Rheology and microstructure of cross-linked waxy maize starch/whey protein suspensions

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Abstract
The objective of the present work was to investigate the effect of the heating process on the structural and rheological properties of whey protein isolate/cross-linked waxy maize starch (WPI/CWMS) blends depending upon the concentration and the starch/whey protein ratio. Starch concentration ranged from 3 to 4% (w/w) and the protein content was of 0.5, 1 and 1.5% (w/w). The blend (pH 7, 100 mM ionic strength) was heated using a jacketed vessel at two pasting temperatures: 90 and 110 °C. The particle size distribution of the WPI suspension (1.5%) displayed three distinct classes of aggregates (0.3, 65 and 220 μm), whereas the size of swollen starch granules varied from 48 to 56 μm according to the pasting temperature. When the two components were mixed together, the peak attributed to swollen starch granules was attenuated and broadened towards higher values (up to 88 μm) due to protein aggregates (260–410 μm). This effect was more pronounced as the protein concentration increased. When compared to starch alone, the rheology of the mixed system was dramatically modified for the flow behaviour as well as for the viscoelastic properties which changed from a solid-like (3–4% starch) to a liquid-like behaviour (3–4% starch/1.5% protein). Microscopic observations showed aggregated proteins located in the continuous phase and swollen starch granules as the dispersed phase. Protein aggregates were of different sizes, part of them appeared adsorbed onto swollen starch granules while another part was unevenly distributed in the continuous phase, yielding discontinuous network which could explain the peculiar viscoelastic behaviour of such suspensions.

1. Introduction

Starch is widely used to control the structure and stability of processed food products due to its thickening and gelling properties after gelatinisation. When a starch-in-water dispersion is heated above the gelatinisation temperature, starch granules loose crystallinity, absorb a large amount of water and swell up to many times their original size leading to the enhancement of viscosity. Cross-linked waxy maize starch (CWMS) is a high amylpectin starch (almost free of amylose), which has been chemically modified to make starch granules to resist to thermal and mechanical treatment. A gelatinized CWMS dispersion can be merely described as a suspension of swollen particles. These systems exhibit a shear-thinning flow behaviour and a solid-like viscoelastic behaviour, these properties being mostly governed by the volume fraction and the deformability of swollen starch granules depending upon concentration (Evans & Haisman, 1979; Evans & Lips, 1992; Loisel, Tecante, & Doublier, 2002; Nayouf, Loisel, & Doublier, 2003; Steeneken, 1989; Tecante & Doublier, 1999). It has been shown that within the pasting temperature range 90–110 °C, the rheological behaviour of CWMS dispersions is dominated by the volume fraction up to 3% concentration, while above 4% concentration deformability of swollen starch granules plays a major role (Loisel et al., 2002; Nayouf et al., 2003).

Apart from starch, whey protein isolates (WPI) can be employed because of their unique functional properties, i.e. gelling, thickening, foaming and water-binding capacity. These ingredients have a high protein content (>95%) in the globular state and consist mostly of β-lactoglobulin (>70% of total protein) and α-lactalbumin (15–20%). Thermal gelation of globular proteins involves an initial denaturation/unfolding step followed by aggregation to finally form a three-dimensional network provided the concentration is high enough. Aggregation of unfolded protein molecules occurs through hydrophobic and/or hydrogen interactions, and formation of disulfide bridges (Mulvihill & Kinsella, 1987). These aggregation and gelation phenomena are strongly dependent upon protein concentration, pH, type and concentration of ions present in the

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aqueous phase. They have been reported to take place from 62 °C for α-lactalbumin to 78 °C for β-lactoglobulin in the pH and ionic conditions of milk (Bryant & Mc Clemens, 1998). For β-lactoglobulin (200 g L⁻¹, pH 7), the denaturation temperature has been found to increase from 69.5 to 76.5 °C while increasing the NaCl content from 0.5 to 2 M (Boye, Ali, & Ismail, 1996).

