JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Stability of Acidic Egg White Protein Emulsions Containing Xanthan Gum

ANTONIOS DRAKOS AND VASSILIS KIOSSEOGLOU*

Laboratory of Food Chemistry and Technology, School of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

The influence of xanthan gum concentration on the physicochemical stability of model oil-in-water emulsions prepared with egg white protein at pH 3.8 and containing 150 mM NaCl was investigated by following droplet aggregate formation, rheological changes, and serum separation with storage time. Egg white emulsions were more strongly flocculated and exhibited higher stability against creaming than those of yolk, irrespective of the presence or absence of xanthan. Depletion effects, originating from the presence in the continuous phase of the emulsions of nonadsorbing xanthan molecules, intensified droplet−droplet flocculation effects and resulted in large droplet flocs. At relatively low xanthan contents, the emulsions exhibited higher stability against creaming compared to the respective control emulsions probably due to the formation of a continuous droplet aggregate network structure. At higher xanthan contents, less extensive droplet interactions, due to slowly evolving microstructure of phase-separated xanthan-rich and xanthan-depleted regions, resulted in emulsions exhibiting increased stability against creaming. The role of interactions between protein molecules adsorbed on neighboring droplets in these changes and their effect on emulsion aging are discussed.

KEYWORDS: Egg white protein; egg yolk; xanthan gum; emulsion; stability

INTRODUCTION

The white fraction of hen's egg, often called albumen, is an aqueous solution containing a number of proteins embedded in a weak gel network structure, made up of ovomucin fibers, which are responsible for the mucous nature of the white (*1*). The main protein constituents of egg white, for example, ovalbumin, ovotransferrin, ovomucoid, and lysozyme, are of globular nature, due to the presence of a number of intramolecular disulfide bonds as well as hydrophobic interactions between nonpolar amino acid groups buried inside the molecular structure. Following heating at temperatures 70−85 °C or upon adsorption at air−water interfaces, the protein molecules denature and interact, leading, respectively, to the development of gel network structures in the bulk or to the formation of interfacial membranes at air bubble surfaces that stabilize food foam systems against bubble coalescence and/or disproportionation (*2*−*4*). Although, however, the white is highly appreciated for its gelation and foaming properties, its emulsifying potential is generally considered rather poor compared to that of yolk. The highly flexible and surface-penetrating apolipoproteins of the latter adsorb at a higher rate and competitively displace the globular egg white proteins or other protein molecules of similar nature from emulsion oil droplet surfaces (*5*, *6*). On the other hand, cholesterol-free salad dressing products, based on egg white, are often encountered on the supermarket shelves, indicating that egg white protein may effectively function under conditions of low pH and relatively high NaCl content and aid in the formation and physical stabilization of these emulsion systems. Additionally, as Kiosseoglou and Sherman (*7*) reported, the presence of white in admixture with yolk in mayonnaise emulsions resulted in a decrease of droplet size and a strengthening of emulsion structure, suggesting that the white proteins might have somehow become involved in the emulsification process.

This investigation aims at clarifying the role of egg white as a functional ingredient in model salad dressing emulsions. Since polysaccharides are usually employed in the preparation of such products to improve their physicochemical stability and rheological characteristics, xanthan gum was incorporated in the systems studied. The presence of polysaccharide molecules in protein emulsions, however, may influence the emulsion structure development and their stability against creaming and/or coalescence depending on the extent of polysaccharide−protein interactions that lead either to droplet bridging, when these interactions result in complex formation, or to thermodynamic incompatibility and phase separation that destabilize the emulsion system through depletion and oil flocculation effects (*8*, *9*).

MATERIALS AND METHODS

Materials. Dehydrated egg white was provided by Sigma Chemical Co. (St. Louis, MO). Its protein content as determined by Kjeldahl

* To whom correspondence should be addressed: tel +302310997834; fax +302310997779; e-mail kiosse@chem.auth.gr.

