JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Improved Sugar Beet Pectin-Stabilized Emulsions through Complexation with Sodium Caseinate

Xiangyang Li,[†] Yapeng Fang,[†] Glyn O. Phillips,[†,‡] and Saphwan Al-Assaf*[,†]

[†]Glyn O Phillips Hydrocolloids Research Centre, Glyndwr University Wrexham, Wrexham LL11 2AW, United Kingdom

[‡]Phillips Hydrocolloid Research Ltd., 45 Old Bond Street, London W1S 4AQ, United Kingdom

ABSTRACT: The study investigates the complexes formed between sodium caseinate (SC) and sugar beet pectin (SBP) and to harness them to stabilize SBP emulsions. We find that both hydrophobic and electrostatic interactions are involved in the complexation. In SC/SBP mixed solution, soluble SC/SBP complexes first form on acidification and then aggregate into insoluble complexes, which disassociate into soluble polymers upon further decreasing pH. The critical pH's for the formation of soluble and insoluble complexes and disappearance of insoluble complexes are designated as pH_c, pH_φ, and pH_d, respectively. These critical pH values define four regions in the phase diagram of complexation, and SC/SBP emulsions were prepared in these regions. The results show that the stability of SBP-stabilized emulsion is greatly improved at low SC/SBP ratios and acidic pH's. This enhancement can be attributed to an increase in the amount of adsorbed SBP as a result of cooperative adsorption to sodium caseinate. Using a low ratio of SC/SBP ensured that all caseinate molecules are completely covered by adsorbed SBP chains, which eliminates possible instability induced by thermal aggregation of caseinate molecules resulting from stress acceleration at elevated temperatures. A mechanistic model for the behavior is proposed.

KEYWORDS: *sugar beet pectin (SBP), sodium caseinate, phase diagram, complexes, emulsion*

1. INTRODUCTION

Pectin is an important hydrocolloid, which has been used extensively in the food industry as gelling agent, thickener, and stabilizer.[1−3] It is mainly extracted from apple pomace and citrus peel, but more recently, pectins have been extracted from other sources such as sugar beet pulp,[4] sunflower,[5] and tomato.[6] In particular, sugar beet pectin (SBP) has been investigated for its strong emulsifying ability,[6−10] because it can produce good emulsions at lower use concentrations than other polymeric emulsifiers such as gum arabic and soybean soluble polysaccharide.[11] Meanwhile, SBP-stabilized emulsions have better acid stability than those stabilized by proteins, which become unstable at around the isoelectric point (IEP).[12,13]

The emulsification mechanism of SBP has been investigated,[8−11,14] and it has been shown that its proteinaceous component contributes an important function in the emulsification process. Funami et al.[9] found that the emulsifying ability of SBP is reduced, significantly following enzymic removal of the protein. Siew et al.[10,14] recovered from the emulsion the absorbed SBP fraction with sodium dodecyl sulfate and found that it contained higher contents of protein and ferulic acid than the initial sample. Thus, as with gum arabic,[10,15] the protein associated with the polysaccharide component might adsorb at the oil−water interface, with the polysaccharide component extending out into the bulk aqueous phase giving the emulsion stability. Recently, such protein−polysaccharide complexes have been visualized by atomic force microscopy.[6,16] Yet despite such emulsification potential, the practical application of SBP for this purpose has been limited because of the low thermal stabilities of the emulsions,[15,16] which can be attributed to its weak adsorption ability.[9−11,14] Only 0.15−0.20 wt % SBP is adsorbed on the droplet surface of emulsions with 15 wt % middle-chain triglyceride (MCT) or 20 wt % limonene oil. A better emulsifying SBP product has been produced using maturation technology.[17] Here, we explore other methods to enhance the stability of SBP-stabilized emulsions.

A possible approach is to use protein−polysaccharide complexes as a new type of food biopolymer to improve functional properties, for example, emulsion stability.[13,18,19] Sodium caseinate and gum arabic or pectin,[20−23] whey proteins and carrageenan or gum arabic,[24,25] bovine serum albumin and gum arabic,[26] β-lactoglobulin, and acacia gum[27] have been investigated. Their applications in the food industry have been well reviewed.[18,24,28−30] However, it should be pointed out that the addition of two emulsifiers often causes competitive adsorption, even for two emulsifiers that have similar ingredients such as α- and β-caseins.[31−33] Previously,[34,35] we investigated the complexation of bovine serum albumin (BSA) with SBP and found that at lower pH's SBP there is a significant increase in adsorbed amounts of SBP at the oil−water interface, thus enhancing the emulsion stability.

In the present study, the acid-stable protein BSA has been replaced by the acid-unstable protein sodium caseinate (SC), which can aggregate and precipitate at IEP. SC contains four main proteins (α_{s1}-, α_{s2}-, β-, and κ-caseins),[13] which have a strong tendency to associate to form supramolecular aggregates such as casein micelles.[21] At neutral pH, κ-caseins on the surface of casein micelles have extended conformation, which can provide strong steric stability. Here, we seek the optimum conditions to enhance the stability of SBP-stabilized emulsions

Received: November 1, 2012
Revised: January 16, 2013
Accepted: January 22, 2013
Published: January 22, 2013

dx.doi.org/10.1021/jf304658q | J. Agric. Food Chem. 2013, 61, 1388−1396

using SBP/SC complexes, and to identify the mechanism for the enhanced emulsion stabilization, which we have achieved.

2. MATERIALS AND METHODS

2.1. Materials. SC was obtained from Rich Products Corp. (USA). Low methoxyl nonamidated SBP (DE of 39%) with a ferulic acid content of 0.29 wt % and protein content of 10.57 wt % (determined by the Lowry method) was supplied from CP Kelco, Denmark. SBP has M_w of 5.3 × 10^5 Da and a radius of gyration (R_g) of 41 nm determined using GPC-MALLS.[36] All of the other chemicals used were purchased from Fisher Scientific UK. They were of analytical grade and used as received.

2.2. Preparation of SC and SBP Stock Solutions. SBP (0.5 wt %) and 0.5 wt % SC solutions were prepared first by dispersing appropriate amounts of SBP or SC into deionized water and leaving on a roller mixer overnight to attain full hydration. After centrifugation for 30 min at 4000*g*, the clear solutions were collected using a needle connected with a syringe and subsequently filtered through a 0.45 μm nylon filter. The hydrodynamic diameter of SC solution was ∼100 nm together with whitish color similar to a milk system. The actual concentrations of SBP and SC solutions were determined by drying aliquots in an oven at 60 °C to constant weight. The stock SBP and SC solutions were diluted to 0.1% with deionized water and the pH's adjusted to 7.0 using 0.1 M NaOH. Sodium azide (0.005 wt %) was also added to prevent microorganism growth. 0.1 wt % SC/SBP mixed solutions were prepared by mixing the 0.1 wt % stock solutions of SBP and SC together with required ratios. 2.5 wt % stock solutions of SBP and SC were also prepared for emulsion preparation using a method similar to that above but without filtration.

2.3. Electrophoretic Mobility. Electrophoretic mobility of 0.1 wt % SBP, 0.1 wt % SC, and 0.1 wt % SBP/SC mixed solutions in a pH range from 8.5 to 2.0 was determined using a Nano-Zetasizer equipped with a MPF-2 multipurpose titrator (Malvern Ltd., UK). Using a standard capillary electrophoresis cell equipped with platinum electrodes, the pH was adjusted using 1 M NaOH, 1 M HCl, or 0.1 M HCl, and when the pH was stabilized the electrophoretic mobility was recorded.

