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Kinetics of Formation and Functional Properties of Conjugates Prepared by Dry-State Incubation of β-Lactoglobulin/Acacia Gum Electrostatic Complexes

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The formation of conjugates between β-lactoglobulin and acacia gum based on electrostatic complexes formed at pH 4.2 was investigated upon dry-state incubation for up to 14 days at 60 °C and 79% relative humidity (RH). By means of SEC-HPLC and RP-HPLC, it was shown that the β-lactoglobulin incubated alone was able to form polymers with molecular masses higher than 200 kDa until 50% of the initial monomeric protein disappeared after 14 days. In the presence of acacia gum at initial protein to polysaccharide weight mixing ratios of 2:1 and 1:2, only 35% of the initial β-lactoglobulin monomers disappeared after 14 days. Using RP-HPLC, an apparent reaction order of 2 was found for the disappearance of monomeric β-lactoglobulin both in the presence or absence of acacia gum. However, the reaction rate was faster in the absence of acacia gum. SDS−PAGE electrophoresis with silver staining confirmed the formation of β-lactoglobulin/acacia gum conjugates. The solubility curves of the incubated β-lactoglobulin showed a minimum around pH 4−5. By contrast, the minimum of solubility of the β-lactoglobulin/acacia gum incubated mixtures shifted to lower pH values compared to initial mixtures. The conjugates exhibited higher foam capacity than the incubated protein as well as lower equilibrium air/water surface tension. Conjugation at ratio 1:2 led to increased interfacial viscosity (300 mN s m^{-1} at 0.01 Hz) compared to β-lactoglobulin alone (100 mN s m^{-1} at 0.01 Hz), but similar interfacial elasticity (30−40 mN m^{-1}). The foam capacity of the conjugates was significantly higher than that of the incubated β-lactoglobulin as well as foam expansion and drainage time, especially at pH 5.3, i.e., higher than the pH of formation of the conjugates.

KEYWORDS: Dry-state incubation; reaction kinetics; protein polymerization; solubility; interfacial properties; foaming properties

INTRODUCTION

Proteins often constitute a key ingredient in processed foods. This is mainly due to their versatile functional properties, including solubility, heat-gelation, emulsification, and foaming (*1*). The chemical composition and the secondary structure of the proteins are primarily responsible for their functional properties. In that respect, globular proteins have shown high capacity to absorb at the air/water interface in order to decrease surface tension and to build interfacial viscoelastic networks after unfolding (*2*). Whey proteins are part of these highly surface-active proteins, and β-lactoglobulin, which represents 50% of the total mass of the whey proteins, is the most widely used (*3*, *4*). β-Lactoglobulin is known to form thick interfacial layers close to its p*I* (5.2), leading to stable foams (*2*, *3*, *5*). It has been shown that partial unfolding of the β-lactoglobulin through heat treatment improved its foaming properties at neutral pH (*6*−*8*). The main limitation of this heat treatment is that it leads to very strong aggregation at pH close to p*I* (hydrophobically mediated aggregation due to overcoming of repulsive electrostatic interactions), leading in the formation of covalently bound protein aggregates (S−S bridging).

A promising way to modulate the heat-denaturation of globular proteins, and thereby their interfacial properties, is to mix them with charged polysaccharides in order to build electrostatic complexes (*9*, *10*). The formation of these complexes is known to improve the heat stability of the proteins but also to improve functionality due to a synergistic effect of the hydrophilic polysaccharide (*11*−*15*). It was recently shown that complexes made of β-lactoglobulin and acacia gum, an anionic arabinogalactan−protein polysaccharide, at pH 4.2 and a ratio of 2:1 exhibited higher interfacial and foam-stabilizing properties compared to β-lactoglobulin alone (*16*). The main limitation in using these electrostatic complexes is their sensitivity regarding pH and ionic strength variation (*17*).

Some authors have proposed to render the electrostatic complex covalent by subsequent dry-heating of the protein−polysaccharide mixture (*18*−*23*). The conjugates that are formed exhibit high interfacial properties. As the formation of the

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conjugates by the Maillard reaction involves the interaction between an amino group from the protein and an reducing aldehyde group from the polysaccharide, it has been hypothesized that a higher yield of conjugation was obtained under conditions where the functional groups from the biopolymers would be sterically close one from the other (*24*−*26*). As the electrostatic complexation between β-lactoglobulin and acacia gum is mainly involving these functional groups (*27*), this study aims at investigating the formation of β-lactoglobulin−acacia gum conjugates made from dry-heating of initial electrostatic complexes built under conditions of strong interaction between the two biopolymers.

MATERIALS AND METHODS

Materials. β-Lactoglobulin (BioPURE, lot JE 001-1-922) was obtained from Davisco Foods International, Inc. (Le Sueur, MN). It was purified from sweet whey using filtration and ion exchange chromatography. The powder composition was (g/100 g of powder): 93.2% protein, 4.3% moisture, 0.2% fat, >0.1% lactose, 2.3% ash (0.019% Ca^{2+}, 0.002% Mg^{2+}, 0.009% K^+, 0.848% Na^+, < 0.04% Cl^-). Using RP-HPLC, it was determined that β-lactoglobulin represented 98.2 wt % of the total proteins, α-lactalbumin 1.6%, and BSA 0.2%. The amount of denatured protein in the powder was 3.8% according to the ratio between the amount of soluble protein at the natural pH of reconstitution (6.8) divided by the amount of soluble protein after centrifugation at pH 4.6. Acacia gum (Instant Gum IRX 40693, lot OS-780) was obtained from the Colloïdes Naturels International Co. (CNI, Rouen, France). The polysaccharide was solubilized from the exuded pellets from *Acacia senegal* trees and filtered before spray-drying. The powder composition was (g/100 g of powder): 4% protein, 80.56% polysaccharide, 12.23% moisture, 3.21% ash (0.552% Ca^{2+}, 0.191% Mg^{2+}, 0.698% K^+, 0.041% Na^+, 0.05% Cl^-). From the mineral contents of the powders, considering the tested protein-to-polysaccharide ratios of 2:1 or 1:2 and the highest total biopolymer concentration used (5 wt %), the ionic strength of our samples was in the range from 0.05 to 1 mM equiv NaCl. Such an amount has been shown to have a negligible effect on complex coacervation (*28*). All reagents used were of analytical grade (Merck, Darmstadt, Germany) unless otherwise stated.

Preparation of the β-Lactoglobulin and Acacia Gum Dispersions. Biopolymer dispersions were prepared (5 wt % for β-lactoglobulin and 2.5 wt % for acacia gum, based on a protein or polysaccharide basis, respectively) by dropwise addition of the required amount of powder into 2 L of MilliQ water (resistivity = 18.2 MΩ cm). The pH was then adjusted to 4.2 by addition of 0.1 or 1 M NaOH or HCl. The protein or polysaccharide concentration was adjusted by addition of MilliQ water. Dispersions were centrifuged at 10 000*g* for 1 h (Sorvall RC5C centrifuge, DuPont, Newtown, CT) to discard insoluble matter and release air bubbles. Dispersions were finally filtered on paper filters (filter type $597^1/_2$, Scleicher and Schuell, Germany) before overnight storage at 4 °C. For the following experiments, we investigated protein/polysaccharide mixtures at 2:1 and 1:2 (w/w) ratios, as well as a protein and polysaccharide controls.

Freeze-Drying and Powder Incubation. The β-lactoglobulin dispersion was mixed with the acacia gum at ratio 2:1 and 1:2 and thoroughly stirred for 1 min in order to generate electrostatic complexes (longer stirring time would lead to macroscopic phase separation). These mixtures as well as the protein and acacia gum dispersions were then introduced in glass balloons and dipped in a liquid nitrogen bath for instantaneous freezing and freeze-dried using a Minilyo II apparatus (Secfroid, Aclens, Switzerland). Around 50 g of each powder (β-lactoglobulin, ratio 2:1 and 1:2) was then spread onto glass Petri dishes that were placed into four sealed glass desiccators containing a saturated solution of KBr (100 g in 50 g of MilliQ water). Desiccators were put into a thermostatic oven at 60 °C with air circulation (Salvis, Verrerie de Carouge, Carouge, Switzerland) until the water activity (A_w) of the powder reached 0.79 (Aqualab CX-2, Decagon Devices Inc.). This incubation temperature of 60 °C was chosen in order to be below the denaturation temperature of β-lactoglobulin ($T_d \sim 85$ °C) to avoid extended protein unfolding. The time leading to an A_w of 0.79 in the powders at 60 °C was considered as time 0 for incubation, and powders were further incubated up to 14 days. Every 2 days, 8-g aliquots were taken out to perform chemical analyses and measure functional properties.

It is worth mentioning that a control experiment carried out by incubating the freeze-dried acacia gum at 60 °C and 79% RH (in case the polysaccharide component could react with the protein fraction) did not result in any significant change on the molecular weight of the acacia gum studied by SDS−PAGE electrophoresis. For this reason, incubated acacia gum alone was not further considered in the following of the study.