10.1021/jf061306d CCC: $33.50
Published on Web 11/30/2006

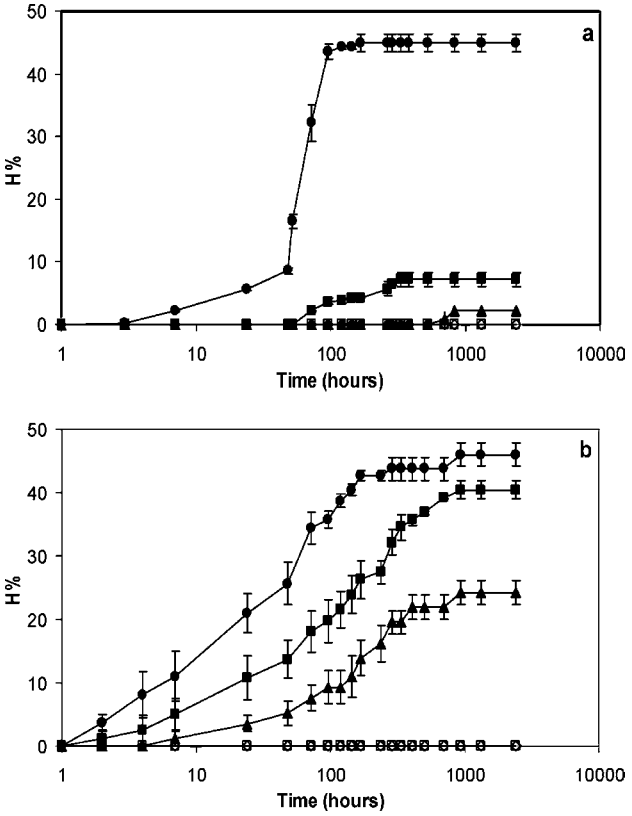

Figure 1. Effect of xanthan on creaming stability of egg white protein (**a**) and yolk (**b**) 30% o/w emulsions. Control emulsions (●); 0.05% (■); 0.1% (▲); 0.2% (◇); 0.4% (□).

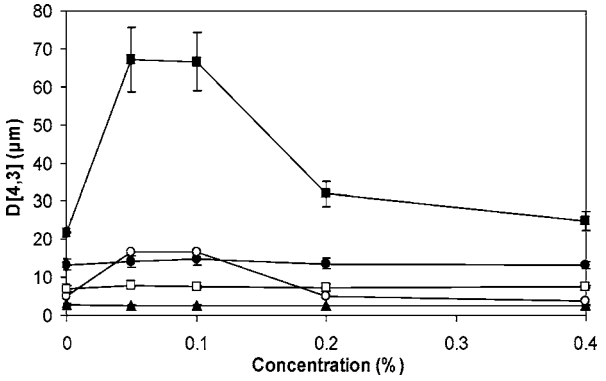

Figure 2. Dependence on xanthan concentration of the mean particle diameter *D*[4, 3] of egg white and yolk 30% o/w emulsions. *D*[4, 3] of the white emulsion was determined following dispersion in water (■) or after treatment with SDS (●) or with SDS plus DTT (□). *D*[4, 3] of the yolk emulsion was determined following dispersion in water (○) or after treatment with SDS (▲).

was 85% (w/w). Refined corn oil was purchased from the local market and used without further purification. Food-grade xanthan gum powder (1253-100G, batch 023K0185), dithiothreitol (DTT), and all the chemicals for the electrophoretic analysis were purchased from Sigma Chemical Co. (St. Louis, MO0,) while sodium dodecyl sulfate (SDS) was obtained from Fluka AG (Buchs, Switzerland).

Solution Preparation. An egg white or yolk solution was prepared by dispersing 5% (w/v) egg white powder or 10% (w/v) liquid yolk in 20 mM citrate buffer (pH 3.8), containing 150 mM NaCI and 0.01% (w/v) NaN_3 (to prevent microbial growth), under continuous stirring for 60 min with the aid of a mechanical stirrer to ensure complete protein dispersion. Under the microscope, the protein solution appeared as a homogeneous system where a number of thin filaments probably corresponding to ovomucin could be detected. A 1% (w/v) xanthan gum solution was prepared by gradually dispersing the polysaccharide powder in the citrate buffer (containing 150 mM NaCI) and stirring for 5 h with the mechanical stirrer to completely disperse the gum.

Emulsion Preparation. A stock oil-in-water (o/w) emulsion was prepared by gradually dispersing 60 mL of oil into 40 mL of egg white protein or yolk solution with the aid of a mechanical stirrer. The resulting crude emulsion was then homogenized for 1 min by employing an Ultra Turrax T25 (IKA Labortechnik, Staufen, Germany) homogenizer equipped with a S25KG-25F dispersion tool and operating at 24 000 rpm. Portions of the stock emulsion were then diluted with appropriate volumes of the citrate buffer and/or the xanthan solution to obtain emulsions with a final oil composition of 30% (v/v), 0−0.4% (w/v) xanthan, and 2.5% (w/v) egg white or 5% (w/v) yolk. The emulsions were stored at 25 °C in closed glass containers and samples were drawn periodically for analysis.