2.4. Construction of Phase Diagram. The complexation between SBP and SC during acidification was monitored using a ZetaSizer 1000HS dynamic light scattering (DLS) apparatus equipped with a 10 mW He−Ne laser (633 nm) (Malvern instruments, UK), a Lambda UV/visible spectrophotometer (PerkinElmer Inc., USA), and a Orion 4 star pH meter (Thermol Scientific Inc., USA). In detail, three portions of 10 g of 0.1 wt % SC/SBP mixed solutions and three portions of the same amounts of glucono-δ-lactone (GDL) power were prepared first. Every portion of the mixed solution was then mixed with one portion of solid GDL for 30 s vigorously. Subsequently, it was transferred to a 10 mm path length plastic cuvette and subjected to measurements immediately using DLS, turbidity, or pH meter. In the aqueous solutions, GDL releases protons ceaselessly, which leads to a slow acidification and molecular complexation. For different ratios of SC/SBP mixed solutions, different amounts of GDL (0.25−1.0 wt %) were used to ensure pH variation over the desired range. Acidification curve was obtained with the pH meter, which can automatically record pH at 1 min interval for the duration of 200 min. The changes in light scattering intensity at 90° (I_{90}) due to complexation were recorded every 1 min during 200 min using DLS. The change in turbidity (τ) during complexation was recorded every 1 min over 200 min using the UV/visible spectrophotometer at a wavelength of 550 nm. The turbidity was defined as:[27]

$$\tau = (1/L)\ln(I_0/I_t) \quad (1)$$

where L is the optical path length (10 mm), I_t is the transmitted light intensity, and I_0 is the incident light intensity. The sharp pH increases in I_{90} and τ were used to determine critical pH's of soluble (pH_c) and insoluble complexes (pH_φ), respectively. Because GDL-induced acidification cannot achieve pH's below 2.0, addition of 5 M HCl was used to decrease the pH to 1.5. The changes of turbidity at 550 nm with pH were obtained at 25 °C after the solutions were circulated into the optical cell using a Bio-Rad EP-1 Econo peristaltic pump.

2.5. Laser Diffraction. Mixed emulsions containing 1.5% SC/SBP mixtures with weight ratio of 2:1 or 1:4 were prepared at various pH's (7.0, 6.0 5.6, 5.0, 4.5, and 3.6). In detail, 80 g of 1.875% SC/SBP mixed solution with weight ratio of 2:1 or 1:4 was prepared with 2.5% stock solutions of SBP and SC, and then was mixed with 15 g of middle-chain triglyceride (MCT), 1 g of 10 wt % benzoic acid, and 4 g of deionized water using a polytron-type at 26 000 rpm for 3 min to prepare pre-emulsions. After the emulsion pH was adjusted to 7.0, the pre-emulsions were passed twice through a high pressure homogenizer (Nanomizer II, Yoshida Co., Japan) at 50 MPa. Finally, the pH's of emulsions were adjusted to the desired pH's using HCl.

Changes in size distributions during acceleration test at 60 °C were determined to evaluate the properties and stability of the mixed emulsions using Malvern Mastersizer 2000 laser diffraction (Malvern Ltd., UK). Mie theory was used to analyze the results using refractive index = 1.45 and adsorption index = 0.001 for MCT, and refractive index 1.33 and adsorption index = 0 for the dispersant (water). The average droplet diameter was characterized by its surface−volume mean diameter $d_{3,2}$ and volume−moment mean diameter $d_{4,3}$. $d_{3,2}$ was used to estimate the specific surface area of freshly made emulsions, while $d_{4,3}$ was used to monitor the changes in size distribution on storage.[37]

2.6. Light Microscope. Photomicrographs of the emulsion droplets were obtained using a BT-1600 image particle size analyzer (Dandong bettersize instrument Ltd., China) consisting of an optical microscope (Nikon YS100) and a CCD camera (HV2001UC). A small drop of emulsion was placed onto a microscopic slide and carefully covered with a coverslip. After being equilibrated for 2 min, the photomicrographs (20× magnification) were taken.

3. RESULTS AND DISCUSSION

3.1. Electrophoretic Mobility. Figure 1 shows the electrophoretic mobility as a function of pH for aqueous

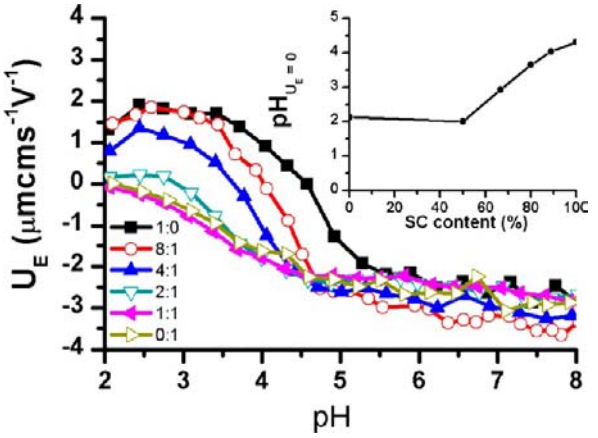

Figure 1. Electrophoretic mobility as a function of pH for 0.1 wt % SC solution, 0.1 wt % SBP solution, and 0.1 wt % SC/SBP mixed solutions at various SC/SBP weight ratios. The inset plot shows the isoelectric point as a function of SC content in SC/SBP mixtures.

solutions of SBP and SC. At pH's higher than 5.6, SC has a constant negative electrophoretic mobility, which increases when the pH is reduced below 5.6. It reaches a positive plateau at pH 3.3 with increasing turbidity and precipitation between pH 5.6 and 3.3. Thus, the reduced electrostatic stability is accompanied by aggregation of caseinate molecules and solution instablility.[20,22] The IEP of SC occurs at pH 4.6 as reported previously.[19,20,38] SBP always has negative values of electrophoretic mobility over the entire pH range, which is different from the behavior of SC. It remains constant above