Determination of Available Free Amino Groups. The amount of available amino groups present on the protein was determined using a modification of the *o*-phthaldialdehyde (OPA) method described earlier by Church et al. (*29*) for milk proteins. The OPA determination was carried out by mixing 200 μL of the protein solution (1.5 g·L^{-1} in 50 mmol·L^{-1} sodium phosphate buffer, pH 7.8), 2 mL of a reducing solution (480 mg of *N*-acetyl-L-cystein dispersed in 200 mL of 0.1 mol·L^{-1} sodium borate buffer, pH 9.3), and 50 μL of a 20% (w/w) SDS solution. Here it is worth noting that the initial reducing compound used for the OPA method was *N*-dimethyl-2-mercaptoethylammonium chloride. However, since this component is no longer commercially available, we replaced it with *N*-acetyl-L-cystein, which has the advantage of being much more time-stable without modifying the reaction (*30*). After mixing and incubation during 10 min at 50 °C, 50 μL of the OPA reagent (prepared by dispersing 170 mg of OPA in 5 mL of methanol) was added. The mixture was further incubated for 30 min at 50 °C and then cooled to room temperature within 30 min before reading the absorbance at 340 nm using an Uvikon 810 spectrophotometer (Flowspek, Basel, CH). The calibration curve was obtained from an L-leucine standard ranging from 0.25 to 2.5 mmol·L^{-1}. All results are mean values resulting from four measurements. Results are expressed as a percentage of the initial available amino groups in the sample. The following products and reagents were used: boric acid, *N*-acetyl-L-cystein, OPA, from Fluka (Chemie GmbH, Buchs, Switzerland); sodium dihydrogen phosphate monohydrate, methanol, and sodium hydroxide from Merck (Darmstadt, Germany); and l-leucine from Sigma (St. Louis, MO).

Reverse-Phase High Performance Liquid Chromatography (RP-HPLC). Incubated protein and protein−polysaccharide mixtures were characterized by reverse-phase high-performance chromatography (RP-HPLC) using the method described by Resmini and co-workers (*31*). This method enables determination of the relative amount of native protein in a sample. The injector system was composed of a Hewlett-Packard series 1050 apparatus equipped with an autosampler and a UV detector at 205 nm. The hydrophobic interaction column 8M-RPS3-124A-115 (150 × 4.6 mm i.d.) was from Polymer Laboratories (Ercatech AG, Bern, Switzerland). It was packed with macroporous polystyrene/divinylbenzene spherical particles (8 μm and 300 Å pores). Elution solvent A was composed of 0.1% TFA in water. Solvent B was 0.1% TFA in 80% acetonitrile. The sample was eluted at 50 °C using the following acetonitrile/water gradient expressed as solvent B proportion: 0−26 min 43%, 26−28 min 63%, 28−35 min 100%, 35−40 min 43%. The flow rate was set to 1 mL min^{-1}. A volume of 20 μL of sample was filtered through 0.45 μm filters (Orange Scientific, Waterloo, Belgium) before injection.

Size Exclusion High Performance Liquid Chromatography (SEC-HPLC). The samples were characterized by size-exclusion high-performance chromatography. This technique allows determining the relative modification of the conformation and molecular weight of proteins through chromatography with a column with defined porosity. The injector system was composed of a Hewlett-Packard series 1050 apparatus equipped with an autosampler and a UV detector at 214 nm. The SEC column TSK-GEL $G2000SW_{XL}$ (300 × 7.8 mm i.d.) was from TosoHaas GmbH (Stuttgart, Germany). It was packed with macroporous silica spherical particles (5 μm and 250 Å pores). The column was calibrated with a series of protein standards having molecular weights ranging from 181 to 660 000 g mol^{-1}. Elution was performed in isocratic mode using a 0.3 M NaCl, 0.05 M phosphate buffer, pH 6.8 solution at a flow rate of 0.5 mL min^{-1}. A volume of

20 μL of sample was filtered through 0.45 μm filters (Orange Scientific, Waterloo, Belgium) before injection.

Gel Electrophoresis. The modification of the molecular weight of the β-lactoglobulin and the formation of conjugates were investigated by gel electrophoresis. Samples were analyzed using NuPAGE Novex 3−8% Tris-acetate gels (Invitrogen) having a molecular mass resolution comprised of 36−400 kDa. Sample protein concentration was 0.3 mg·mL^{-1}, and reduction treatment was achieved by adding 50 μL of sample buffer (LDS) and 10 μL of Nu-PAGE sample reducing agent (NP0004) to 50 μL of protein solution followed by incubation for 2−5 min at 85 °C. After incubation, the samples were cooled to 25 °C, and 10 μL of the solutions was loaded on the gel as well as a molecular standard (5 μL) of a reduced unstained electrophoresis calibration kit (17-0446-01, Pharmacia). The running buffer for migration, Tris-acetate SDS running buffer (20×) (LA0041), was diluted 10 times with MilliQ water, and migration was performed at a constant voltage of 125 V, for 60−90 min. Gels were silver stained according to the method described by Hochstrasser and co-workers (*32*).

Solubility. Samples were evaluated for solubility by reconstitution of 1 wt % protein-based dispersions in a 50 mM citrate−phosphate buffer at pH ranging from 3.0 to 7.0. After stirring at room temperature for 1 h, the dispersions were then centrifuged at 3100*g* for 30 min using a Sorvall RC5C centrifuge (Kendro, Carouge, CH). Protein amount was determined before and after centrifugation using the Bradford's colorimetric method (*33*). An aliquot of 40 μL of the protein dispersion was mixed with 2 mL of the Bradford's reagent (Bio-Rad protein assay, Bio-Rad, München, Germany), and absorbance at 595 nm was read after 15 min using an Uvikon 810 spectrophotometer (Flowspek, Basel, CH). Experiments were run in duplicate. Solubility was calculated as being the ratio between the protein content after centrifugation over the initial protein content.

Surface Properties and Foam Stability. *Interfacial Rheology.* Dynamic surface tension ($\sigma_{a/w}$) was measured in β-lactoglobulin and β-lactoglobulin/acacia gum dispersions using a dynamic pendant-drop Tracker tensiometer (ITConcept, Longessaigne, France) as described previously (*34*). Basically, the principle is to form an axisymmetric air bubble at the tip of a needle of a syringe. A computer drives the plunger position of the syringe via a motor drive, into a thermostated optical glass cuvette containing 7 mL of biopolymer dispersion. The image of the bubble is taken from a CCD camera and digitized. The interfacial tension $\sigma_{a/w}$ is calculated by analyzing the profile of the bubble according to the Laplace equation. When equilibrium surface tension was obtained, the dynamic elastic modulus $|E|$ was determined by fluctuating sinusoidally the area of the bubble at a frequency varying from 0.01 to 0.1 Hz and a relative amplitude ($\Delta A/A$) of 0.1 to stay in the linear region of response. $|E| = A(\Delta\sigma_{a/w}/\Delta A) = E' + iE''$ where $\eta_d = E''/\omega$, A is the bubble area (mm^3), $\sigma_{a/w}$ the surface tension (mN m^{-1}), E' the real part of the dilational elasticity (mN m^{-1}), E'' the imaginary part of the dilational elasticity (mN m^{-1}), η_d the dilational viscosity (mN s m^{-1}), and ω the frequency (Hz). Experiments were performed at 24 ± 0.5 °C. The biopolymer concentration was 0.5 wt % and the surface of the bubble was set to 16 mm^2.

Foaming and Foam Stability. Determination of the foaming properties of aqueous dispersions is dependent on the technique and apparatus used. To avoid these problems, we have chosen to use a standardized foaming method developed by Guillerme et al. (*35*). The principle is to foam a defined quantity of solution by gas sparging through a glass frit (porosity and gas flow are controlled). The generated foam rises along a glass column, where its volume is followed by image analysis using a CCD camera. The amount of liquid incorporated in the foam and the foam homogeneity are followed by measuring the conductivity in the cuvette containing the liquid and at different heights in the column by means of electrodes (*36*). The commercial Foamscan apparatus (ITConcept, Longessaigne, France) has been used to perform these experiments. The foaming properties of 0.5 wt % dispersions in 50 mM citrate−phosphate buffer were measured by injecting 20 mL of the dispersions at pH 4.2, 5.3, and 7.0 into the cuvette and sparging air at 80 mL·min^{-1}. This flow rate was found to allow investigations on mixtures at different biopolymer concentrations. The porosity of the glass frit used for testing these foaming properties allows formation of air bubble having diameters comprised between 10 and 16 μm. Bubbling was stopped after a volume of 120 cm^3 of foam was obtained (i.e. two-thirds of the column height). At the end of the bubbling, the foaming capacity ($FC = V_{final-foam}/V_{final-gas}$) and the foam expansion ($FE = V_{final-foam}/V_{initial-liquid} - V_{final-liquid}$) were calculated.[37] Finally, the time stability of the foam was characterized by measuring the time needed for the liquid in the foam to drain by half of its value at the end of bubbling. All experiments were duplicated.

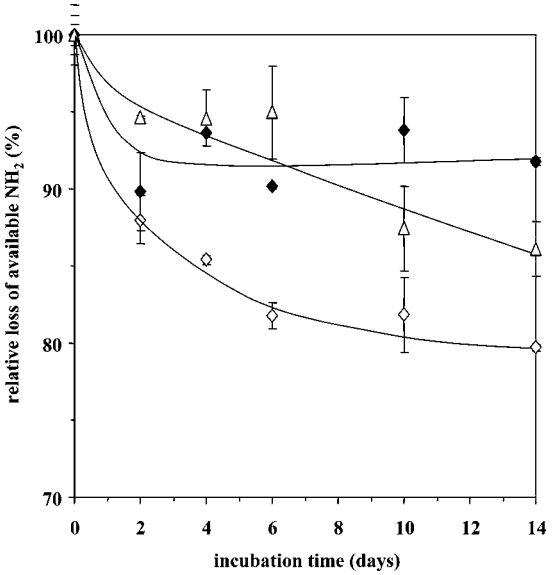

Figure 1. Variation of the available NH_2 groups determined by the OPA method in β-lactoglobulin and β-lactoglobulin/acacia gum powders after incubation from 0 to 14 days at 60 °C and 79% RH (the vertical bars represent the standard deviation): (◇) β-lactoglobulin, (◆) β-lactoglobulin/acacia gum ratio 2:1, and (△) β-lactoglobulin/acacia gum ratio 1:2.