β-Lactoglobulin has been found to form gels for concentration above 10 g L⁻¹ in 100 mM NaCl and pH 7 (Durand, Gimel, & Nicolai, 2002). As for isolated β-lactoglobulin, the critical concentration for gelation to take place in the case of WPI heated to 90 °C has been found to be 11 g L⁻¹ (Puyol, Perez, & Horne, 2001). Increasing the ionic strength to 60 and 100 mM NaCl resulted in a lower gelling temperature for a WPI concentration ranging from 2.5 to 10% at pH 6.7 (Puyol et al., 2001).

In the food industry, mixed systems of proteins and polysaccharides are often used to improve the textural properties. Consequently, investigation of gel formation of whey protein/starch mixed system has received considerable attention, particularly on the basis of rheological methods (Aguilera & Baffico, 1997; Fitzsimons, Mulvihill, & Morris, 2008; Matser & Steeneken, 1997; Okechukwu & Rao, 1997; Ravindra, Genovese, Foegeding, & Rao, 2004). The CWMS/WPI mixtures can be described as swollen starch granules (in WPI) dispersed in the continuous phase constituted by the protein network, provided the protein concentration is high enough. If no interaction occurs between these two biopolymers, the proteins are located in the intergranular phase between the starch granules. Upon heating, the whey protein concentration in the intergranular phase would increase as a result of the exclusion by swollen starch granules and the protein contributes to the properties of the continuous phase (Matser & Steeneken, 1997), as it has been suggested in the case of starch/polysaccharide mixtures (Alloncle, Lefebvre, Llamas, & Doublier, 1989; Tecante & Doublier, 1999). It is then possible to calculate the effective protein concentration in the intergranular phase, from the volume fraction of starch in the mixture.

The aim of this study was to describe the rheological properties and the structure of a CWMS/WPI mixture at fixed pH (7) and ionic strength (100 mM) and for different CWMS/WPI ratios, for two pasting temperatures 90 and 110 °C. Two starch concentrations, 3 and 4%, were used. The WPI nominal concentrations (0.5, 1.0, 1.5%) were chosen in order to lie under (0.5%) or at the vicinity (1.5%) of the critical gelling concentration.

2. Materials and methods

2.1. Materials

The cross-linked waxy maize starch (CWMS, adipate cross-linked and acetate stabilized) was supplied by Roquette Frères (Lestrem, France); the whey protein isolate WPI (Prolacta 90, 92.4% protein) was obtained from Lactalis (Laval, France).

2.2. Preparation of the mixtures

The WPI powder was first dispersed in demineralised water at 40 °C under strong mechanical stirring (1300 rpm), to obtain 0.5, 1.0 or 1.5% concentration, then submitted to milder stirring conditions (300 rpm) for 1 h; the dispersion was then cooled down to 25 °C. NaCl was added up to 100 mM and the pH adjusted to pH 7 with 1 M NaOH; the preparation was left overnight at 4–5 °C under magnetic stirring.

Starch at 3 or 4% (w/w) was slurried in the WPI solution previously prepared at 0.5, 1.0, or 1.5% (w/w) at 60 °C under mechanical stirring. The thermal treatment was then applied to these suspensions in a jacketed vessel (capacity 2 L, stirring rate 400 rpm) pre-heated at 90 or 110 °C. This temperature was maintained for 10 min. The suspension was then cooled down to 70 °C before sampling. It is to be emphasized that the dispersion was continuously stirred during the preparation procedure. Concentrations were then controlled by weighing aliquots overnight at 102 °C (accuracy of the order of 0.5%).

2.3. Particle size determination

Particle size determination was performed at room temperature using a Malvern Master sizer (Malvern Instrument, Ltd) with a 300 nm Fourier cell (range 0.05–879 µm) using 1.529 for the refractive index and 0.1 absorption for starch and 1.545 and 0.001 for WPI respectively. The starch paste was diluted (1/10) with demineralised water, then dispersed in the sample dispersion unit (1/100 ml water) and fed into the measuring cell. From each characterization, the median volume diameter D(v, 0.5) defined as the diameter for which 50% of the particles (by volume) are larger than this value was chosen to allow comparisons with the literature (Okechukwu & Rao, 1995). When the size distribution was poly-disperse as for WPI solutions or for the WPI/starch mixtures the peaks of the particle size distribution were favoured to describe the curves. The refractive index used for the mixture was the same as for starch alone. Each experiment was repeated twice with an accuracy of about 0.5%.