dx.doi.org/10.1021/jf304658q | J. Agric. Food Chem. 2013, 61, 1388−1396

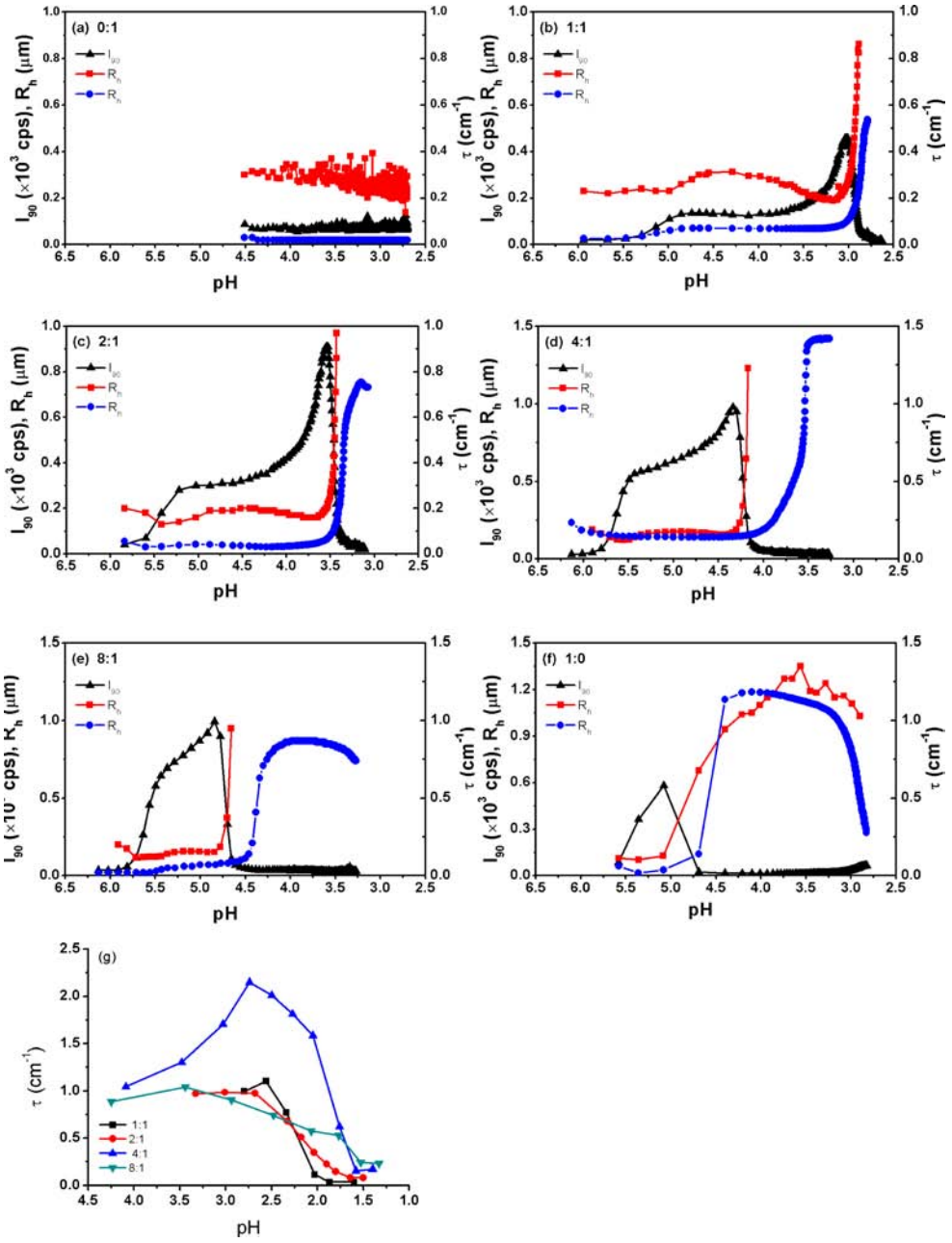

Figure 2. Changes in light scattering intensity at 90° (I_{90}) and turbidity (τ) during acidification with GDL for 0.1 wt % SC/SBP mixed solutions with various SC/SBP weight ratios: (a) SC/SBP = 0:1, (b) SC/SBP = 1:1, (c) SC/SBP = 2:1, (d) SC/SBP = 4:1, (e) SC/SBP = 8:1, and (f) SC/SBP = 1:0. (g) Changes in turbidity (τ) during acidification with HCl for 0.1 wt % SC/SBP mixed solutions with various SC/SBP weight ratios.

pH 4.5, but starts to increase thereafter. At pH 2.0, it approaches zero. The change in the electrophoretic mobility with pH could be related to the complete dissociation of the SBP chains into polyanion and cations at pH > 4.5. Dissociation of SBP is partially suppressed below pH 4.5, leading to a decrease in charge density, and at pH 2.0, SBP is fully protonated such that the system approaches neutrality. Electrostatic repulsion prevails at pH values higher than pH 4.6 because SC and SBP then both carry net negative charges, whereas electrostatic attractive is dominant between pH 2.0 and 4.6 because they would have net opposite charges. Below pH 2.0, the electrostatic interaction becomes negligible, because the charge density of the pectin chain approaches zero. The electrophoretic mobility for mixed SBP/SC solutions was also plotted in Figure 1 as a function of pH. When the content of SC is smaller than 50%, the electrophoretic mobility profile is similar to that of SBP, which indicates that SBP dominates the electrophoretic mobility of mixture solutions. When the content of SC is above 50%, the role of SC appears gradually, and the net charge of mixture can be positive at lower pH's. The isoelectric point as a function of SC content is also summarized in Figure 1. IEP is fixed at pH 2.0 when SC content is lower than 50% but increases with SC content thereafter. This implies that only when the SC content is higher than 50% could positively charged SC molecules be neutralized with negatively charged SBP molecules.

3.2. Phase Diagram of Complexation. Figure 2 follows the complexation between SBP and SC in 0.1% SC/SBP mixed solutions using light scattering at 90° (I_{90}), average diameter of complexes R_h, and turbidity (τ). For comparison, changes in I_{90}

dx.doi.org/10.1021/jf304658q | J. Agric. Food Chem. 2013, 61, 1388−1396

and τ in SBP and SC solutions were also given. For SBP, I_{90} and τ remain low and constant, indicating the absence of aggregation, due probably to the high proportion of acetyl groups in SBP preventing molecular aggregation.[39,40] As for SC, I_{90} and τ start to increase at pH's 5.6 and 4.7, respectively. The increases in I_{90} and τ for SC solutions at pH's 5.6 and 4.7 are probably due to the effect of pH on the stability resulting from change in hydrodynamic size, predominantly stabilized by the hairy layer of κ-casein at the surface.[22,41] The κ-casein has an extended conformation at pH > 5.6, which gives the micelles strong steric stability.[22] When the solution pH is below 5.6, the extended conformation collapses.[22] The reduction in the size of micelle like-structure, possibly present in the commercial SC used in this study, induces an increase in I_{90}. When the pH is further decreased to below the IEP, insoluble aggregates form because of the loss in the electrostatic stability, resulting in the increased turbidity. For SC/SBP mixed solutions, although I_{90} also increases at around pH 5.6, it reaches a plateau quickly and remains unchanged thereafter over a wide pH range, which indicates that SBP can adsorb on to the surface of caseinate micelle and form soluble complexes through electrostatic attraction as proposed by Ye et al.[20] Probably, the adsorption of SBP makes up for the reduction in the stability via electrostatic interactions due to the decrease in the charge density of caseinate molecules during acidification, thus enhancing stability at low pH's. However, solution turbidity eventually increases at a lower pH, for example, pH 3.5, at SC/SBP = 2, indicating the formation of insoluble SC/SBP complexes. Likely, the net negative charges carried by complexes decrease with a reduction in pH. At a certain pH, soluble complexes could not prevent aggregation due to weak electrostatic stability. In this study, the pH's corresponding to sharp increases in I_{90} and τ were determined and taken as critical pH's of soluble (pH_c) and insoluble complexes (pH_φ), respectively. The complexation process at lower pH was also studied through dropwise addition of HCl. At around pH 1.6, the solutions became clear again, implying dissociation of insoluble complexes (see Figure 2g). As shown in Figure1, the electrostatic interaction between SBP and SC becomes negligible when pH < 2.0 due to full protonation of SBP. This pH can be regarded as the third critical complexation pH (pH_d). Here, it should be noted that the turbidity obtained by HCl is higher than the value obtained by GDL. For the method with GDL, insoluble complexes precipitate with gravity, leading to decrease in the observed turbidity. For the method with GDL, insoluble complexes disperse in the solution due to the slow acidification and mechanical circulation.