RESULTS AND DISCUSSION

Kinetics of Formation of β-Lactoglobulin/Acacia Gum Conjugates. The kinetics of formation of conjugates was followed using chemical and chromatographic techniques. The amount of free NH_2 remaining on the β-lactoglobulin has been determined as a function of the incubation time (**Figure 1**). Considering the β-lactoglobulin incubated alone, the amount of free NH_2 groups decreased rapidly by almost 20% of the initial value during the first 10 days and reached a plateau value. This result is surprising, as this loss would correspond to an average of three lysine residues per β-lactoglobulin molecule (*38*, *39*). Since the β-lactoglobulin powder contained less than 1% lactose and no browning of the powder was observed after incubation, the initial β-lactoglobulin/lactose ratio should not allow the Maillard reaction to take place (*39*). More likely, another reaction is able to take place within the β-lactoglobulin powder (oxidation, deamidation, unfolding of the protein), leading to an underestimation of the NH_2 via the OPA assay (*38*, *40*). To check this hypothesis, SDS−PAGE gel electrophoresis was conducted under reducing conditions (**Figure 2**). As can be seen in lanes 2−5, corresponding to incubation times from 0 to 10 days, the bands corresponding to the initial β-lactoglobulin are decreasing in intensity with incubation time. In addition, more discrete bands can be observed in the molecular range from 43 to 67 kDa and larger than 94 kDa. As these oligo- or polymeric protein bands were still present on the reduced gel, the polymerization was not due to the classical disulfide bridging that is commonly described for heat-treated whey protein powders (*41*). On the basis of these observations, protein polymerization could be attributed to the formation of

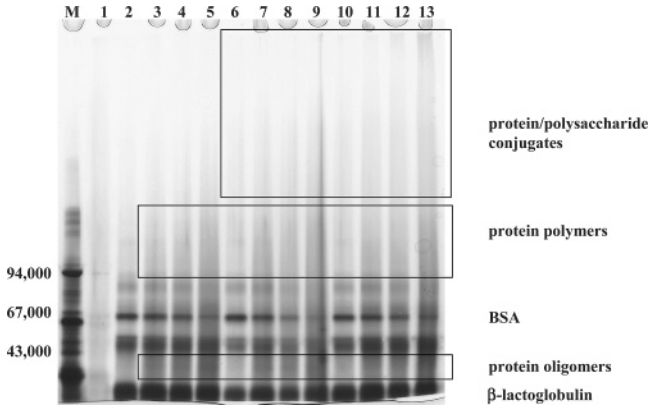

Figure 2. Silver-stained SDS–PAGE electrophoresis gel obtained under reducing conditions for β-lactoglobulin and β-lactoglobulin/acacia gum powders incubated at 60 °C and 79% RH: Lane M, molecular markers; lane 1, acacia gum at day 0; lane 2, β-lactoglobulin at day 0; lane 3, β-lactoglobulin at day 2; lane 4, β-lactoglobulin at day 4; lane 5, β-lactoglobulin at day 10; lane 6, β-lactoglobulin/acacia gum ratio 2:1 at day 0; lane 7, β-lactoglobulin/acacia gum ratio 2:1 at day 2; lane 8, β-lactoglobulin/acacia gum ratio 2:1 at day 4; lane 9, β-lactoglobulin/acacia gum ratio 2:1 at day 10; lane 10, β-lactoglobulin/acacia gum ratio 1:2 at day 0; lane 11, β-lactoglobulin/acacia gum ratio 1:2 at day 2; lane 12, β-lactoglobulin/acacia gum ratio 1:2 at day 4; and lane 13, β-lactoglobulin/acacia gum ratio 1:2 at day 10.

covalent bonding or hydrophobic bridging (*42*, *43*). This polymerization process could modify the conformation of the β-lactoglobulin, leading to a reduced accessibility of some NH_2 groups for the OPA method. A possible chemical reaction that may lead to the decrease of the NH_2 functions in the heated protein sample is deamidation (*44*). Several authors have found that proteins rich in glutamine and asparagine were able to polymerize during dry-heating; however, it was generally at lower water activities than 0.79. This was the case for ovalbumin, which reached a deamidation degree of 12% upon incubation for 10 days at 80 °C under a RH of 5.7% (*43*). Mine (*45*) reported similar deamidation results after dry-heating egg-white proteins at 75 °C and basic pH. To be able to attribute the decrease of the free NH_2 functions from the protein to the formation of conjugates in protein−polysaccharide mixtures, it is thus mandatory to carry out these necessary control experiments.

Similarly to the protein alone, the variation of the free NH_2 content in the β-lactoglobulin−acacia gum mixtures showed a gradual decrease with incubation time (**Figure 1**). However, the extent of NH_2 loss reached after 14 days was lower than for the β-lactoglobulin alone, namely 15% for the 1:2 ratio and 8% for the 2:1 ratio. Thus, it can already be concluded that the presence of acacia gum in the mixture reduces the extent of protein polymerization. Whether this was effectively due to the formation of β-lactoglobulin/acacia gum conjugates or only due to the protective effect of polysaccharide (less water available around protein molecules compared to polysaccharide ones) was checked by SDS−PAGE gel electrophoresis using silver staining. This technique allows simultaneous detection of native and glycated proteins. Lanes 6−13 show the modifications in protein structure in the mixtures incubated from 0 to 10 days (**Figure 2**). Clearly, the intermediate band of protein oligomers that was found for the β-lactoglobulin alone was limited for the two tested ratios, indicating that protein polymerization was reduced by the presence of acacia gum. However, a continuum of species having molecular weights larger than 94 kDa was clearly visible, especially for the ratio of incubation 1:2. In addition, the intensity of the bands increased with the incubation time, meaning that the polymerization reaction was kinetically driven. Interestingly, these polymers were characterized by concomitant brown and yellow colors on the gel, revealing the presence of both protein and polysaccharide. As the reduction of the samples did not allow separating the polymers, it could be fairly concluded that they were β-lactoglobulin/acacia gum conjugates.

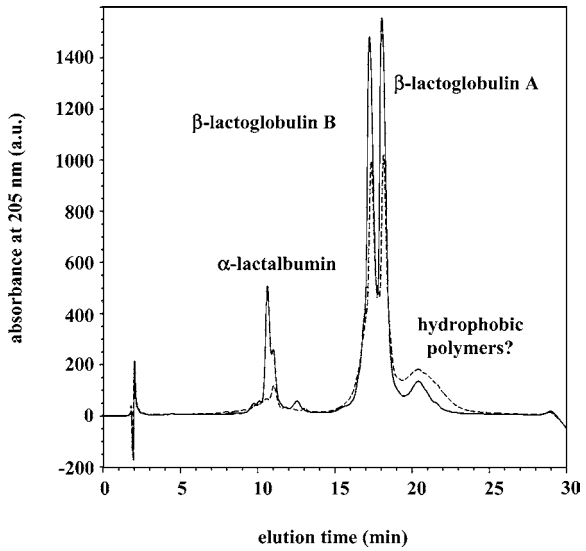

Figure 3. RP-HPLC chromatogram obtained from 0.5 wt % β-lactoglobulin dispersions after incubation of powder for 0 and 14 days at 60 °C and 79% RH: (full line) incubation for 0 day and (dashed line) incubation for 14 days.

As we previously showed that the determination of the free amino groups of the protein using the OPA method could be misleading in following the conjugation reaction, the reaction kinetics was further investigated by means of RP-HPLC. The disappearance of the β-lactoglobulin monomers (variant A and B) was followed as a function of the incubation time. **Figure 3** clearly shows that the initial peaks corresponding to the elution of the two major variants of the β-lactoglobulin decreased in intensity upon incubation. This result confirms above-mentioned interpretations on β-lactoglobulin polymerization when incubated alone. Even α-lactabumin was affected by this loss of native form upon incubation. The kinetics of conversion of the monomeric β-lactoglobulin into oligomers or polymers is reported in **Figure 4**. The conversion rate was higher for β-lactoglobulin than for the two tested mixing ratios. At longer incubation times, the reaction rate seemed to approach a plateau value, resulting in 50% conversion of β-lactoglobulin when incubated alone and 35% when incubated in the presence of acacia gum. The reactions rates for monomer disappearance have been calculated for the three systems by assuming that the apparent order of the reaction was 1, 1.5, or 2. These reaction orders are likely to occur for glycation of proteins as recently described for the lactosylation of α-lactlabumin, where pseudo-orders ranging from 1 to 2 were found (*39*). Some authors reported reactions orders of 2 rather than the expected 1 for the deamidation of caseinate (*46*). In addition, disulfide/sulhydryl exchange occurring during heat treatment of whey proteins is known to follow reaction orders ranging from 1.5 to 2 for β-lactoglobulin and 1 for α-lactalbumin (*47*, *48*). As a result, linear fits giving the highest regression coefficients ($R^2 > 0.96$) have been retained for our set of data. As can be seen in **Figure 5**, the best fit was obtained for a reaction order of 2. Interestingly, reactions rates were similar for the β-lactoglobulin/acacia gum mixtures, $r = 0.04-0.045\ s^{-1}$, but the rate was much

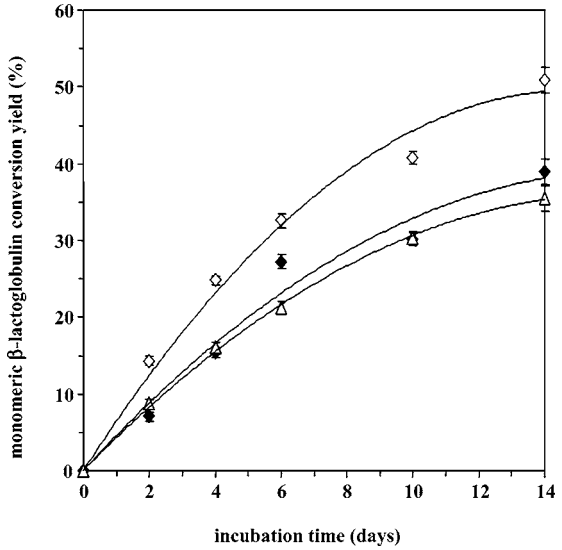

Figure 4. Conversion yield of initial monomeric β-lactoglobulin as determined by RP-HPLC after incubation of powders from 0 to 14 days at 60 °C and 79% RH (the vertical bars represent the standard deviation): (◇) β-lactoglobulin, (◆) β-lactoglobulin/acacia gum ratio 2:1, and (△) β-lactoglobulin/acacia gum ratio 1:2. Lines are only guides for the eyes.