2.4. Rheological measurements

Flow and viscoelastic properties of the suspensions were measured at 60 °C using a controlled stress rheometer (AR1000, TA Instruments) with the cone/plate geometry (6 cm/2°). The exposed edge of the sample was coated with a thin layer of paraffin oil in order to minimize moisture loss during the measurements. For flow curves, an up–down shear scan from 0.01 to 300 s⁻¹ (30 s) was applied, followed by a logarithmic stepwise decrease from 300 to 0.01 s⁻¹ after equilibrium for each shear rate. For viscoelastic measurements in oscillatory shear, the samples were subjected to a frequency scan from 40 to 0.2 rad/s at 4% strain deformation (within the linearity limits of viscoelasticity). Each experiment was repeated twice with an accuracy of about 5%.

2.5. Microscopic observation

2.5.1. Light microscopy

Optical images were obtained on a microscope equipped with a CCD camera (Leica Microscope). The samples were diluted and dyed with “Fast green FCF” before being observed with a magnification of 400.

2.5.2. Confocal laser scanning microscopy (CLSM)

CLSM was used in the fluorescent mode. Observations were made with a Carl Zeiss LSM 410 Axiovert (Le Pecq, France) using a water-immersed ×40 objective. The proteins were labelled by adding 100 µL of a 2 g L⁻¹ ANS (8-anilino-1-naphthalene sulphonic acid) solution to 2 mL of the mixture. The labelled solution was then treated at 95 °C during 10 min, poured warm onto the slide, and then left during 24 h at ambient temperature. ANS is known to become fluorescent in the UV light when it is adsorbed onto hydrophobic zones. This allows one to observe proteins without covalently bound fluorescent labelling (Bourriot, Garnier, & Doublier, 1999). The excitation using the UV laser was performed at 364 nm and the emission was recorded between 450 and 497 nm.
2.5.3. Phase-contrast light microscopy

The samples were observed without any dilution on a Nikon Eclipse E 400 microscope with a ×20 objective.

3. Results

3.1. Particle size distributions

Typical results for particle size distribution are shown in Fig. 1–3. Fig. 1 shows the particle size distribution curves of the WPI solution (1.5%), the CWMS suspension (3%) and WPI/CWMS mixtures all heated to 90 °C. For WPI alone, three populations were observed corresponding to three sizes of whey protein aggregates. The most important fraction was centred at around 0.3 μm. It is to be noticed that the size of the β-lactoglobulin dimer is about 6 nm. Therefore, the size of 0.3 μm is to be ascribed to protein aggregates likely owing to the association of primary aggregates as described by Mahmoudi (2007) in the same pH and ionic environment. The two other peaks were centred at ~65 μm and 220 μm. They may be due to the growth or aggregation of the former aggregates into larger ones.

For the 3% CWMS dispersion, there was only one peak (D (v, 0.5) = 48 μm), representing the swollen starch granules in agreement with previous results (D (v, 0.5), ~45 μm) (Nayouf et al., 2003). The profile displayed by the 0.5% WPI/3% CWMS mixture was almost superimposed to that of CWMS alone. There was no evidence of whey protein aggregates below 1 μm and the size of the swollen starch granules appeared unchanged. There was however a slight shift of the maximum of the peak from 48 to 56 μm. For the 1.5% WPI/3% CWMS mixture, dramatic changes were experienced: the main peak was still centred at 56 μm but this was broadened while a shoulder appeared at around 260 μm. This could be ascribed to the presence of large protein aggregates in the same size range as for 1.5% WPI solutions alone (3rd peak). It is to be noticed that the main peak (56 μm) can be ascribed to swollen starch granules as well as to protein aggregates (2nd peak centred at 65 μm).

