatant is 2.6 mg/mL which indicates that about 10% of dry starch put in 30 mg/mL suspension is released in the continuous phase. The statistical analysis of the results obtained demonstrated that centrifugation time, rotation

speed and the interaction of both parameters do not influence either viscosity or dry matter weight and, as a consequence, do not influence the release of substance from the starch granule in the continuous media.

3.3. Differential scanning calorimetry

The complexation of the initial modified waxy maize starch and of its supernatant with LPC was investigated using DSC and the thermograms obtained were compared. In Fig. 1a (initial starch + LPC) the first heating ramp (1) presents a large and high peak at a temperature of ≈77 °C which corresponds to the gelatinization of the Novation 2600 starch sample, as expected. The large peak at 56 °C in Fig. 1b (supernatant + LPC) could be explained by the melting of a retrograded structure of the molecules released from starch and present in the supernatant, as the sample was left for five days at ambient temperature.

In Fig. 1a, it is speculated that an interaction might have occurred between the initial starch and LPC (see peak at a temperature range of 112–127 °C) which appeared as a slight endotherm in the three heating scans. Despite the fact that these endotherms were not considerable, the possibility that melting of a thermal event is detected cannot be ruled out. The detection of the melting of the (starch supernatant)-LPC complex was more evident (Fig. 1b): clear endotherms were observed in the three heating scans. These dissociation endotherms demonstrate the presence of a complex formed between the lipid and a linear chain (ΔH = 0.126 J/g for the second heating ramp, ΔH = 0.117 J/g for the 3rd heating ramp in Fig. 1b). As far as waxy maize starch is supposed to be pure amylopectin, therefore the supernatant collected should be mainly composed of sufficiently linear amylopectin segments that were able to complex LPC.

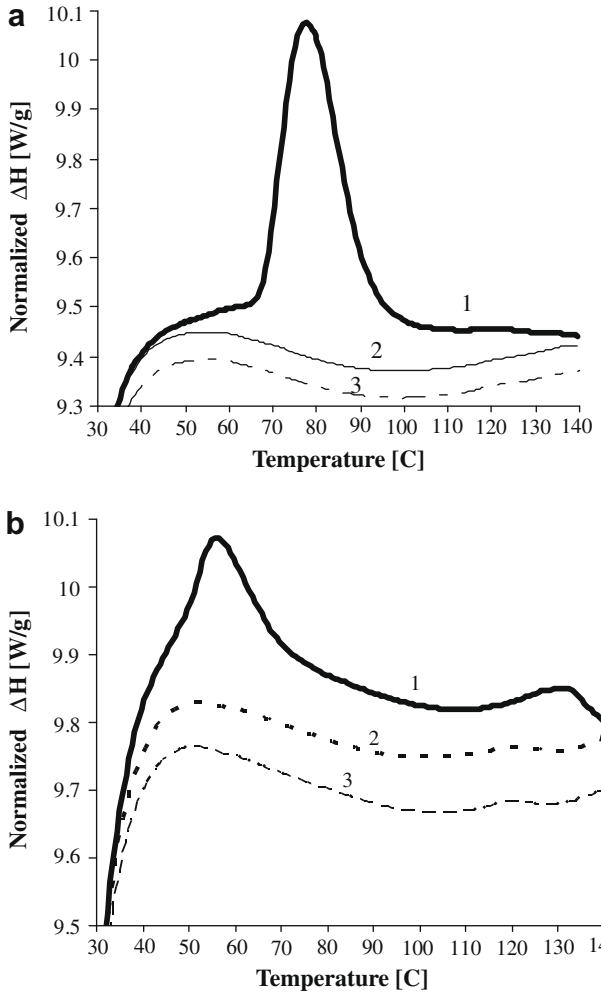

Fig. 1. Thermograms obtained from DSC for (a) initial dry starch mixed with LPC (5%) and (b) supernatant from a 50 mg/mL starch suspension mixed with LPC (5%). 1st run (1), 2nd run (2), 3rd run (3).

3.4. Iodine staining

The spectrograms of the iodine stained samples are presented Fig. 2. Pure amylopectin sample (line 2) has an absorbance peak around 555 nm which is consistent with literature (Banks & Greenwood, 1975). The supernatant sample from a 50 mg/mL initial starch suspension (line 1) has similar absorbance behaviour (540 nm) indicating the presence of amylopectin in solution. The absorbance wavelength value for the pure amylopectin is slightly higher than that of the supernatant because of the difference of the origin of the starches: the amylopectin used is from potato whereas the supernatant is obtained from a waxy maize starch. The maximum absorbance peak position is dependent on the linear chain length (Santacruz, Anderson, & Aman, 2005): the higher the DP, the larger the absorption wavelength. We can thus assume that the pure amylopectin from potato has a slightly higher DP than the amylopectin of the supernatant sample. The absorbance value of the supernatant is much higher than that of the pure amylopectin solution due to difference in polymer concentration (see details on sample preparation, Section 2.2.5).