The critical complexation pH's at various SC/SBP ratios are summarized in Figure 3. These three critical pH's define four regions in the phase diagram. In regions A ($pH > pH_c$) and D ($pH < pH_d$), only free polymers exist. In regions B ($pH_\varphi < pH < pH_c$) and C ($pH_d < pH < pH_\varphi$), soluble complex and insoluble complexes exist, respectively. The phase diagram is different from that for BSA/SBP,[42] where the main driving force for the formation of soluble complex is electrostatic interactions. Only when the solution pH is close to or below the IEP can soluble complexes then be formed. For the SC/SBP system, soluble complexes can be formed at a higher pH (5.6) than the IEP of SC (4.6), where hydrophobic associations occur but with net electrostatic repulsive interactions. However, without electrostatic attraction between SBP and SC, SBP cannot adsorb on to the surface of the caseinate to inhibit caseinate molecules from further aggregation. The heterogeneous charge distribution in SC micelles could account for the formation of electrostatic complexation at high pH's. As mentioned above, negatively charged macropeptides of κ-casein can extend from the micellar surface to create a hairy layer, with the remainder of the molecule carrying positive charges covered by the hairy layer.[43,44] When the hairy layer collapses, SC/SBP complexes can form through electrostatic attraction between the negatively charged SBP and the exposed positive patches on the surface of the caseinate aggregates as occurs during complexation between sodium caseinate and gum arabic.[20] Thus, both hydrophobic interactions and electrostatic interactions contribute to complexation between SC and SBP. Moreover, the two complexes have different structures. Small BSA molecules can adsorb on the segments of a SBP molecule and form an intramolecular complex. However, SBP would adsorb on the outside of the casein micelle.[20]

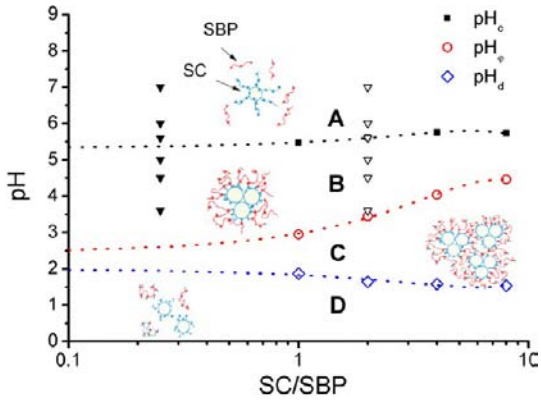

Figure 3. Phase diagram of complexation between SC and SBP. "▼" and "▽" represent mixed solutions with 4:1 and 2:1, respectively, for emulsion preparation.

3.3. Application of Complexation in Emulsions. To identify the best conditions to improve the stability of SBP-stabilized emulsions with SC, emulsions were prepared in different regions of the phase diagram. Figure 3 shows the emulsions prepared with SC/SBP ratio of 2:1 or 1:4 at pH's 7.0, 6.0, and 5.6 located in region A, and also at pH's 5.0, 4.5, and 3.6 located in region B.

3.3.1. Emulsions Prepared with 1.5 wt % Mixed SC/SBP Solution at SC/SBP Ratios of 2:1. SC/SBP mixed emulsions containing 0.5 wt % SBP and 1.0 wt % SC at various pH's have two distinct size distributions (Figure 4a). At pH's 7.0, 6.0, and 5.6 and at pH's 5.0, 4.5 and 3.6, the size distributions are similar with an average droplet size of 0.22 μm for the former and above 7 μm for the latter group. Below pH 5.6 complexes can be formed (see Figure 2c), and this accounts for the increase in droplet size. Figure 4b compares the size distributions of three emulsions prepared at pH 7.0 with 0.5 wt % SBP, 1.0 wt % SC, and a mixture of 0.5 wt % SBP and 1.0 wt % SC, respectively. The behavior of the mixed system mirrors that of the SC-stabilized emulsion, indicating that the mixed emulsion is dominated by the caseinate molecules. The size distribution of SC-stabilized emulsion is bimodal, and emulsion droplets of size >2 μm can be clearly seen from the distribution, which suggests that even 1% sodium caseinate is not enough to produce a good emulsion. The stability of these mixed emulsions was evaluated by accelerated testing at 60 °C for 1 week (Figure 4c). The emulsions at pH's 5.6 and 5.0 have a lower stability than the other emulsions.

dx.doi.org/10.1021/jf304658q | J. Agric. Food Chem. 2013, 61, 1388−1396

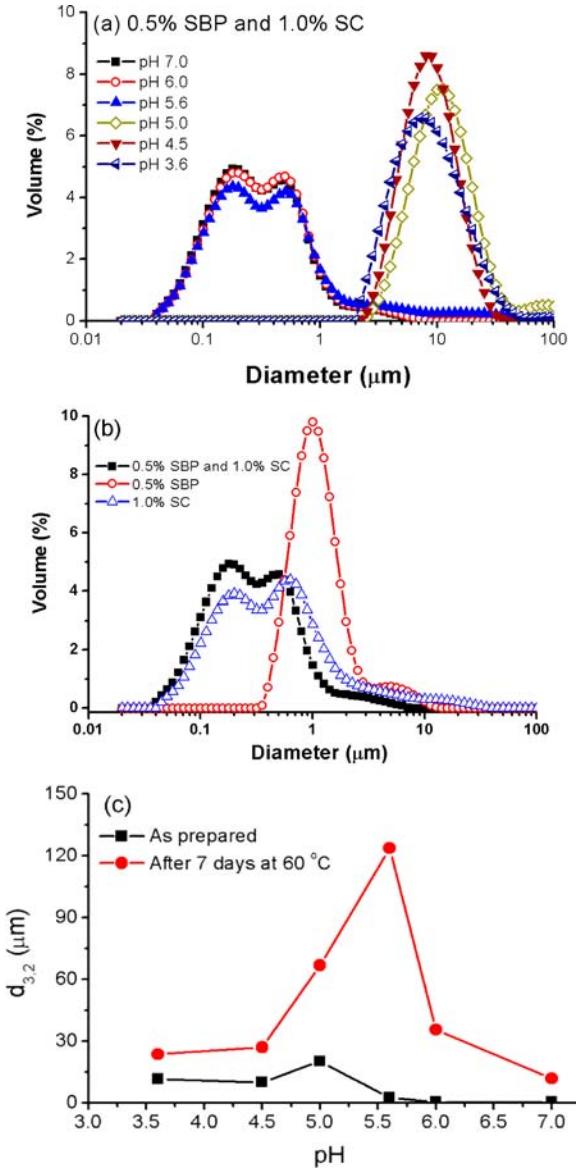

Figure 4. (a) Droplet size distributions for emulsions containing 0.5 wt % SBP and 1.0 wt % SC at various pH's. (b) Size distributions of three emulsions prepared at pH 7.0 with 0.5 wt % SBP, 1.0 wt % SC, and a mixed solution of 0.5 wt % SBP and 1.0 wt % SC, respectively. (c) Comparison of $d_{4,3}$ values for emulsions containing 0.5 wt % SBP and 1.0 wt % SC as prepared and emulsions after heating at 60 °C for 7 days.

Figure 5 shows the micrographs for these freshly made mixed emulsions, which consist of small droplets. Small-scale flocculation occurs at or above pH 5.6, while large-scale flocculation is observed below pH 5.6. Depletion flocculation is probably above pH 5.6 as found when a small amount of nonadsorbing polysaccharides such as pectin[13] or guar gum[45] is added to a SC-coated emulsion at neutral pH. Pectin and caseinate micelles are thermodynamically incompatible above pH 5.6.[21,46] Because of thermodynamic incompatibility, SBP molecules could similarly become depleted from the adsorbed SC layer, and when the depletion layers overlap, the effective osmotic pressure in the overlapped volume is lowered, resulting in an effective attraction between the two droplets. The effective attraction would then cause depletion flocculation. Large-scale flocculation was also reported in SC-coated