Particle Size Measurements. Emulsion samples were extensively diluted with distilled water and the particle size distribution was measured by laser light scattering on a Malvern Mastersizer 2000 unit (Malvern Instruments Ltd., Malvern, Worcestershire, U.K.). The particles represent, depending on the pretreatment applied to the sample, either single droplets or droplet aggregates. The following optical parameters were applied: corn oil refractive index 1.4673, absorption 0.002, and water refractive index 1.3300. Particle size data are reported either in the form of particle size distribution curves or as weight-average or volume−surface mean diameters, $D[4, 3]$ ($= \Sigma_i n_i d_i^4/\Sigma_i n_i d_i^3$) and $D[3, 2]$ ($= \Sigma_i n_i d_i^3/\Sigma_i n_i d_i^2$), respectively, where n_i is the number of particles with diameter d_i. Mean particle diameter were calculated as the average of three measurements made on at least three separately prepared samples. Additionally, to investigate the role of hydrophobic interactions and disulfide bonds in droplet flocculation, emulsion samples were also treated with 1% SDS and/or 20 mM DTT for 2 h before the application of the laser scattering technique.

Emulsion Stability Measurements. To determine emulsion stability against creaming, a quantity of 10 mL of each emulsion was transferred into a cylindrical glass container (internal diameter 18 mm, height 65 mm) sealed with a plastic cap and stored at room temperature for a time period of several days, and the height of the visible serum separation layer, H_t, was recorded with storage time. The extent of creaming was expressed as % serum $= (H_t/H_0) \times 100$, where H_0 represents the initial emulsion height.

Emulsion stability against coalescence was evaluated by comparing the mean droplet diameters of fresh and aged emulsions. The droplet size was determined following sample treatment with 1% SDS plus 20 mM DTT.

Optical Microscopy. The structural characteristics of selected emulsion samples were determined by use of an Axiolab A reflected light microscope (Zeiss, Berlin, Germany) equipped with a Power Shot G2 photographic camera (Canon, Tokyo, Japan). Following agitation in order to obtain a homogeneous emulsion sample, a drop was placed on the slide and covered by a coverslip. Alternatively, the samples were diluted with appropriate xanthan buffer solutions to obtain diluted emulsions containing 0.5% (v/v) oil and 0−0.4% (w/v) xanthan.

Rheological Measurements. The emulsion rheological properties were determined with the aid of a Brookfield DVII, LV viscometer (Brookfield Engineering Laboratories Inc.) combined with a SC4-18/13R small-sample adapter (concentric cylinder geometry). All the measurements were conducted at 25 °C, 30 min after the sample was loaded. Mean viscosity values were calculated as the average of three measurements made on at least three separately prepared samples.

Electrophoretic Analysis of Adsorbed Protein. Emulsion samples (15 mL) were diluted with 45 mL of citrate buffer and the diluted emulsions were subjected to centrifugation at 3500*g* for 30 min. The cream was collected and 45 mL of buffer was added, followed by centrifugation. This process was repeated until no traces of protein, as determined by the Lowry method (*10*), were detected in the subnatant. The washed cream was then recovered and subjected to repeated freeze−thaw cycles until all the oil was released and removed while the protein residue was lyophilized. The lyophilized adsorbed protein was analyzed by SDS−PAGE under reducing conditions, according to Laemmli (*11*), by employing a vertical electrophoresis apparatus V2-II-20 (Scie-plas, Warwickshire, U.K.). Acrylamide separating and

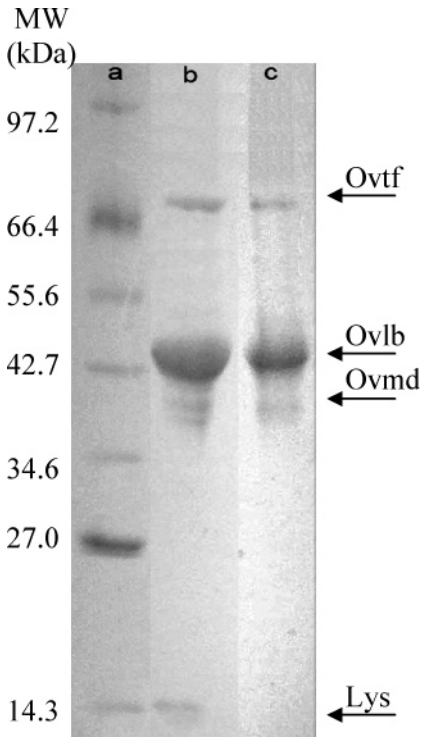

Figure 3. SDS–PAGE patterns of the protein fraction adsorbed to droplet surfaces of 30% o/w emulsion prepared with egg white protein (**c**); (**a**) MW standard; (**b**) egg white protein.