Fig. 2 shows the distribution profiles with 4% CWMS at the same pasting temperature (90 °C). The pattern exhibited by the 0.5% WPI/3% CWMS mixture was slightly shifted from 48 to 56 μm as expected (Nayouf et al., 2003). For 1.5% WPI alone, a unique peak at 220 μm was observed showing that increasing the heating temperature from 90 to 110 °C dramatically modified the size distribution of whey protein aggregates. The smallest aggregates (from 0.1 to 1 μm) seen at 90 °C completely disappeared and a unique population of about 200 μm was observed. This in agreement with the fact that increasing the temperature enhances the denaturation rate of the WPI and consequently the size of the aggregates (Kazmierski & Corredig, 2003). For the 1.5% WPI/3% CWMS mixture, two distinct peaks centred, respectively, at 56 and 410 μm were clearly identified instead of the broad one observed at 90 °C. Table 1

![Graph](image1)

Fig. 1. Particle size distribution of 1.5% WPI alone ( ), 3% CWMS alone ( ), 0.5% WPI/3% CWMS ( ) and 1.5% WPI/3% CWMS ( ) mixtures pasted at 90 °C.

![Graph](image2)

Fig. 2. Particle size distribution of 1.5% WPI alone ( ), 4% CWMS alone ( ), 0.5% WPI/4% CWMS ( ) and 1.5% WPI/4% CWMS ( ) mixtures pasted at 90 °C.

![Graph](image3)

Fig. 3. Particle size distribution of 1.5% WPI alone ( ), 3% CWMS alone ( ) and 1.5% WPI/3% CWMS ( ) mixtures pasted at 110 °C.
summarises the overall data in terms of peak diameters obtained in 1.5% WPI alone, 3 or 4% CWMS and mixed WPI/CWMS dispersions heated to 90 °C or 110 °C. We attempted to deconvolute the distribution curves; unfortunately this could not be performed accurately since the populations for Figs. 1 and 2 were too close to each other. Finally, increasing starch concentration in the WPI/CWMS mixture from 3 to 4% also shifted the peaks to larger values suggesting whey protein aggregates of a larger size. From these data, it appears that the size of the swollen starch granules was mostly determined by the pasting temperature and apparently was not strongly influenced by the presence of proteins.

3.2. Rheological properties

All rheological measurements have been performed at the end of the cooling period at 60 °C. All flow curves at 60 °C in linear scales of the CWMS alone suspensions as well as of the WPI/CWMS mixtures exhibited a non-Newtonian shear-thinning behaviour with an anti-clockwise loop ("anti-thixotropic" behaviour) (not showed). Such a behaviour has been reported for CWMS suspensions (Genovese & Rao, 2003; Nayouf et al., 2003; Tecante & Dublier, 1999). This may be ascribed to a rearrangement of close-packed deformable particles under shearing to yield a higher organization of the system (Barnes, Hutton, & Walters, 1989). Therefore, adding WPI to the starch suspension did not change the anti-thixotropic character of the CWMS alone suspensions.

Figs. 4, 5 and 6 show the equilibrium flow curves obtained by stepwise measurements in the presence of 3% starch pasted at 90 °C, 4% at 90 °C and 3% at 110 °C, respectively. Regarding the starch suspensions alone, the entire flow curves could be fitted using the Herschel–Bulkley model according to Eq. (1), as suggested by Evans and Haisman (1979) in the case of CWMS.

\[
\tau = \tau_0 + K\dot{\gamma}^n
\]  

(1)