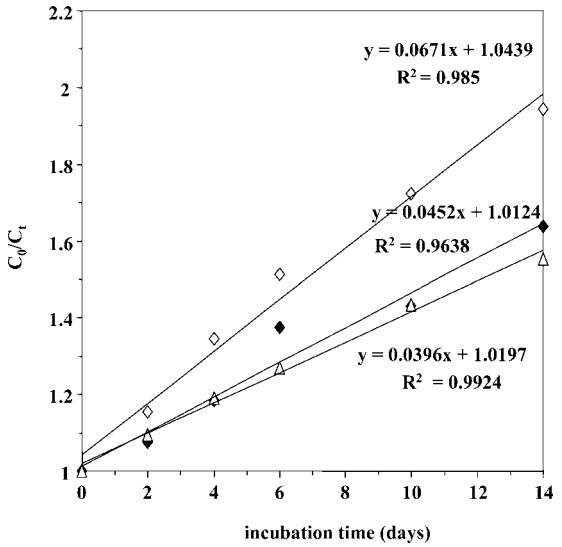

Figure 5. Second-order kinetic plot of the loss of monomeric β-lactoglobulin as determined by RP-HPLC after incubation of powders from 0 to 14 days at 60 °C and 79% RH: (◇) β-lactoglobulin, (◆) β-lactoglobulin/acacia gum ratio 2:1, and (△) β-lactoglobulin/acacia gum ratio 1:2. Lines represent linear fit of the experimental data.

higher for β-lactoglobulin alone ($r = 0.067\ s^{-1}$). As disulfide/sulfhydryl are not likely to occurs under the experimental conditions (low pH, no reduction of the bounds in SDS−PAGE electrophoresis), the reaction orders of 2 that were observed are likely due to protein polymerization, maybe due to partial deamidation and to protein/polysaccharide conjugation in the case of mixtures.

The determination of the molecular weight of the soluble fraction of the incubated powders was carried out under physiological conditions (pH 6.8) using SEC−HPLC. For β-lactoglobulin alone, it is clear that part of the native protein was transformed into oligomers and polymers having molecular weight higher than 40 000 $g \cdot mol^{-1}$ (β-lactoglobulin dimer) as well as larger polymers that could have been removed during

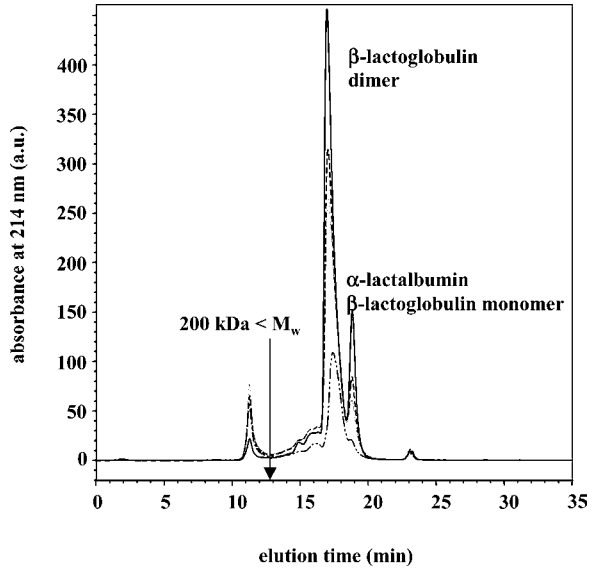

Figure 6. SEC-HPLC chromatogram of a 0.5 wt % β-lactoglobulin/acacia gum at ratio 1:2 dispersed in 50 mM phosphate buffer, pH 6.8, after various incubation time at 60 °C and 79% RH: (–) after 0 day of incubation, (-----) after 4 days of incubation, (···) after 6 days of incubation, and (–•–•) after 14 days of incubation.

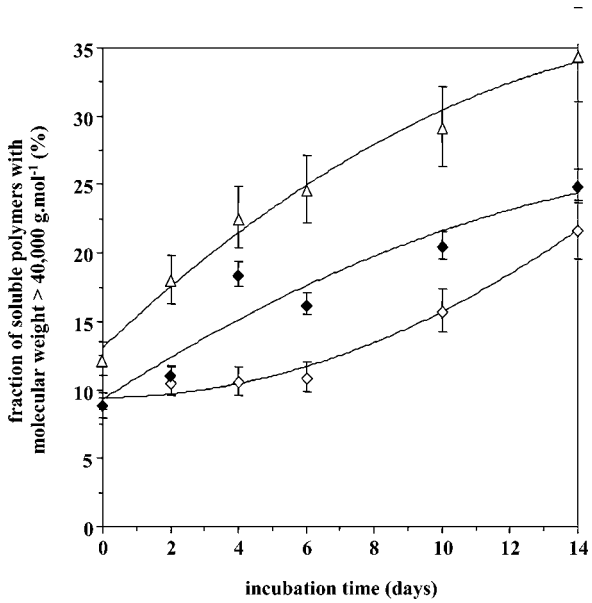

Figure 7. Fraction of polymers having an apparent molecular weight higher than 40 000 g mol^{-1} as determined by SEC-HPLC as a function of the incubation time: (◇) β-lactoglobulin, (◆) β-lactoglobulin/acacia gum ratio 2:1, and (△) β-lactoglobulin/acacia gum ratio 1:2. The vertical bars represent the standard deviation.

the filtration step (**Figure 7**). Interestingly, more than 75% of the initial β-lactoglobulin amount was still present after 14 days. The discrepancy with the 50% obtained with RP-HPLC can be easily explained by the fact that it is not possible to distinguish between β-lactoglobulin monomer and α-lactalbumin using SEC−HPLC under the tested conditions (**Figure 6**). As well, SEC−HPLC does not separate covalent and electrostatic dimers, so native β-lactoglobulin content is likely to be overestimated. Considering the two protein/polysaccharide mixtures, it can be seen that the amount of protein polymers having molecular weight higher than the β-lactoglobulin dimer increased with the incubation time (**Figure 7**). Interestingly, more than 35% of polymers were detected by SEC−HPLC for the ratio 1:2, which

fits with the RP-HPLC values. Only 25% were measured for the 2:1 protein-to-polysaccharide ratio, which is lower than the 35% calculated by RP-HPLC. No clear explanation can be given here; however, as the formation of conjugates leads to an increase of the amide bonds detected at 214 nm, this could be an explanation for the discrepancies found between the two analytical methods. A possible way to get through this problem might be to work at a second wavelength that is more specific for proteins (280 nm, for example).

Functional Properties of β-Lactoglobulin/Acacia Gum Conjugates. The water solubility of the incubated β-lactoglobulin and β-lactoglobulin-acacia gum powders was investigated at pH ranging from 3 to 7. Before incubation, protein water solubility was close to 100% for all samples for the whole pH range except between 4 and 5 (data not shown). This narrow pH range is known to correspond to the region of the isolectrical point of β-lactoglobulin (*3*). The protein solubility values of 85−90% could be explained by favored protein−protein interactions that may lead to partial aggregation/insolubilization of the β-lactoglobulin. After 2 days of dry incubation at 60 °C, the solubility curves changed drastically (**Figure 8**A). The β-lactoglobulin exhibited a clear minimum of solubility at pH 4.5 that corresponded to 25% of soluble protein. Solubility reached again values >90% for pH <3.5 and >6.0, respectively. Similar observations were made by Chevalier et al. (*38*) after heating a β-lactoglobulin solution for 72 h at 60 °C (i.e., below the denaturation temperature of the protein). Incubation clearly led to the formation of large oligomers and polymers that readily insolubilized in the region close to the β-lactoglobulin isoelectrical pH. The 2:1 mixture showed a similar response, except that the minimum of solubility was around pH 4.0, where 30% protein was soluble (**Figure 8A**). Around this lower pH of solubility, protein was more soluble by about 20% compared to the β-lactoglobulin alone. Hence, the presence of acacia gum during incubation was limiting the formation of the protein oligomers, as described in the previous section, and conjugate formation might be a reason. The 1:2 mixture was totally different from the previous samples. No minimum of solubility was detected in the pH range investigated, the lowest value being 50% at pH 3.0. Here again, conjugation with acacia gum can explain this shift of the minimum of solubility. After 14 days of incubation, the solubility profile for β-lactoglobulin remained unchanged (**Figure 8B**). However, the mixtures showed a slight decrease of solubility, especially for ratio 1:2, which gave a minimum of 35% at pH 3−3.5. When compared to values obtained after 2 days, these changes could be due to additional conjugation reactions between the protein and the polysaccharide. The solubility results obtained after 2 days are interesting to interpret from the side of the shift of the minimum value. Hence, the decrease of the minimum of solubility toward acidic value is because NH_2 groups are disappearing on the protein surface. This is especially true and well-correlated with the decrease of the NH_2 functions that was measured using the OPA method for the β-lactoglobulin−acacia gum mixtures (**Figure 1**).