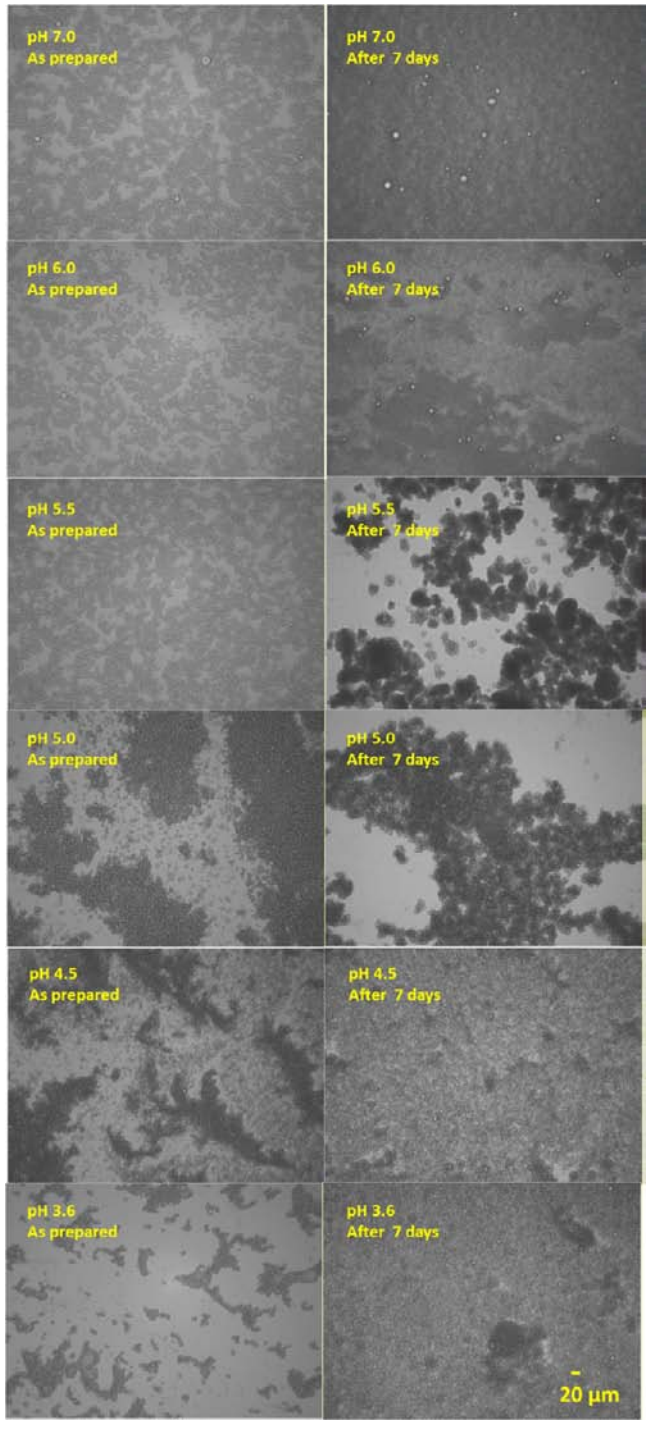

Figure 5. Micrographs for fresh emulsions and emulsions following acceleration test by incubating at 60 °C for 7 days at different pH's containing 0.5 wt % SBP and 1.0 wt % SC.

emulsion after very small added amounts of pectin or sodium alginate at acidic pH.[13,19] The amount of polysaccharide added was then not sufficient to saturate the droplet surface, such that one polysaccharide chain was shared by two or more droplets, leading to bridging flocculation. Also, the negative charges carried by adsorbed polysaccharides neutralized the positive charges on the adsorbed protein molecules at acidic pH's, leading to a significant decrease in the emulsion electrostatic stability promoting further flocculation. The large-scale aggregation in the mixed SC/SBP emulsion below pH 5.6

dx.doi.org/10.1021/jf304658q | J. Agric. Food Chem. 2013, 61, 1388−1396

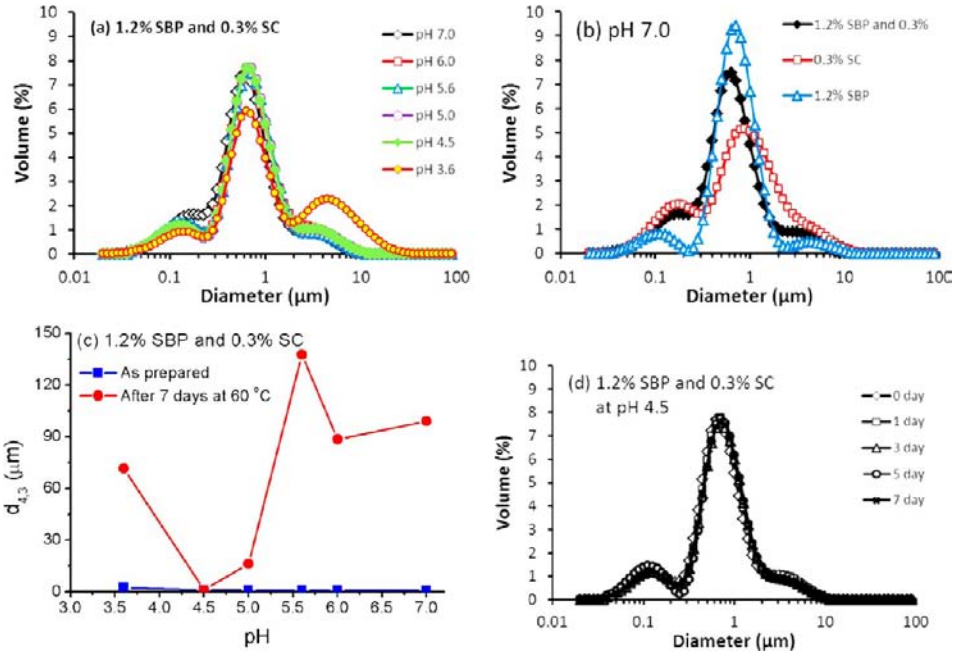

Figure 6. (a) Droplet size distributions for emulsions containing 1.2 wt % SBP and 0.3 wt % SC at various pH's. (b) Comparison in droplet size distributions of emulsions prepared at pH 7.0 with 1.0 wt % SBP, 0.3 wt % SC, and a mixed solution of 1.2 wt % SBP and 0.3 wt % SC, respectively. (c) Comparison of $d_{4,3}$ data for mixed emulsions "as prepared" and after 7 days and (d) changes in the size distributions of the mixed emulsion at pH 4.5 during heating at 60 °C for 7 days.

might then also be attributed to bridging flocculation and charge neutralization. It should be pointed out that self-aggregation of the adsorbed SC molecules on different droplets might also occur. The micrographs of the emulsions after 7 days at 60 °C (Figure 5) confirm the rapid increase in droplet size due to bridging flocculation, charge neutralization, and/or hydrophobic aggregation of caseinate molecules. The emulsions at other pH's have a slightly better stability than at pH's 5.6 and 5.0 but are still not stable enough for practical applications.

3.3.2. Emulsions Prepared with 1.5 wt % Mixed SC/SBP Solution at SC/SBP Ratios of 1:4. Figure 6a shows the size distributions of emulsions containing 1.2 wt % SBP and 0.3 wt % SC at various pH's. These mixed emulsions have similar size distributions except the emulsions at pH 3.6. It is well established that emulsions stabilized by sodium caseinate become unstable due to increased hydrophobic interactions with decreasing pH.[18] When emulsion pH is below IEP of SC (pH 5.6), large-scale aggregation of emulsion droplets occurs, leading to an appearance of precipitation, which is prevented at this condition. Increased proportions of SBP might suppress self-aggregation of caseinate molecules at pH < 5.6, forming complexes that favor the formation of good emulsions. The result also implies that the electrostatic stability of SBP has an important influence on the mixed emulsions. Above 4.5, SBP molecules are fully charged, and the strong electrostatic stability could prevent bridging flocculation. Below pH 4.5, the electrostatic stability is reduced significantly due to protonation of SBP (see Figure 1), leading to the formation of flocs at pH 3.6. Figure 6b shows a comparison of the size distributions of the three emulsions prepared at pH 7.0 containing, respectively, 1.2 wt % SBP, 0.3 wt % SC, and a mixture of 1.2 wt % SBP and 0.3 wt % SC. The mixture seems only a superimposition of the other two emulsions, which indicates that both SBP and SC would contribute to the mixed emulsion performance. Recently, we investigated the competitive adsorption between SBP and hydroxypropyl methylcellulose (HPMC) at the oil/water interface.[36] At only 0.1 wt %, HPMC can dominate the emulsion properties even in the presence of 1.5 wt % SBP, indicating the weak adsorption ability of SBP. Also, it was reported the maximum adsorbed SBP concentrations in emulsions are only about 0.2 wt %.[9,14] Thus, even a small amount of SC could then promote emulsification.