Where \(\tau\) is the shear stress (Pa), \(\dot{\gamma}\) is the shear rate (s\(^{-1}\)), \(\tau_0\) is the yield stress (Pa), \(K\) is the consistency index (Pa s\(^n\)) and \(n\) is the flow behaviour index. The values of the Herschel–Bulkley parameters (\(\tau_0, K, n\)) for the different concentrations are given in Table 2. It is to be emphasized that the equation could be applied for the whole curve in the case of CWMS, while it was only valid within the shear rate range 0.01–2 s\(^{-1}\) for the mixtures as explained in the following. The steady state flow curves of the mixtures exhibited a rather different pattern from the ones of CWMS alone. The shape of the curves was similar to that of CWMS within the shear rate range 2–300 s\(^{-1}\) but was dramatically shifted to lower shear stress values in the low shear rate range (10\(^{-2}\)–2 s\(^{-1}\)). Therefore, the apparent viscosity of the mixtures was much lower at low shear rates (10\(^{-2}\)–2 s\(^{-1}\)) than the one of the starch CWMS suspension. The reverse was seen at high shear rate (2–300 s\(^{-1}\)) particularly in Figs. 4 and 6, the apparent viscosity being higher for the mixture than for CWMS alone. As WPI concentration increased (from 0.5 to 1.5%), the decrease of apparent viscosity at low shear rate was even more pronounced. It is also evident that at low shear rate, the flow curve tends to a plateau suggesting a yield stress. However, from the shape of these flow curves, it is obvious that the Herschel–Bulkley equation is not obeyed over the entire shear rate range in the case of the mixtures. Whichever, it was possible to apply this equation over the range 10\(^{-2}\)–2 s\(^{-1}\), allowing one to estimate the extrapolated yield stress of the different suspensions. The Herschel–Bulkley parameters obtained in these conditions are also given in Table 2. The yield stress decreased from 2.57 Pa (3% CWMS) to

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>Peaks (μm) of the particle size distribution for WPI, CWMS suspensions and their mixtures pasted at 90 °C and 110 °C.</td>
</tr>
<tr>
<td>Cooking temperature (°C)</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>90</td>
</tr>
<tr>
<td>3%</td>
</tr>
<tr>
<td>4%</td>
</tr>
<tr>
<td>110</td>
</tr>
<tr>
<td>3%</td>
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</table>

- not determined.
0.68 Pa (0.5% WPI/3% CWMS) and 0.44 Pa (1.5% WPI/3% CWMS) when heated at 90 °C. In case of 4% CWMS, the yield stress was dramatically lowered from 11.7 Pa for 4% CWMS alone to 1.2 Pa and 0.7 Pa with 0.5% WPI and 1.5% WPI, respectively. This parameter decreased from 2.6 Pa (3% CWMS) down to 0.24 Pa (1.5% WPI/3% CWMS) when the dispersions were heated at 110 °C.

From these overall results, it is clear that adding WPI (0.5 to 1.5%) to starch suspensions dramatically modified the flow behaviour with a marked decrease of the yield stress whatever the CWMS concentration (3 or 4%) and pasting temperature (90 or 110 °C). Increasing the WPI concentration from 0.5 to 1.5% lowered the yield stress slightly more. These dramatic modifications in the flow behaviour at low shear rate reflect important structural differences between the CWMS suspensions and the WPI/CWMS mixtures.

In Figs. 7–9, the viscoelastic behaviour of these different suspensions is illustrated by means of the mechanical spectra (\(G'\) and \(G''\) as a function of frequency) measured at 60 °C. The starch suspensions (3% or 4%) heated to 90 °C or 110 °C exhibited a solid-like behaviour with \(G' > G''\) and \(G'\) almost independent of frequency. This is typical behaviour of cross-linked starch suspensions which is to be ascribed to close-packing of swollen starch granules (Nayouf et al., 2003; Tecante & Doublier, 1999). The mere increase of starch concentration from 3% to 4% has been to increase \(G'\), the storage modulus, from 8 to 80 Pa when pasting at 90 °C (Figs. 7 and 9). This is related to the increase of the volume fraction occupied by swollen starch granules which is the determining parameter of the rheological properties at this pasting temperature. Increasing the temperature from 90 °C to 110 °C (at 3% CWMS) enhanced the \(G'\) values from 8 to about 20 Pa (Fig. 9). This is also to be ascribed to an increase of the volume fraction occupied by swollen starch granules, as indicated by the increase of the median diameter which varied from 48 to 56 \(\mu\)m (Table 1).