Surface Tension and Interfacial Viscoelasticity. Table 1 presents the equilibrium air/water surface tension determined at pH 4.2 for powders incubated for 0 and 14 days, respectively. Without incubation, equilibrium values of $\sigma_{a/w}$ were close (= 43−44 mN m^{-1}) for the β-lactoglobulin alone and for the mixture at ratio 2:1. A higher value was obtained for the mixture at ratio 1:2. This surface tension decrease is correlated with the ability of proteins to absorb at the air/water interface and to unfold. This is especially true for β-lactoglobulin, which is able

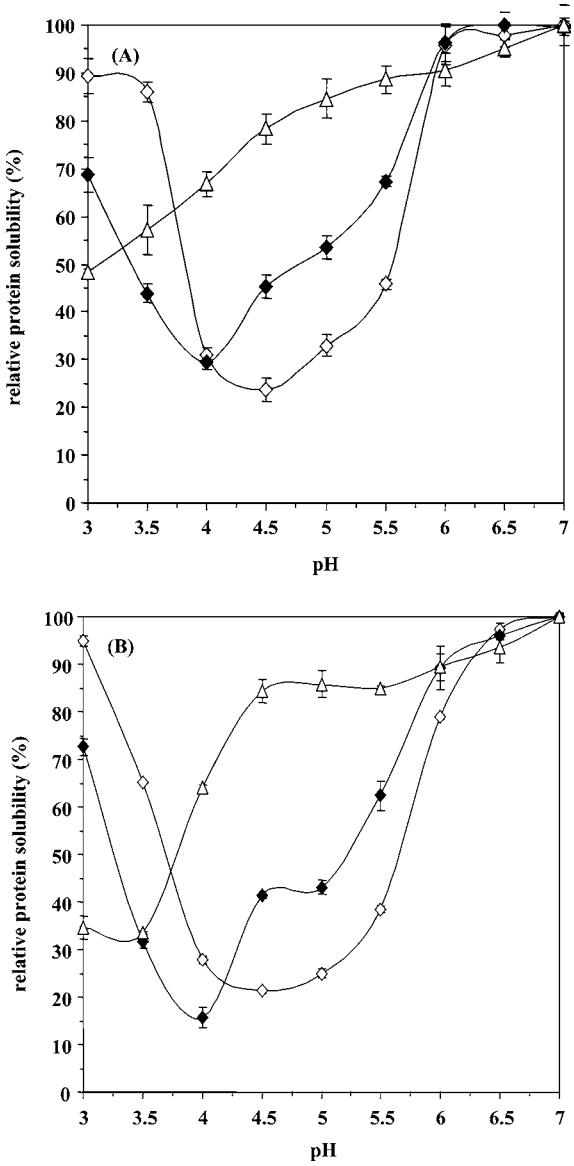

Figure 8. (**A**) Relative protein solubility obtained from a 1 wt % dispersion after incubation for 2 days at 60 °C and 79% RH: (◇) β-lactoglobulin, (◆) β-lactoglobulin/acacia gum ratio 2:1, and (△) β-lactoglobulin/acacia gum ratio 1:2. The vertical bars represent the standard deviation. (**B**) Relative protein solubility obtained from a 1 wt % dispersion after incubation for 14 days at 60 °C and 79% RH: (◇) β-lactoglobulin, (◆) β-lactoglobulin/acacia gum ratio 2:1, and (△) β-lactoglobulin/acacia gum ratio 1:2. Vertical bars represent the standard deviation.

to expose many hydrophobic regions originally buried in the β-sheet structure (*1*, *6*). The lower decrease observed for the ratio 1:2 could be attributed to the contribution of the acacia gum that is interacting electrostatically with the β-lactoglobulin, leading to soluble complexes with larger size (molecular weight and diameter) than the protein alone (*16*). The fact that the equilibrium surface tension is lower at ratio 2:1 might be due to complete charge neutralization, leading to lower repulsion between adsorbed complexes and also more favorable interfacial spreading conditions ($\sigma_{complexes/water}$ is on the order of several μN m^{-1}, so that spreading is favored) (*49*). After 14 days of incubation, the equilibrium surface tension of β-lactoglobulin alone significantly increased to 49 mN m^{-1} (note the large standard deviation, too; **Table 1**), whereas it remained constant for the two mixtures. Such a results might be explained by the polymerization of the β-lactoglobulin (more than 50% from the

Table 1. Equilibrium Surface Tension Obtained at pH 4.2 and Foam Capacity and Expansion Obtained at pH 4.2, 5.3, and 7.0 from 0.5 wt% Aqueous Dispersions Prepared from β-Lactoglobulin and β-Lactoglobulin/Acacia Powders after Dry-State Incubation for 0 and 14 Days at 60 °C and 79% RH

		β-lactoglobulin		β-lactoglobulin/acacia gum			
				ratio 2:1		ratio 1:2	
	pH	0 days	14 days	0 days	14 days	0 days	14 days
$\sigma_{a/w}$ (mN·m^{-1})[a]	4.2	43.15 ± 0.02	48.95 ± 0.58	43.89 ± 0.05	43.67 ± 0.07	46.80 ± 0.03	46.95 ± 0.05
foam capacity	4.2	1.29 ± 0.01	1.16 ± 0.00	1.26 ± 0.08	1.27 ± 0.03	1.22 ± 0.02	1.19 ± 0.01
	5.3	1.33 ± 0.01	1.00 ± 0.04	1.32 ± 0.03	1.20 ± 0.01	1.23 ± 0.03	1.19 ± 0.01
	7.0	1.32 ± 0.01	0.99 ± 0.03	1.33 ± 0.01	1.24 ± 0.03	1.20 ± 0.02	1.21 ± 0.00
foam expansion	4.2	7.25 ± 0.35	26.15 ± 3.04	7.30 ± 0.28	10.00 ± 0.85	8.50 ± 0.28	14.05 ± 2.05
	5.3	7.00 ± 0.14	66.05 ± 1.06	7.00 ± 0.14	9.90 ± 0.00	8.55 ± 0.07	14.40 ± 0.71
	7.0	6.55 ± 0.49	46.70 ± 6.65	6.85 ± 0.07	7.20 ± 0.57	10.55 ± 0.07	9.15 ± 0.49

[a] Determined after 9600 s.

RP-HPLC results), leading to fewer surface-active polymers. This observation has been already reported for β-lactoglobulin upon heat-denaturation (*7*, *50*) or upon mixture of native β-lactoglobulin and heat-polymerized β-lactoglobulin (*8*). Such a decrease of surface activity upon incubation was not detected in β-lactoglobulin/acacia gum mixtures that led to conjugate formation rather than protein polymerization.

The interfacial properties of the adsorbed layer obtained at equilibrium were probed using pendant drop tensiometry. **Figure 9A** presents the variation of the interfacial elasticity, E', and the interfacial viscosity, η_d, before and after incubation of the β-lactoglobulin at pH 4.2. Surface elasticity clearly increased upon incubation, passing from 20 to 35 mN·m^{-1} at a frequency of 0.01 Hz. Interestingly, one could note the low frequency dependence of E' after incubation for 14 days, compared to no incubation. Hence, the behavior of the interface was characterized as solidlike, which could be explained by the presence of protein polymers at the interface. This is not the case with no incubation, where the frequency dependence of E' was marked, expressing the reorganization of the single protein molecules at the interface upon deformation (*2*). Interfacial viscosity was more frequency-dependent than E', exhibiting an overall decrease upon frequency increase. This behavior is well-known for protein films and is related to relaxation phenomena within the film (*51*). However, as η_d was lower after 14 days, one can conclude that protein aggregates were probably more flexible than the native protein and also adsorbed at a lower interfacial concentration (*8*). Finally, the phase angle measured at the interface clearly depicted the higher solidlike character of the incubated β-lactoglobulin. Hence, the value was constant around 12−15° (major elastic contribution) compared to 25−35° for the native protein (major viscous contribution) (*51*). The surface properties of the interface stabilized at a β-lactoglobulin/acacia gum mixture at ratio 2:1 were different from those of the protein alone (**Figure 9B**). Without incubation, elasticity was higher than for protein alone and the phase angle lower over the whole frequency range. This showed the more elastic behavior of the β-lactoglobulin/acacia gum film at pH 4.2 that has been described recently (*16*). Upon 14 days of incubation, the film elasticity decreased by 10 mN·m^{-1} and the interfacial viscosity dropped from 75 mN·s·m^{-1} at a frequency of 0.01 Hz. The phase angle slightly decreased from 22° to 16°, proving that the film was more elastic upon incubation. From these results, it can be concluded that native β-lactoglobulin was still able to reach the interface (frequency dependence of η_d), which correlates well with the fact that only 35% of the initial protein monomers disappeared upon incubation. However, β-lactoglobulin/acacia gum conjugates were probably present too, which explains the decrease of the phase angle (*52*). At ratio 1:2, no significant difference of the interfacial elasticity was found before and after 14 days of incubation at pH 4.2 (**Figure 9C**). Interestingly, the surface elasticity was higher than for the protein alone or the 2:1 ratio (>30 mN·m^{-1}). Interfacial viscosity exhibited strong frequency dependence, with an increase after incubation. Values as high as 300 mN·s·m^{-1} (225 mN·s·m^{-1} for BLG alone, 180 mN·s·m^{-1} for ratio 2:1) were obtained at a frequency of 0.01 Hz. This strong viscous contribution was visible in the variation of the phase angle, which was around 30° over the frequency range tested. Thus, one could surmise that mainly monomeric and nonconjugated β-lactoglobulin was adsorbed at the interface (less elastic than ratio 2:1).