The stability of these mixed emulsions was also determined by heating at 60 °C for 7 days. Figure 6c shows the comparison of $d_{4,3}$ values for these mixed emulsions "as prepared" and after 7 days. Although the solution pH has no significant influence on the emulsification properties, the stability is significantly affected. The emulsions at pH's 5.6 had the worst stability and at 4.5 the best. The size distribution remains unchanged, which indicates a strong stability of the emulsion at pH 4.5 (Figure 6d). The micrographs shown in Figure 7 showed large flocs in the emulsions at pH's 7.0, 6.0, and 5.6 after 7 days at 60 °C, which indicates that the SC concentration used in emulsification is too low to prevent the coalescence of the emulsion droplets. The emulsions at pH's 5.0, 4.5, and 3.6 have relatively good stability with only a few large particles forming after heating at 7 days at 60 °C. The stability of emulsions stabilized by only 1.5% SBP at various pH's has been reported recently by our group.[30] The droplet size distribution changed significantly after 1 week acceleration at 60 °C, as the average diameter of emulsion droplets increased from 0.75 to 5.0 μm. Therefore, it can be concluded that the stability of SBP-stabilized emulsions can be improved by small addition of SC at acidic pH's. Pallandre et al.[19] also found that good SC-coated emulsions could only be produced when the concentration of added sodium alginate was close to or larger than that of SC, which is consistent with our findings.

Overall, good stable emulsions can then only be made at low SC/SBP ratios. On the basis of our results, a schematic diagram

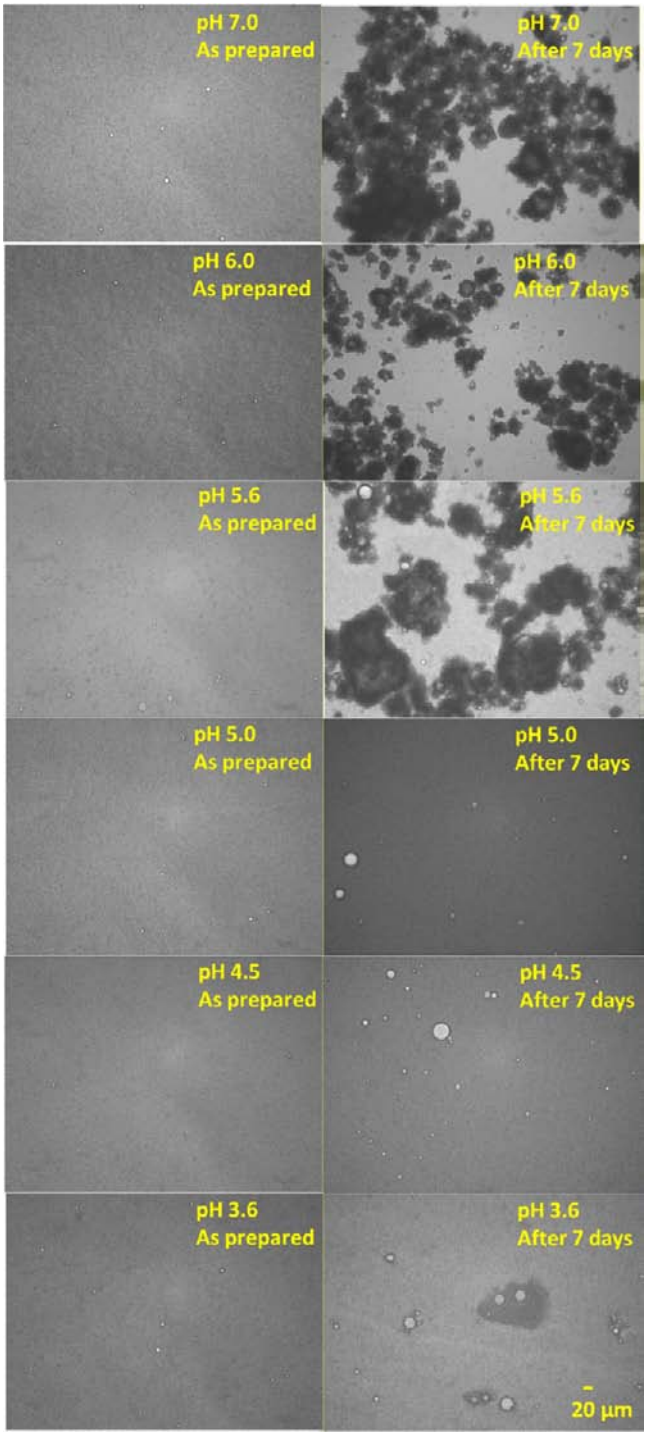

Figure 7. Micrographs for the SC/SBP mixed emulsions containing 1.2 wt % SBP and 0.3 wt % SC at various pH's after 7 days at 60 °C.

of interfacial structures for various emulsions is proposed in Figure 8.

We propose that both α- and β-caseins are adsorbed on the oil droplet surface and covered by κ-caseins to give caseinate micelle like structure as shown in Figure 8. Above pH 5.6, SBP and SC are thermodynamically incompatible, leading to a competitive adsorption. The result of competitive adsorption depends on their relative adsorbed amounts and surface activity.[36] In the presence of large concentrations of SC, for example, the SC/SBP mixed emulsion containing 1.0 wt % SC and 0.5 wt % SBP at pH 7.0, the amount of adsorbed SC is more than that of adsorbed SBP because of the strong adsorption ability of SC. Therefore, SC dominates the overall emulsion properties. For SBP, it is difficult to adsorb onto the adsorbed SC layer because of thermodynamic incompatibility as shown in Figure 8a. The nonadsorbing SBP molecules in the emulsion induce depletion flocculation and reduce the emulsion stability. In the presence of a small amount of SC, for example, the SC/SBP mixed emulsions containing 0.3 wt % and 1.2 wt % at pH 7.0, the mixed emulsions are controlled by both SC and SBP because the adsorbed amounts of SC and SBP are similar as illustrated in Figure 8b. The small amount of adsorbed polymer cannot prevent coalescence. Below pH 5.6, SBP and SC may form complexes through electrostatic attractions between them. For the emulsions having a higher SC/SBP ratio, such as the SC/SBP mixed emulsion containing 1.0 wt % SC and 0.5 wt % SBP at pH 4.5, the small amount of SBP cannot completely cover the adsorbed layer. Therefore, large-scale bridging flocculation occurs due to self-aggregation of adsorbed SC molecules on the different droplets as illustrated in Figure 8c. However, for the emulsions having a lower SC/SBP ratio, for example, the SC/SBP mixed emulsions containing 0.3 wt % and 1.2 wt % at pH 4.5, the adsorbed SC is covered completely, as shown in Figure 8d. At this condition the bridging flocculation (Figure 5), coalescence (Figures 4c, 6c, and 7), and self-aggregation (Figure 6a) are reduced significantly or eliminated. More importantly, more SBP molecules may be adsorbed due to cooperative adsorption with adsorbed caseinate molecules as found in the BSA/SBP system,[34] leading to the improvement of the emulsion stability. In the BSA/SBP system, we successfully determined the amounts of adsorbed SBP and BSA using GPC. It was found that the amount of adsorbed SBP was increased significantly as compared to the emulsion stabilized by SBP only at acidic pH. However, the same method could not be applied to the SC/SBP system due to the overlap of SBP and SC peaks.