Adding WPI to the starch suspension yielded a dramatic change in the viscoelastic properties of the system. This is clearly illustrated for 1.5 WPI/3% CWMS whatever the pasting temperature (90 °C or 110 °C) (Figs. 7 and 9) and also for 1.5 WPI/4% CWMS pasted at 90 °C (Fig. 8). The solid-like behaviour of CWMS alone tends to disappear. Instead, a liquid-like behaviour with a pronounced dependency of \(G'\) and \(G''\) as a function of frequency, \(G' < G''\) at low frequency and a cross-over of \(G''(\omega)\) and \(G'(\omega)\) which was between 2 and 4 rad/s. Such mechanical spectra are reminiscent of those exhibited by macromolecular solutions. For macromolecular solutions, below the cross-over, the terminal zone is accessed with theoretical slopes of 2 for \(G''(\omega)\) and 1 for \(G'(\omega)\) while, beyond the \(G''(\omega)\) and \(G'(\omega)\) cross-over, the so-called plateau zone is accessed. It is noteworthy that slopes of 2 and 1 for \(G''(\omega)\) and \(G'(\omega)\), respectively, in the terminal zone are approached in the cases of 1.5 WPI/4% CWMS at 90 °C (Fig. 8) and 1.5 WPI/3% CWMS at 110 °C (Fig. 9). On another hand, the plateau zone is described only in its first part. Such a rather surprising behaviour is to be paralleled with the changes in the flow properties above-described, particularly the decrease of the yield stress.

All these results demonstrate that WPI/CWMS mixtures were much less structured than the starch suspensions. It is to be noticed that the mixtures were white and granular while the CWMS suspensions appeared translucent and more homogeneous.

### Table 2

<table>
<thead>
<tr>
<th>Pasting temperature (°C)</th>
<th>CWMS % (w/w)</th>
<th>WPI % (w/w)</th>
<th>(\gamma (s^{-1}))</th>
<th>(\tau_0) (Pa)</th>
<th>(K)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>3</td>
<td>0</td>
<td>0.01–300</td>
<td>2.57</td>
<td>0.85</td>
<td>0.51</td>
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<td></td>
<td>0.5</td>
<td></td>
<td>0.01–2</td>
<td>0.68</td>
<td>1.93</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td></td>
<td>0.01–2</td>
<td>0.44</td>
<td>1.66</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.01–300</td>
<td>11.75</td>
<td>1.60</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>0.01–2</td>
<td>1.19</td>
<td>8.85</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>0.01–2</td>
<td>0.70</td>
<td>8.15</td>
<td>0.52</td>
</tr>
<tr>
<td>110</td>
<td>3</td>
<td>0</td>
<td>0.01–300</td>
<td>2.60</td>
<td>0.92</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td></td>
<td>0.01–2</td>
<td>0.24</td>
<td>3.40</td>
<td>0.45</td>
</tr>
</tbody>
</table>

3.3. Microscopic observations

Fig. 10 shows the photomicrographs (light microscopy) of the mixtures cooked at 90 °C (Fig. 10a–c) and 110 °C (Fig. 10d). In these...
proteins appeared whose size was around 80–200 nm in accordance with the size determination (Fig. 1). For the higher pasting temperature (110 °C; Fig. 10d), the dark zones indicating the presence of proteins were more contrasted suggesting the WPI aggregates were larger. From these observations, the WPI/CWMS suspensions could be described as a co-existence of protein-rich zones and starch-rich zones. The repartition of protein aggregates throughout the starch dispersion varied with WPI concentration. At 0.5%, the protein spots appeared to be randomly distributed over the starch dispersion (Fig. 10a). At 1.0% and 1.5% WPI (Fig. 10b and c), clusters of protein aggregates were formed that seemed to be localised mostly in the intergranular space between the swollen starch granules although some of the proteins could be localised onto the starch granules. These effects were accentuated at 110 °C (Fig. 10d), the strands of protein aggregates being intensified partly imprisoning starch-rich zones.