Foamability and Foam Stability. The foamability of the incubated β-lactoglobulin and β-lactoglobulin/acacia gum mixtures was evaluated from the calculation of the foam capacity and foam expansion parameters upon nitrogen sparging within a 0.5 wt % dispersion at pH 4.2. It can be seen that the foam capacity was identical (FC = 1.27) for the β-lactoglobulin alone and ratio 2:1 without incubation (**Table 1**). The foam capacity of the 1:2 mixture was slightly lower, FC = 1.20. These results are in line with the equilibrium surface tension values obtained before under the same condition. Hence, lower equilibrium surface tension correlated with a higher capability of the surface-active material to stabilize the air bubble interface because of its conformation or interfacial concentration (*5*). These first results showed also that 0.33 wt % β-lactoglobulin (at ratio 2:1) was enough to stabilize the created interface, whereas 0.17 wt % protein was too low (at ratio 1:2). Upon incubation for 14 days, the foam capacity of the β-lactoglobulin decreased by almost 30% compared to the nonincubated one. This result fits with the formation of 50% protein polymers after 14 days (polymers that are characterized by a lower surface activity compared to native β-lactoglobulin) (increase of equilibrium surface tension after 14 days) (*8*). Foaming capacities obtained for the two mixtures after incubation for 14 days were lower than before incubation but significantly higher than for the protein incubated alone (**Table 1**). Here again, these results correlate well with the stability of the surface tension of the mixture after incubation and the lower amount of protein polymers that are formed compared to β-lactoglobulin incubated alone. The likely explanation is that native β-lactoglobulin (65%) can diffuse faster at the interface to stabilize it compared to polymeric protein (*8*). The effect of pH on the foam capacity was clearly evidence before and after incubation. β-Lactoglobulin incubated alone exhibited a marked decrease of FC from 1.32 to 0.99 at pH 7.0 after 14 days. For the ratio 2:1 and 1:2, decrease of foaming capacity was very limited at pH 5.3 and 7.0, even after 14 days of incubation (**Table 1**). A similar stability trend was found considering the foam expansion

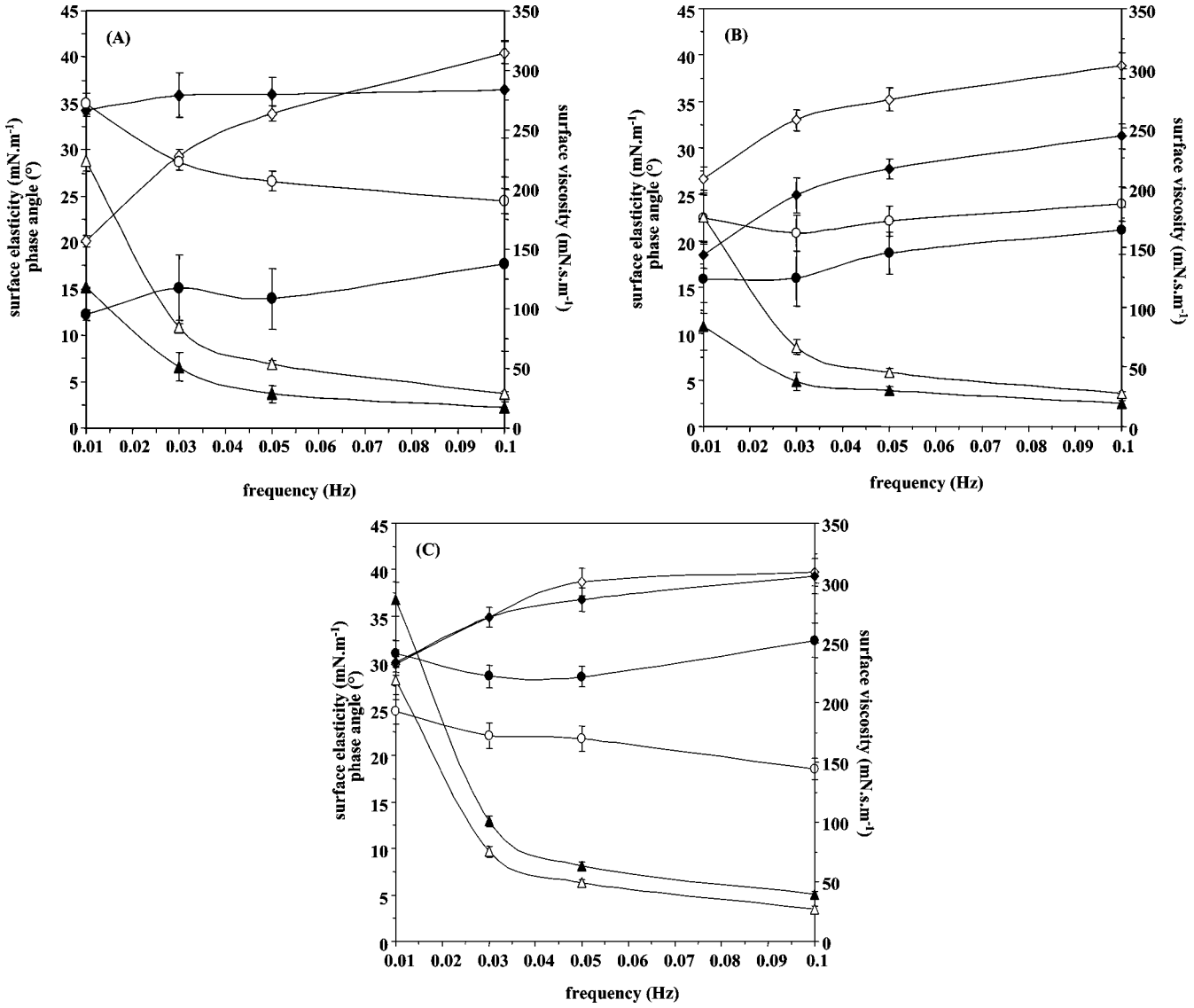

Figure 9. (**A**) Surface elasticity, viscosity, and phase angle determined from pendant drop tensiometry at pH 4.2 for a 0.5 wt % β-lactoglobulin dispersion incubated for 0 or 14 days at 60 °C and 79% RH: (◇) surface elasticity, E', after 0 day; (◆) surface elasticity, E', after 14 days; (△) surface viscosity, η_d, after 0 day; (▲) surface viscosity, η_d, after 14 days; (○) phase angle after 0 day; (●) phase angle after 14 days. The vertical bars represent the standard deviation. (**B**) Surface elasticity, viscosity, and phase angle determined from pendant drop tensiometry at pH 4.2 for a 0.5 wt % β-lactoglobulin/acacia gum dispersion at ratio 2:1 incubated for 0 or 14 days at 60 °C and 79% RH: (◇) surface elasticity, E', after 0 day; (◆) surface elasticity, E', after 14 days; (△) surface viscosity, η_d, after 0 day; (▲) surface viscosity, η_d, after 14 days; (○) phase angle after 0 day; (●) phase angle after 14 days. The vertical bars represent the standard deviation. (**C**) Surface elasticity, viscosity, and phase angle determined from pendant drop tensiometry at pH 4.2 for a 0.5 wt % β-lactoglobulin/acacia gum at ratio 1:2 dispersion incubated for 0 or 14 days at 60 °C and 79% RH: (◇) surface elasticity, E', after 0 day; (◆) surface elasticity, E', after 14 days; (△) surface viscosity, η_d, after 0 day; (▲) surface viscosity, η_d, after 14 days; (○) phase angle after 0 day; (●) phase angle after 14 days. The vertical bars represent the standard deviation.

parameter at pH 4.2. Hence, values were almost similar around 7.5 for the three samples before incubation. This was a proof that all foams contained a similar amount of liquid (the lower the FE, the higher the amount of liquid in the foam), which could be due either to small air bubbles (increase of the number of Plateau border), to an increased surface viscosity, or to a combination of these two parameters. After incubation, the FE of the β-lactoglobulin reached 26, whereas it stayed around 10 and 14 for the ratio 2:1 and 1:2, respectively. Much drier foams were then produced after polymerization of the β-lactoglobulin, which is well-correlated with both the low foam capacity and significant increase of the equilibrium surface tension. By contrast, conjugate formation enables one to keep constant the wetness of the foams, especially for the protein-to-polysaccharide ratio of 2:1. The effect of pH was really important on the foam expansion parameter after incubation, since FE = 66 for the incubated β-lactoglobulin at pH 5.3 and 46.70 at pH 7.0. Both high FE values demonstrate that the β-lactoglobulin polymer strongly aggregates in the bulk at pH 5.3 (low protein solubility) or leads to weak interfacial layers at pH 7.0. A very high stability of FE was obtained at ratio 2:1, both for pH 5.3 and 7.0, after 14 days (**Table 1**). For the 1:2 ratio, the highest FE stability was obtained at pH 7.0, where the solubility of the conjugates combined with their interfacial viscosity must be optimal.