The 60% amylopectin/40% amylose sample (line 3) shows a shift of the absorbance peak towards higher wavelengths. This is attributed to the presence of amylose in the sample: the amylose/iodine complex is known to absorb at 620 nm (Santacruz et al., 2005). The mixture of amylose and amylopectin absorbs at an intermediate wavelength between amylose and amylopectin (595 nm). The iodine staining method confirmed the presence of amylopectin dissolved in the continuous phase.

3.5. Size exclusion chromatography coupled to multi-angle laser light scattering and analytical ultracentrifugation

SEC chromatograms show a single wide peak for each supernatant solution collected from 10, 30 and 50 mg/mL initial starch suspensions (Fig. 3). The heights of the peaks (concentration detector) increase non-linearly with increasing initial starch concentration;

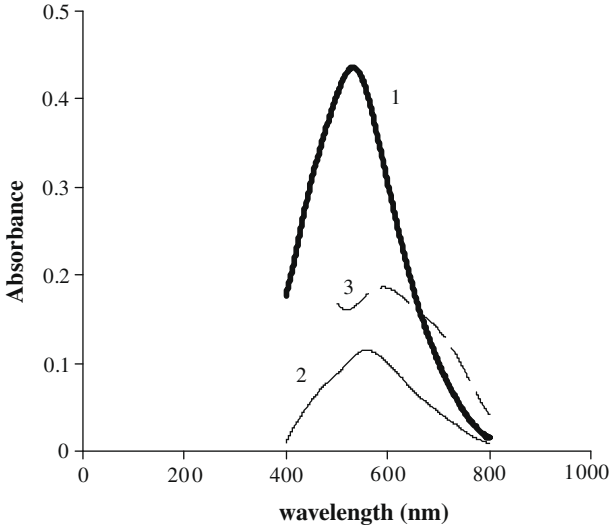

Fig. 2. Spectrogram of iodine stained samples: supernatant (1), 100% amylopectin (2) and 60% amylopectin/40% amylose (3).

Table 1
Summary of results from AUC, SEC-MALLS and intrinsic viscosity determination.

Initial starch suspension concentration, mg/mL	Amylopectin concentration estimate in the supernatant, mg/mL		$M_w \times 10^6$, g/mol	$s_{20,w}$,S	$[\eta]$, mL/g
	AUC	SEC-MALLS			
10	0.58 ± 0.01	0.63 ± 0.02	1.2 ± 0.03	15.9 ± 0.1	22 ± 1
30	2.49 ± 0.02	2.68 ± 0.01	1.54 ± 0.02	16.4 ± 0.1	21 ± 1
50	6.77 ± 0.05	6.67 ± 0.02	1.72 ± 0.02	19.0 ± 0.1	29.5 ± 0.3

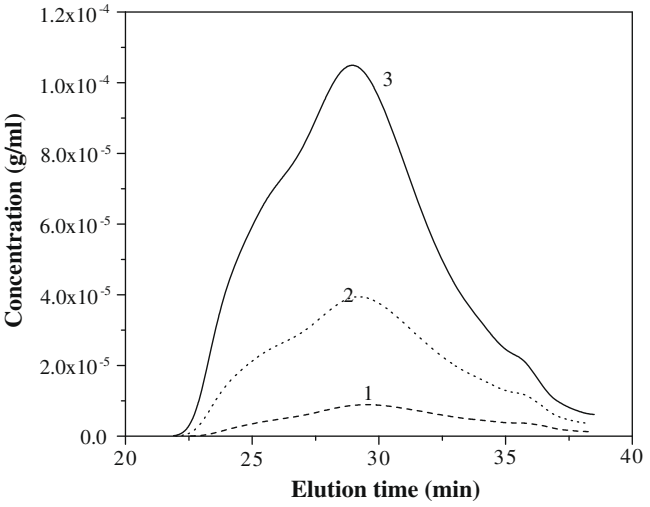

Fig. 3. SEC-MALLS elution profile: concentration vs. time for supernatants collected from the initial 10 mg/mL (1); 30 mg/mL (2) and 50 mg/mL (3) starch suspensions.

also there is a slight increase in weight average molecular weight (Table 1).

Results obtained with analytical centrifugation are summarised in Table 1 for the same three supernatants. The evolution of the sedimentation coefficient of the supernatant with initial starch concentration is not linear. The sedimentation coefficient values between 15.9 and 19S (Table 1) allow an estimation of the molecular weight of around 10^6 g/mol, which slightly increases with initial starch concentration, in good agreement with estimates from SEC-MALLS. Moreover, the peaks were quite wide hence indicating a polydisperse population of macromolecules. It is worth noting that supernatant concentrations obtained through dry matter weight, AUC and SEC-MALLS methods yielded the same values (see Section 3.2 and Table 1 for 30 mg/mL suspension).