A clearer insight into the localisation of the whey protein aggregates is brought by CLSM microscopy. It is to be emphasized that ANS is used in order to visualize the proteins. Fig. 11 shows the photomicrographs of the different dispersions pasted at 90 °C. At 1.5% WPI without starch (Fig. 11a), small bright spots were seen indicating the presence of relatively small protein aggregates. Fig. 11b shows the result obtained with starch alone (3%). Starch granules were clearly seen in black while the protein aggregates appeared distributed in the intergranular phase (grey zones) and brighter spots were seen in the vicinity of swollen starch granules. At 1.5% WPI/3% CWMS (Fig. 11d and f), this distribution appeared more clearly with larger spots corresponding to large protein aggregates. Therefore, part of the proteins was apparently adsorbed onto the surface of starch granules while the other part was located in the intergranular phase. It was not possible however to evaluate the relative ratio of the two populations. This interpretation is corroborated by phase-contrast microscopy as illustrated in Fig. 12 for 0.5% WPI/3% CWMS (Fig. 12a) and 1.5% WPI/3% CWMS (Fig. 12b) pasted at 90 °C. Particularly in Fig. 12b, protein aggregates are clearly seen on the surface of the swollen starch granules.

4. Discussion

The WPI/CWMS mixture can be described as a composite system of swollen starch granules dispersed in a continuous phase containing whey proteins. Due to the composition of starch (99% amylopectin) together with cross-linking, no diffusion of starch macromolecules is expected towards the surrounding phase at these pasting temperatures (90–110 °C). Regarding the starch alone suspension, the rheological behaviour is governed by the volume fraction occupied by the swollen starch granules and their deformability. The volume fraction varies from ~0.50 (3%, 90 °C) to 0.65–0.70 (4%, 90 °C) (Nayouf et al., 2003).

When heating a WPI/CWMS mixture, two concomitant phenomena are expected to take place in the same temperature range (60–80 °C), namely protein denaturation, on one side, starch pasting (swelling), on the other side. From the comparison of size distributions of CWMS alone and WPI/CWMS mixtures, we can suggest the swelling of starch granules upon pasting is not modified by the presence of WPI. This result has been also postulated by Fitzsimons et al. (2008) for WPI/CWMS suspensions. The intergranular phase of the system is composed of denatured whey
proteins that form aggregates whose size depends on the pasting conditions (heating kinetics, shearing rate, final heating temperature) and on the protein concentration, this later parameter being itself governed by the available volume. Since swollen starch granules occupy 50% to 65–70% of the available volume, the protein concentration is more than twice the nominal concentration. Therefore, for 1.5% WPI/3–4% CWMS mixture, the protein concentration is theoretically higher than the critical gelling concentration (Cc ≈ 1% according to Puyol et al., 2001) for WPI. However, from the microscopic observations, it is clear that the proteins form large aggregates in the continuous phase but do not result in a continuous protein network although the WPI concentration is high enough to yield a gel. This is likely due to the fact that the mixture is continuously stirred during pasting in the jacketed vessel. The kinetics of aggregation may be slowed down upon continuous stirring compared to the static conditions as it has been showed in the case of a WPI/xanthan mixture (Walkenström, Nielsen, Windhab, & Hermansson, 1999).