SBP has advantages over other polysaccharides in forming protein/polysaccharide complexes to prepare emulsions. First, SBP extends the pH range for producing stable emulsions. For most protein/polysaccharide systems such as SC/LM-pectin[13] and SC/guar gum,[45] high ratios of protein/polysaccharide mixtures have to be used to produce good and low viscosity emulsions. As shown in the phase diagram of complexation, a little change in pH at high protein/polysaccharide ratios easily forces the system to enter region A (soluble polymers) or C (insoluble complexes), where competitive adsorption or large-scale bridging flocculation occurs, thus decreasing the emulsion stability. The high emulsifying ability of SBP enables low ratios of protein/polysaccharide blends to be used, extending the range of region B (soluble complexes). Second, unlike other polysaccharides such as LM-pectin or gelation, SBP has weak aggregating ability at acidic pH's because of the high content of acetyl groups, and therefore can provide better steric stability at low pH's.

In this study, acid-unstable protein sodium caseinate was used to improve the stability of emulsions stabilized by SBP. Unlike previously reported systems such as acid-stable protein BSA,[34,35] hydrophobic interactions are also involved in the complexation between SC and SBP, and stable emulsions can be obtained with low ratios of SC/SBP blends at acidic pH's ($4.5 < pH < 5.6$). Acidic pH's can increase the amount of adsorbed SBP through cooperative adsorption with SC. Moreover, high contents of SBP can cover completely the

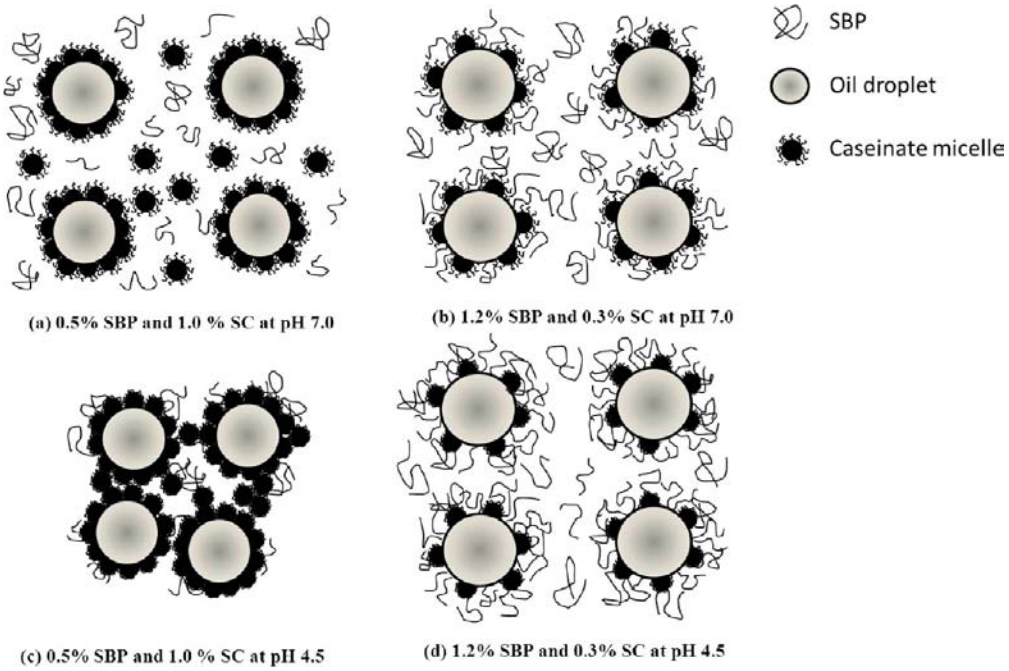

Figure 8. Schematic illustration of possible interfacial structures for emulsions prepared at pH 7.0 with (a) a mixed solution of 0.5 wt % SC and 1.0 wt % SBP or (b) a mixed solution of 0.3 wt % SC and 1.2 wt % SBP and emulsions prepared at pH 4.5 with (c) a mixed solution of 0.5 wt % SC and 1.0 wt % SBP and (d) a mixed solution of 0.3 wt % SC and 1.2 wt % SBP.

adsorbed caseinate layer on the oil/water interface, preventing instability induced by hydrophobic aggregation of sodium caseinate at low pH's.

AUTHOR INFORMATION

Corresponding Author

*Tel.: +44 (0)1978 29 3321. Fax: +44 1978 (0)293370. E-mail: s.alassaf@glyndwr.ac.uk.

Notes

The authors declare no competing financial interest.

We acknowledge financial support from Phillips Hydrocolloids Research Ltd. (U.K.) and the tuition fees waiver awarded to X.L. by Glyndwr University Wrexham.

REFERENCES

(1) Mohnen, D. Pectin structure and biosynthesis. *Curr. Opin. Plant Biol.* **2008**, *11*, 266−277.

(2) Voragen, A. G. J.; Pilnik, W.; Thibault, J.-F.; Axelos, M. A. V.; Renard, C. M. G. C.; Stephen, A. M. Pectins. *Food Sci. Technol.* **1995**, *67*, 287−339.

(3) Thakur, B. R.; Singh, R. K.; Handa, A. K. Chemistry and uses of pectin - A review. *Crit. Rev. Food Sci.* **1997**, *37*, 47−73.

(4) Fishman, M. L.; Chau, H. K.; Cooke, P. H.; Hotchkiss, A. T., Jr. Global structure of microwave-assisted flash-extracted sugar beet pectin. *J. Agric. Food Chem.* **2008**, *56*, 1471−1478.

(5) Iglesias, M. T.; Lozano, J. E. Extraction and characterization of sunflower pectin. *J. Food Eng.* **2004**, *62*, 215−223.

(6) Kirby, A. R.; MacDougall, A. J.; Morris, V. J. Atomic force microscopy of tomato and sugar beet pectin molecules. *Carbohydr. Polym.* **2008**, *71*, 640−647.

(7) Gromer, A.; Kirby, A. R.; Gunning, A. P.; Morris, V. J. Interfacial structure of sugar beet pectin studied by atomic force microscopy. *Langmuir* **2009**, *25*, 8012−8018.

(8) Williams, P. A.; Sayers, C.; Viebke, C.; Senan, C.; Mazoyer, J.; Boulenguer, P. Elucidation of the emulsification properties of sugar beet pectin. *J. Agric. Food Chem.* **2005**, *53*, 3592−3597.

(9) Funami, T.; Zhang, G. Y.; Hiroe, M.; Noda, S.; Nakauma, M.; Asai, I.; Cowman, M. K.; Al-Assaf, S.; Phillips, G. O. Effects of the proteinaceous moiety on the emulsifying properties of sugar beet pectin. *Food Hydrocolloids* **2007**, *21*, 1319−1329.

(10) Siew, C. K.; Williams, P. A. Characterization of the surface-active components of sugar beet pectin and the hydrodynamic thickness of the adsorbed pectin layer. *J. Agric. Food Chem.* **2008**, *56*, 8111−8120.

(11) Nakauma, M.; Funami, T.; Noda, S.; Ishihara, S.; Al-Assaf, S.; Nishinari, K.; Phillips, G. O. Comparison of sugar beet pectin, soybean soluble polysaccharide, and gum arabic as food emulsifiers. 1. Effect of concentration, pH, and salts on the emulsifying properties. *Food Hydrocolloids* **2008**, *22*, 1254−1267.

(12) Rangsansarid, J.; Fukada, K. Factors affecting the stability of O/W emulsion in BSA solution: Stabilization by electrically neutral protein at high ionic strength. *J. Colloid Interface Sci.* **2007**, *316*, 779−786.

(13) Surh, J.; Decker, E. A.; McClements, D. J. Influence of pH and pectin type on properties and stability of sodium-caseinate stabilized oil-in-water emulsions. *Food Hydrocolloids* **2006**, *20*, 607−618.

(14) Siew, C. M.; Williams, P. A. Role of protein and ferulic acid on the emulsification properties of sugar beet pectin. *J. Agric. Food Chem.* **2008**, *56*, 4164−4171.