The foam stability of the three sample dispersions has been followed after 0, 6, and 14 days of incubation and at pH 4.2, 5.3, and 7.0 by measuring the time needed for the liquid in the

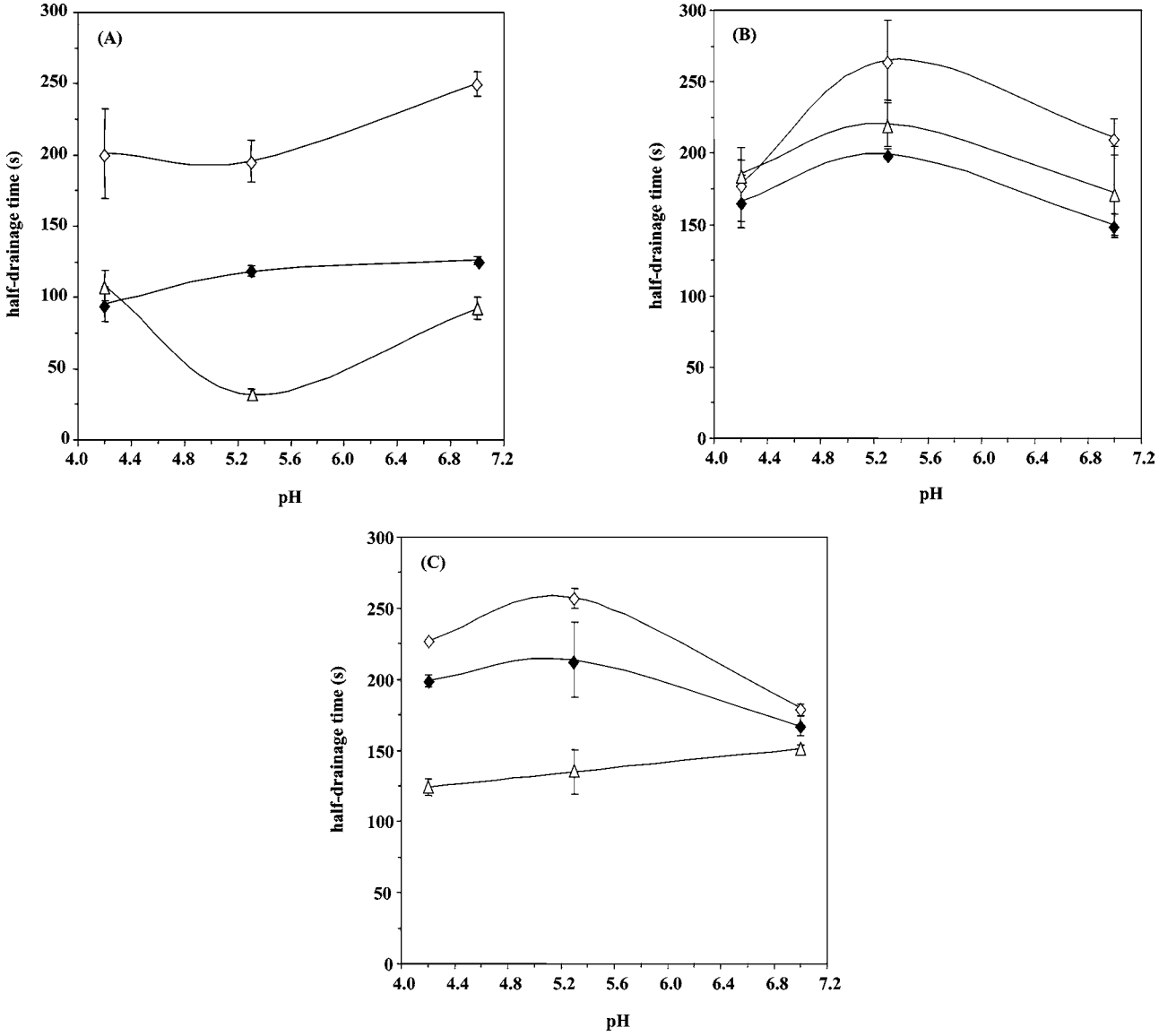

Figure 10. (**A**) Half-drainage time obtained from foams based on a 0.5 wt % dispersion of β-lactoglobulin as a function of pH after incubation for 0, 6, and 14 days at 60 °C and 79% RH: (◇) after 0 day of incubation, (◆) after 6 days of incubation, and (△) after 14 days of incubation. The vertical bars represent the standard deviation. (**B**) Half-drainage time obtained from foams based on a 0.5 wt % dispersion of β-lactoglobulin/acacia gum at ratio 2:1 as a function of pH after incubation for 0, 6, and 14 days at 60 °C and 79% RH: (◇) after 0 day of incubation, (◆) after 6 days of incubation, (△) after 14 days of incubation. The vertical bars represent standard deviation. (**C**) Half-drainage time obtained from foams based on a 0.5 wt % dispersion of β-lactoglobulin/acacia gum at ratio 1:2 as a function of pH after incubation for 0, 6, and 14 days at 60 °C and 79% RH: (◇) after 0 day of incubation, (◆) after 6 days of incubation, and (△) after 14 days of incubation. The vertical bars represent the standard deviation.

foam to drain by half its value. **Figure 10A** presents results obtained for the β-lactoglobulin alone. If pH had only little effect on the liquid stability in the foam at 0 and 6 days of incubation, the effect was amplified after 14 days. Hence, very low drainage stability was found at pH 5.3, i.e., close to the isoelectrical point of the β-lactoglobulin dimer (*3*). It is generally recognized that most stable foams of nonheated globular proteins are obtained around their isoelectrical pH, because of the packed viscoelastic network that can be formed (*53*−*55*). In the present case, it seems that protein polymerization led to a minimum of solubility, inducing a low water binding capacity. As far as the incubation time effect on drainage stability is concerned, a significant decrease from around 200 to 100 s was visible after 6 days (**Figure 10A**). The half-drainage time reached 25 s at pH 5.3 after 14 days of incubation. In the presence of acacia gum during incubation at ratio 2:1, the liquid stability of the foams greatly improved, since all drainage values were above 150 s, whatever the pH or the incubation time (**Figure 10B**). Interestingly, maximum values of drainage stability were all obtained for pH 5.3, which can be probably explained by the higher protein solubility compared to the protein alone. This result is particularly interesting, as it means that it is possible to heat-treat β-lactoglobulin close to its IEP without losing its foam-stabilizing properties, providing that conjugates are formed with acacia gum. **Figure 10C** shows the foam drainage stability obtained at ratio 1:2. Here stability was intermediate between that of β-lactoglobulin alone and ratio 2:1. Nevertheless, improvement was clear upon conjugate formation as the foam drainage half-time was higher after 14 days than for pure β-lactoglobuline after 6 days of incubation (**Figure 10A**). It is important to notice that the highest drainage stability was here again obtained at pH 5.3, as for the 2:1 ratio.

CONCLUSIONS

It has been shown that β-lactoglobulin was able to polymerize under dry heating at 60 °C, pH 4.2, and a relative humidity of 79%. This polymerization was detrimental for protein solubility close to its p*I* and as well for the air/water interfacial properties of β-lactoglobulin (increase in surface tension, lower viscoelastic properties). Hence, foam stability (volume and liquid) was very poor, especially around pH 5.3, which is close to the p*I* of β-lactoglobulin. Upon conjugation with acacia gum, β-lactoglobulin showed a shift of its minimum of solubility towards acidic pH. Interfacial elasticity was higher for conjugates (ratio 1:2), but the highest viscoelastic character (high E', low η_d, low phase angle) was obtained at ratio 2:1. Foam stability was significantly improved when conjugates were used, especially at pH 5.3. Conjugates obtained at ratio 2:1 exhibited the highest foam volume stabilization properties as well as highest capacity to retain liquid in the foam. Dry incubation of electrostatic protein/polysaccharide complexes should be considered as a technological mean to render these complexes less sensitive to pH or ionic strength variation in finished food product.