4. Discussion

It is known that when starch is heated, linear amylose leaches out of the granule. However, in the case of modified waxy maize used in the study the amylose is nearly inexistent (traces). Hence no polymeric material should be dissolved in the water, which was demonstrated not to be the case. Our physico-chemical study revealed the presence of a slightly surface active component in the supernatant and starches have been shown to be surface active (Prochaska, Kedziora, Le Thanh, & Lewandowicz, 2007). Fast scan DSC measurements allowed detecting the presence of a small portion of linear chains (which could belong to amylopectin) and the iodine staining method confirmed the presence of amylopectin chains in the medium. The results obtained with the AUC and SEC-MALLS showed that it was in fact low molecular weight polymer of about 10^6 g/mol (usually amylopectin is above 10^7 g/mol) that was dissolved in the continuous phase with concentrations varying from 0.6 to 6.7 mg/mL for initial 10 mg/mL and 50 mg/mL starch suspensions, respectively.

The values of intrinsic viscosity (around 25 mL/g) seem quite low compared to those reported for the waxy maize amylopectin dissolved in water/dimethylsulfoxide mixtures e.g. around 200 mL/g, for molecular weights around $M_w = 10^7 - 10^8$ g/mol (Bello-Perez, Roger, Colonna, & Paredes-Lopez, 1998; Carrière, 1998; Chamberlain & Rao, 2000; Fiedorowicz & Rebilas, 2002; Fishman, Rodriguez, & Chau, 1996). Banks, Geddes, Greenwood, & Jones (1972) reported values in the same range as ours (35 mL/g) when the starch was stirred and boiled in water. The "low" (when considering amylopectin molecules) intrinsic viscosity suggests that in addition to a lower molecular weight, the polymer present in the continuous phase is also highly branched and has a random-coil conformation. Solution conformation can be estimated semi-quantitatively using the translational frictional ratio, f/f_0 (Tanford, 1961). As a general approximation $f/f_0 \sim 1-2$; 2 – 5 and >6 for compact spheres, random-coils and rigid rod type conformations, respectively:

$$\frac{f}{f_0} = \frac{M_w\left(1-\overline{v}-\rho_{20,w}\right)}{\left(N_A 6\pi\eta_{20,w}s^0_{20,w}\right)}\left(\frac{4\pi N_A}{3\overline{v}M_w}\right)^{1/3}$$

where $\overline{v}$ is the partial specific volume of amylopectin (0.64 mL/g); N_A is Avogadro number and $\rho_{20,w}$ and $\eta_{20,w}$ are the densities and viscosities of water at 20.0 °C, respectively. For the starch supernatants we find an average translational frictional ratio of 3.8 ± 0.3, consistent with a random-coil conformation. This value also depends on hydration of the macromolecule and on molecular weight and therefore more hydrated and/or higher molecular weight polymers may appear to have a less compact structure than is truly the case.

Different hypotheses can be proposed to explain the release of low molecular weight amylopectin from modified waxy maize starch into the continuous phase. The starch used in our experiments shows inhibited swelling but no evidence of cross-linking. The granules do not burst even with severe heating, so the possibility of the amylopectin being released through bursting is highly unlikely. The hypothesis is that low molecular weight amylopectin chains are able to leach out of the granules due to their small size. The small size can also be because of high branching, which is reflected by low intrinsic viscosity values. A second hypothesis is that clusters of amylopectin chains on the surface of the granule are "ripped" during stirring. This suggestion is based on the results reported in (Stark & Lynn, 1991) which stipulates that amylopectin clusters constitute the beginning of the next growth ring, and compares the granule to a hairy ball. The amylopectin clusters are linked to other amylopectin molecules via glucosidic bonds; the dissociation energy of the latter is of 77 kJ/mol (Jang et al., 2005) which is weak enough to be broken with mechanical energy and heat. If the "hairy particle model" were the only the reason for the presence of amylopectin in the continuous phase, amylopectin concentration in the supernatant should be roughly proportional to the initial starch concentration in power 2/3, which is not the case (it is proportional to the initial starch concentration in power 1.5). Thus we assume that both hypotheses are valid for the case studied.

5. Conclusions

The study of the continuous phase of swollen-in-water Novation 2600 starch granules suspension revealed the presence of low molecular weight amylopectin. The molecular weight values obtained suggest that only small and/or branched chains are released. The presence of amylopectin in suspension continuous phase should be taken into account if preparing mixtures with other (bio)polymers or interpreting the rheological properties of starch suspensions.

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