(15) Funami, T. Z. G.; Noda, S.; Nakauma, M.; Asai, I.; Al-Assaf, S. P. G. O. Does pectin emulsification mechanism operate via an AGP (arabinogalactan protein) type fraction? *Foods Food Ingredients J. Jpn.* **2006**, *211*, 255−263.

(16) Kirby, A. R.; MacDougall, A. J.; Morris, V. J. Sugar beet pectin-protein complexes. *Food Biophys.* **2006**, *1*, 51−56.

(17) Funami, T. N. M.; Noda, S.; Ishihara, S.; Al-Assaf, S.; Phillips, G. O. Enhancement of the performance of sugar beet pectin as an emulsifier. *Foods Food Ingredients J. Jpn.* **2008**, *213*, 346.

(18) Ye, A. Q. Complexation between milk proteins and polysaccharides via electrostatic interaction: principles and applications - a review. *Int. J. Food Sci. Technol.* **2008**, *43*, 406−415.

(19) Paliandre, S.; Decker, E. A.; McClements, D. J. Improvement of stability of oil-in-water emulsions containing caseinate-coated droplets by addition of sodium alginate. *J. Food Sci.* **2007**, *72*, 518−524.

(20) Ye, A. Q.; Flanagan, J.; Singh, H. Formation of stable nanoparticles via electrostatic complexation between sodium caseinate and gum arabic. *Biopolymers* **2006**, *82*, 121−133.
(21) Rediguieri, C. F.; de Freitas, O.; Lettinga, M. P.; Tuinier, R. Thermodynamic incompatibility and complex formation in pectin/caseinate mixtures. *Biomacromolecules* **2007**, *8*, 3345−3354.
(22) Tromp, R. H.; de Kruif, C. G.; van Eijk, M.; Rolin, C. On the mechanism of stabilisation of acidified milk drinks by pectin. *Food Hydrocolloids* **2004**, *18*, 565−572.
(23) Jensen, S.; Rolin, C.; Ipsen, R. Stabilisation of acidified skimmed milk with HM pectin. *Food Hydrocolloids* **2010**, *24*, 291−299.
(24) de Kruif, C. G.; Weinbreck, F.; de Vries, R. Complex coacervation of proteins and anionic polysaccharides. *Curr. Opin. Colloid Interface Sci.* **2004**, *9*, 340−349.
(25) Weinbreck, F.; Nieuwenhuijse, H.; Robijn, G. W.; de Kruif, C. G. Complexation of whey proteins with carrageenan. *J. Agric. Food Chem.* **2004**, *52*, 3550−3555.
(26) Vinayahan, T.; Williams, P. A.; Phillips, G. O. Electrostatic interaction and complex formation between gum arabic and bovine serum albumin. *Biomacromolecules* **2010**, *11*, 3367−3374.
(27) Mekhloufi, G.; Sanchez, C.; Renard, D.; Guillemin, S.; Hardy, J. pH-induced structural transitions during complexation and coacervation of beta-lactoglobulin and acacia gum. *Langmuir* **2005**, *21*, 386−394.
(28) Turgeon, S. L.; Schmitt, C.; Sanchez, C. Protein-polysaccharide complexes and coacervates. *Curr. Opin. Colloid Interface Sci.* **2007**, *12*, 166−178.
(29) de Kruif, C. G.; Tuinier, R. Polysaccharide protein interactions. *Food Hydrocolloids* **2001**, *15*, 555−563.
(30) Turgeon, S. L.; Beaulieu, M.; Schmitt, C.; Sanchez, C. Protein-polysaccharide interactions: phase-ordering kinetics, thermodynamic and structural aspects. *Curr. Opin. Colloid Interface Sci.* **2003**, *8*, 401−414.
(31) Dickinson, E.; Rolfe, S. E.; Dalgleish, D. G. Competitive adsorption of alpha(s1)-casein and beta-casein in oil-in-water emulsions. *Food Hydrocolloids* **1988**, *2*, 397−405.
(32) Dickinson, E.; Rolfe, S. E.; Dalgleish, D. G. Competitive adsorption in oil-in-water emulsions containing alpha-lactalbumin and beta-lactoglobulin. *Food Hydrocolloids* **1989**, *3*, 193−203.
(33) Damodaran, S.; Sengupta, T. Dynamics of competitive adsorption of alpha(s)-casein and beta-casein at planar triolein-water interface: Evidence for incompatibility of mixing in the interfacial film. *J. Agric. Food Chem.* **2003**, *51*, 1658−1665.
(34) Li, X.; Fang, Y.; Al-Assaf, S.; Phillips, G. O.; Jiang, F. Complexation of bovine serum albumin and sugar beet pectin: stabilizing oil-in-water emulsions. *J. Colloid Interface Sci.* **2012**, *388*, 103−111.
(35) Li, X.; Fang, Y.; Al-Assaf, S.; Phillips, G. O.; Yao, X.; Zhang, Y.; Zhao, M.; Zhang, K.; Jiang, F. Complexation of bovine serum albumin and sugar beet pectin: Structural transitions and phase diagram. *Langmuir* **2012**, *28*, 10164−10176.
(36) Li, X.; Al-Assaf, S.; Fang, Y.; Phillips, G. O. Competitive adsorption between sugar beet pectin (SBP) and hydroxypropyl methylcellulose (HPMC) at the oil/water interface. *Carbohydr. Polym.* **2013**, *91*, 573−580.
(37) Akhtar, M.; Dickinson, E.; Mazoyer, J.; Langendorff, V. Emulsion stabilizing properties of depolymerized pectin. *Food Hydrocolloids* **2002**, *16*, 249−256.
(38) Anal, A. K.; Tobiassen, A.; Flanagan, J.; Singh, H. Preparation and characterization of nanoparticles formed by chitosan-caseinate interactions. *Colloids Surf., B* **2008**, *64*, 104−110.
(39) Kuuva, T.; Lantto, R.; Reinikainen, T.; Buchert, J.; Autio, K. Rheological properties of laccase-induced sugar beet pectin gels. *Food Hydrocolloids* **2003**, *17*, 679−684.
(40) Oosterveld, A.; Beldman, G.; Searle-van Leeuwen, M. J. F.; Voragen, A. G. J. Effect of enzymatic deacetylation on gelation of sugar beet pectin in the presence of calcium. *Carbohydr. Polym.* **2000**, *43*, 249−256.
(41) Chen, J. S.; Dickinson, E.; Edwards, M. Rheology of acid-induced sodium caseinate stabilized emulsion gels. *J. Texture Stud.* **1999**, *30*, 377−396.
(42) Li, X.; Fang, Y.; Al-Assaf, S.; Phillips, G. O.; Yao, X.; Zhang, Y.; Zhao, M.; Zhang, K.; Jiang, F. Complexation of bovine serum albumin and sugar beet pectin: Structural transitions and phase diagram. *Langmuir* **2012**, *28*, 10164−10176.
(43) Dalgleish, D. G.; Corredig, M.; Doyle, M. P.; Klaenhammer, T. R. The structure of the casein micelle of milk and is changes during processing. *Annu. Rev. Food Sci. Technol.* **2012**, *3*, 449−467.
(44) Griffin, M. C. A.; Roberts, G. C. K. A H-1-NMR study of casein micelles. *Biochem. J.* **1985**, *228*, 273−276.
(45) Neirynck, N.; Van lent, K.; Dewettinck, K.; Van der Meeren, P. Influence of pH and biopolymer ratio on sodium caseinate-guar gum interactions in aqueous solutions and in O/W emulsions. *Food Hydrocolloids* **2007**, *21*, 862−869.
(46) Maroziene, A.; de Kruif, C. G. Interaction of pectin and casein micelles. *Food Hydrocolloids* **2000**, *14*, 391−394.