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LITERATURE CITED

(1) Damodaran, S. Protein stabilisation of emulsions and foams. *J. Food Sci.* **2005**, *70*, 54−66.
(2) Murray, B. S. In *Proteins at liquid interfaces*; Miller, R., Möbius, D., Eds.; Elsevier: Amsterdam, 1998; pp 179−220.
(3) Kinsella, J. E. Milk proteins: Physicochemical and functional properties. *Crit. Rev. Food Sci. Nutr.* **1984**, *21*, 197−262.
(4) Phillips, L. G.; Whitehead, D. M.; Kinsella, J. *Structure−function properties of food proteins*; Academic Press: San Diego, 1994.
(5) Wilde, P. J.; Clark, D. C. Foam formation and stability. In *Methods of testing protein functionality*; Hall, G. M., Ed.; Chapman and Hall: London, 1996; pp 110−152.
(6) Kim, D. A.; Cornec, M.; Narsimhan, G. Effect of thermal treatment on interfacial properties of β-lactoglobulin. *J. Colloid Interface Sci.* **2005**, *285*, 100−109.
(7) Bals, A.; Kulozik, U. Effect of preheating on the foaming properties of whey protein isolate using a membrane foaming apparatus. *Int. Dairy J.* **2003**, *13*, 903−908.
(8) Davis, J. P.; Foegeding, E. A. Foaming and interfacial properties of polymerized whey protein isolate. *J. Food Sci.* **2004**, *69*, 404−410.
(9) Poole, S. Review: The foam-enhancing properties of basic biopolymers. *Int. J. Food Sci. Technol.* **1989**, *24*, 121−137.
(10) 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.
(11) Hansen, P. M. T.; Black, D. H. Whipping properties of spray-dried complexes from whey protein and carboxymethylcellulose. *J. Food Sci.* **1972**, *37*, 452−456.
(12) Ahmed, M.; Dickinson, E. Foaming of aqueous solutions of protein + propylene glycol alginate. *Food Hydrocolloids* **1991**, *4*, 395−402.
(13) Mann, B.; Malik, R. C. Studies on some functional characteristics of whey protein−polysaccharide complex. *J. Food Sci. Technol.* **1996**, *33*, 202−206.
(14) Dickinson, E.; Izgi, E. Foam stabilisation by protein-polysaccharide complexes. *Colloids Surf. A* **1996**, *113*, 191−201.
(15) Shih, F. F. Interaction of soy protein isolate with polysaccharide and its effcet on film properties. *JAOCS* **1994**, *71*, 1281−1285.
(16) Schmitt, C.; Kolodziejczyk, E.; Leser, M. E. Interfacial and foam stabilization properties of β-lactoglobulin−accaia gum electrostatic complexes. In *Food Colloids: Interactions, microstructure and processing*; Dickinson, E., Ed.; Royal Society of Chemistry: Cambridge, 2005; pp 284−300.
(17) Schmitt, C.; Sanchez, C.; Desobry-Banon, S.; Hardy, J. Structure and technofunctional properties of protein−polysaccharide complexes. A review. *Crit. Rev. Food Sci. Nutr.* **1998**, *38*, 689−753.
(18) Kato, A. Functional protein−polysaccharide conjugates. *Comments Agric. Food Chem.* **1996**, *3*, 139−153.
(19) Delben, F.; Stefancich, S. Interaction of food polysaccharides with ovalbumin. *Food Hydrocolloids* **1998**, *12*, 291−299.
(20) Hattori, M.; Okada, Y.; Takahashi, K. Functional changes in β-lactoglobulin upon conjugation with carboxymethyl cyclodextrin. *J. Agric. Food Chem.* **2000**, *48*, 3789−3.
(21) Song, Y.; Babiker, E. E.; Usui, M.; Saito, A.; Kato, A. Emulsifying properties and bactericidal action of chitosan−lysozyme conjugates. *Food Res. Int.* **2002**, *35*, 459−4.
(22) Diftis, N.; Kiosseoglou, V. Improvement of emulsifying properties of soybean protein isolate by conjugation with carboxymethyl cellulose. *Food Chem.* **2003**, *81*, 1−6.
(23) Einhorn-Stoll, U.; Ulbrich, M.; Sever, S.; Kunzek, H. Formation of milk protein-pectin conjugates with improved emulsifying properties by controlled dry heating. *Food Hydrocolloids* **2005**, *19*, 329−340.
(24) Einhorn-Stoll, U.; Salazar, T.; Jaafar, B.; Kunzek, H. Thermodynamic compatibility of sodium caseinate with different pectins. Influence of the milieu conditions and pectin modifications. *Nahrung* **2001**, *45*, 332−337.
(25) Tolstoguzov, V. Some thermodynamic considerations in food formulation. *Food Hydrocolloids* **2003**, *17*, 1−23.
(26) Galazka, V. B.; Dickinson, E.; Ledward, D. A. Influence of high pressure on protein-polysaccharide interactions in emulsions. In *Food Colloids: Fundamentals of Formulation*; Dickinson, E., Miller, R., Eds.; Royal Society of Chemistry: Cambridge, 2001; pp 315−322.
(27) Schmitt, C.; Sanchez, C.; Despond, S.; Renard, D.; Robert, P.; Hardy, J. Structural modification of β-lactoglobulin as induced by complex coacervation with acacia gum. In *Food Colloids: Fundamentals of Formulation*; Dickinson, E.; Miller, R., Eds.; Royal Society of Chemistry: Cambridge, 2001; pp 332−341.
(28) Weinbreck, F.; de Vries, R.; Schrooyen, P.; de Kruif, C. G. Complex coacervation of whey proteins and gum arabic. *Biomacromolecules* **2003**, *4*, 293−303.
(29) Church, F. C.; Swaisgood, H. E.; Porter, D. H.; Catignani, G. L. Spectrophotometric assay using *o*-phthaldialdehyde for determination of proteiolysis in milk and isolated milk proteins. *J. Dairy Sci.* **1983**, *66*, 1219−1227.
(30) Medina Hernandez, M. J.; Bonet Domingo, E.; Villanueva Camanas, R. M.; Garcia Alvarez-Coque, M. C. Use of the *o*-phthalaldehyde and *N*-acetyl-l-cysteine reagent in the evaluation of milk proteins. *J. Dairy Sci.* **1991**, *74*, 1779−1785.
(31) Resmini, P.; Pellegrino, L.; Hogenboom, J. A.; Andreini, R. Thermal denaturation of whey protein in pasteurized milk. Fast evaluation by HPLC. *Ital. J. Food Sci.* **1989**, *3*, 51−62.
(32) Hochstrasser, D. F.; Patchornik, P.; Merril, C. R. Development of polyacrylamide gels that improve the separation of proteins and their detection by silver staining. *Anal. Biochem.* **1988**, *173*, 412−423.
(33) Braford, M. M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(34) Benjamins, J.; Cagna, A.; Lucassen-Reynders, E. H. Viscoelastic properties of triacylglycerol/water interfaces covered by proteins. *Colloids Surf. A* **1996**, *114*, 245−254.

(35) Guillerme, C.; Loisel, W.; Bertrand, D.; Popineau, Y. Study of foam stability by video image analysis: Relationship with the quantity of liquid in the foams. *J. Text. Stud.* **1993**, *24*, 287−302.
(36) Kato, A.; Takahashi, A.; Matsudomi, N.; Kobayashi, K. Determination of foaming properties of proteins by conductivity measurements. *J. Food Sci.* **1983**, *48*, 62−65.
(37) Carrera-Sanchez, C.; Rodriguez-Patino, J. M. Interfacial, foaming and emulsifying characteristics of sodium caseinate as influenced by protein concentration. *Food Hydrocolloids* **2005**, *19*, 407−416.
(38) Chevalier, F.; Chobert, J. M.; Popineau, Y.; Nicolas, M. G.; Haertlé, T. Improvement of functional properties of β-lactoglobulin glycated through the Maillard reaction is related to the nature of the sugar. *Int. Dairy J.* **2001**, *11*, 145−152.
(39) Nissen Lund, M.; Olsen, K.; Sorensen, J.; Skibsted, L. H. Kinetics and mechanism of lactosylation of α-lactalbumin. *J. Agric. Food Chem.* **2005**, *53*, 2095−2102.
(40) Matsudomi, N.; Nakano, K.; Soma, A.; Ochi, A. Improvement of gel properties of dried egg white by modification with galactomannan through the Maillard reaction. *J. Agric. Food Chem.* **2002**, *50*, 4113−4118.
(41) Shimada, K.; Cheftel, J. C. Sulfhydryl group/disulfide bond interchange reaction during the heat-induced gelation of whey protein isolate. *J. Agric. Food Chem.* **1989**, *37*, 161−168.
(42) Aeschbach, R.; Amado, R.; Neukom, H. Formation of dityrosine cross-links in proteins by oxidation of tyrosine residues. *Biochim. Biophys. Acta* **1976**, *439*, 292−301.
(43) Matsudomi, N.; Takahashi, K.; Miyata, T. Some structural properties of ovalbumin heated at 80 °C in the dry state. *Food Res. Int.* **2001**, *34*, 229−235.
(44) Zhang, J.; Lee, T. C.; Ho, C. T. Thermal deamidation of proteins in a restricted water environment. *J. Agric. Food Chem.* **1993**, *41*, 1840−1843.
(45) Mine, Y. Effect of dry heat and mild alkaline treatment on functional properties of egg white proteins. *J. Agric. Food Chem.* **2005**, *45*, 2924−2928.
(46) Metwalli, A. A. M.; van Boekel, M. A. J. S. On the kinetics of heat-induced deamidation and breakdown of caseinate. *Food Chem.* **1998**, *1*−*2*, 53−61.
(47) Dannenberg, F.; Kessler, H. G. Reaction kinetics of the denaturation of whey proteins in milk. *J. Food Sci*. **1988**, *53*, 258−263.
(48) Roefs, S. P. F. M.; de Kruif, C. G. A model for the denaturation and aggregation of β-lactoglobulin. *Eur. J. Biochem.* **1994**, *226*, 883−889.
(49) Scholten, E.; Sagis, L. M. C.; Van der Linden, E. Bending rigidity of interfaces in aqueous phase-separated mixtures. *J. Phys. Chem. B* **2004**, *108*, 12164−12169.
(50) Zhu, H.; Damodaran, S. Heat-induced conformational changes in whey protein isolate and its relation to foaming properties. *J. Agric. Food Chem.* **1994**, *42*, 846−855.
(51) Bos, M. A.; Van Vliet, T. Interfacial rheological properties of adsorbed protein layers and surfactants: A review. *Adv. Colloids Interface Sci.* **2001**, *91*, 437−471.
(52) Dickinson, E.; Galazka, V. B. Emulsion stabilisation by protein-polysaccharide complexes. In *Gums and* Stabilisers *for the Food Industry 6*; Phillips, G. O., Williams, P. A., Wedlock, D. J., Eds.; IRL Press: Oxford, 1992; pp 351−362.
(53) Damodaran, S. Adsorbed layers formed from mixtures of proteins. *Curr. Opin. Colloid Interface Sci.* **2004**, *9*, 328−339.
(54) Yu, M. A.; Damodaran, S. Kinetics of protein foam destabilization: Evaluation of a method using bovine serum albumin. *J. Agric. Food Chem.* **1991**, *39*, 1555−1562.
(55) Petkova, V.; Sultanem, C.; Nedyalkov, M.; Benattar, J.; Leser, M. E.; Schmitt, C. Structure of a free standing film of β-lactoglobulin. *Langmuir* **2003**, *19*, 6942−6949.

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