Disruption of the WPI network by the presence of swollen starch granules has been reported by several authors (Aguilera & Rojas, 1996; Ravindra et al., 2004; Shim & Mulvaney, 2001). Aguilera and Rojas (1996) observed that the rigidity of the WPI/corn starch gels (storage modulus) decreased as the starch mass fraction increased (from 2 to 7% for a total solid content of 10%). They attributed this effect to the disruptive effect of starch granules in the continuous WPI matrix and ascribed this to structural incompatibility. Shim and Mulvaney (2001) also described two separated networks for WPI and corn starch with diffusion of amylose in the matrix (at pH 7, 95 °C, ratio WPI:corn starch 0.5, total solids 15%). The structure of the mixed system can then be described as a filled gel where the continuous phase is a network, itself a mixture of amylose and protein. In the case of WPI/CWMS systems (pH 7, 75 mM NaCl, pasting temperature 85 °C, total solids 5%), Ravindra et al. (2004) also showed that at starch concentrations higher than 3% (and 2% WPI) the swollen starch granules become the predominant phase interfering with the formation of a continuous WPI network and weakening the structure of the system. It should be noticed that in these different studies a moderate stirring was applied during the heating period which would explain why a continuous protein network cannot be formed (Fitzsimons et al., 2008). According to these authors, a continuous protein network can be obtained in the case of WPI/CWMS mixtures only if no shear is applied during the thermal treatment, the heating process as well as the cooling one. In the present work, strong stirring was applied (400 rpm) during the whole preparation process as would occur in an industrial process. It is therefore expected that the protein network cannot be formed in the continuous phase. As a result, the WPI/CWMS system in the present work can be merely described as swollen starch granules dispersed in a suspension of WPI aggregates. The continuous phase appears itself discontinuous with protein aggregates of different sizes as showed by size distribution measurements and microscopic observations. On another hand, part of the largest size aggregates appears stuck onto the swollen starch granules. These structural features can explain the peculiar rheological properties of these mixed systems. The solid-like character of CWMS suspensions is well documented in the literature (Evans & Lips, 1992; Nayouf et al., 2003). This appears beyond a critical phase volume (Φc) where close-packing is approached (Φc > 0.50). This obviously does not imply that a continuous network is obtained but means that the swollen starch granules interact between each other due to their vicinity. It is also beyond Φc that a yield stress is displayed in the flow curves (Nayouf et al., 2003). Both characteristics, solid-like viscoelastic behaviour, on one side, and existence of a yield stress in flow measurements, on the other side, reflect the behaviour of suspensions of close-packed particles. It might be expected that the addition of WPI would give rise to a reinforcement of the system with a higher G' modulus and a higher yield stress as was reported by Fitzsimons et al. (2008). Actually, totally different and unusual behaviours have been observed. Indeed, the apparent viscosity at high shear rate of the 0.5–1.5% WPI/3% CWMS mixture was increased with respect to CWMS alone. This is the expected effect of increasing the protein...
concentration in the continuous phase. In contrast, however, the yield stress was decreased. This means that interactions between the swollen starch granules are weakened by the presence of WPI aggregates in the continuous phase. In the case of the 1.5% WPI/4% CWMS stronger changes were seen but the apparent viscosity was lowered whatever the shear rate range. The changes in the viscoelastic behaviour were far more dramatic since the solid-like behaviour was lost and a typical liquid-like behaviour was observed. The viscoelastic spectrum covered part of the terminal zone ($G' < G''$) towards the low frequencies and the beginning of the plateau zone ($G' > G''$) at high frequency. Such a behaviour is well documented for entangled macromolecular systems as well as wormlike micelles originating from surfactants. However, so far as we are aware, this has not been reported for suspensions of deformable particles dispersed in a suspension of denser particles.

We suggest that the presence of large WPI particles in contact with swollen starch granules modifies the friction forces between them. This might reduce the number of contact zones between starch granules or facilitate their relative movement under low shear forces. The overall consequence would be a destabilization of the packed structure formed by starch granules. This would occur at 3 and 4%, that is beyond $\Phi_c$. At 4%, the volume fraction of starch granules is about 0.65–0.70 which means that the swollen starch granules are tightly packed (Evans & Lips, 1992). It has been showed

Fig. 11. CLSM micrographs of 1.5% WPI (a), 3% CWMS alone (b), 0.5% WPI/3% CWMS (c) and (d), and 1.5% WPI/3% CWMS (e) and (f) pasted at 90 °C. Scale bars refer to 50 μm (a, b, c and e) and 10 μm (d and f).
that the deformability of starch granules plays a major role in the rheological properties. Under these conditions (4% starch), the presence of protein aggregates of larger size contributes to lower the apparent viscosity over the entire shear rate range. The extent of disorder brought about by protein aggregates to the organised structure of micellar casein–guar gum mixtures. Food Hydrocolloids, 13, 43–49.