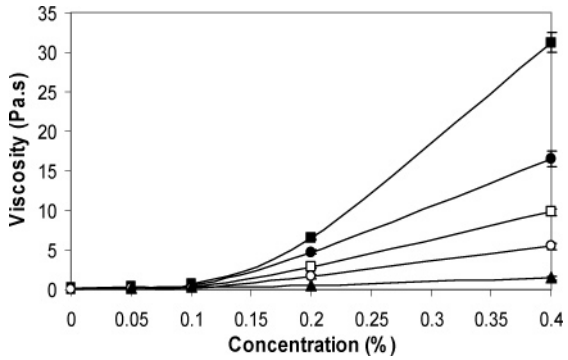

Figure 4. Effect of xanthan concentration on viscosity of egg white (■, □) and yolk emulsions (●, ○), determined at shear rates 1.5 (solid symbols) and 6 s^{-1} (open symbols); xanthan solution (▲).

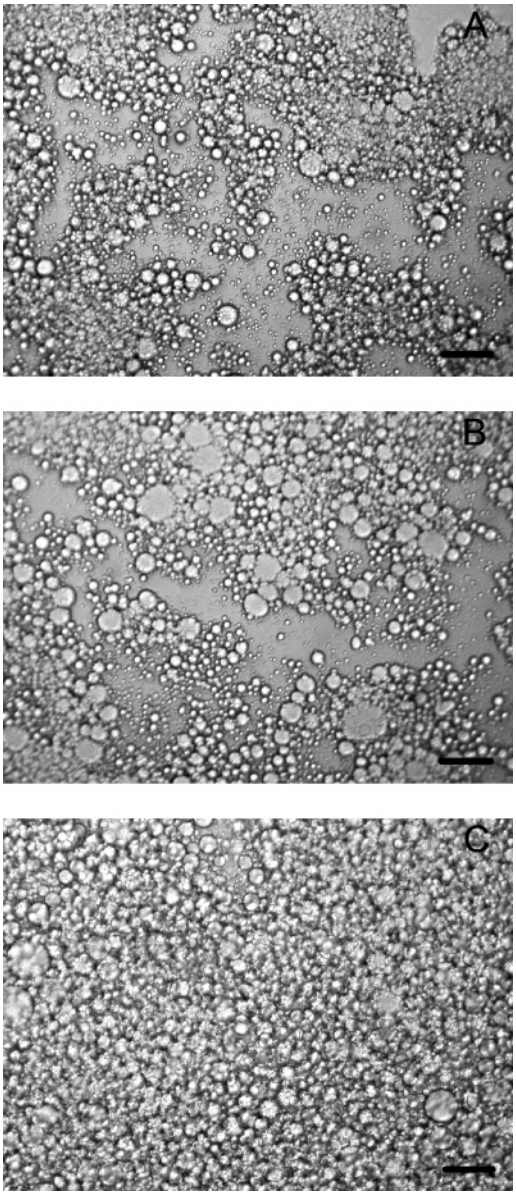

Figure 5. Photomicrographs of 30% o/w emulsions prepared with egg white and containing 0 (**A**), 0.1 (**B**), and 0.4% (**C**) xanthan. Bar length is 30 μm. Photographs were taken without previous dilution.

stacking gels were 3% and 10%, respectively, and Coomassie Brilliant Blue G250 was used to stain the gel sheets for protein. Photographs of the electrophoretic patterns were processed with the Gel Pro Analyzer v. 3.0 (Media Cybernetics, Silver Spring, MD) scanning densitometer software to determine the percentage of protein of each band.

Statistical Analysis. All experiments were repeated at least three times and the data were analyzed with the one-way ANOVA program. The level of confidence was 95%. Significant differences between means were identified by the LSD procedure with the statistical software package SPSS v. 8.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The effect of xanthan content on creaming behavior of emulsions prepared with either egg white or yolk is shown in **Figure 1a,b**, respectively. In the absence of xanthan, the two types of emulsions exhibited different rates of serum separation, with those based on egg white being more stable. However, the final levels of the separated serum did not differ significantly ($p > 0.05$). In the presence of xanthan, both the rate of creaming and the final serum height, and also the time needed for the appearance of the first sign of serum at the bottom of the container, differed significantly ($p < 0.05$) with the egg white emulsions being more stable. Additionally, as was expected, the stability of the samples increased with the xanthan content and, irrespective of the emulsifier used, all the emulsions were practically stable above a xanthan content of 0.10%. Since the emulsions did not exhibit any droplet coalescence over the time period studied, as preliminary experiments showed, and the initial droplet size of yolk-based emulsions was significantly lower ($p < 0.05$) compared to that of egg white emulsions (**Figure 2**), the differences in creaming behavior between the two types of emulsions should be sought in the way the emulsion droplets interacted to form a droplet network and the strength of droplet−droplet interactions that in turn affected the process of droplet rearrangement and network collapse during storage (*12*−*14*).

In the absence of xanthan, the droplets of egg white emulsions appeared to interact more strongly compared to those of yolk−based emulsions and produced aggregates of a larger size when dispersed in water (**Figure 2**). When globular proteins such as

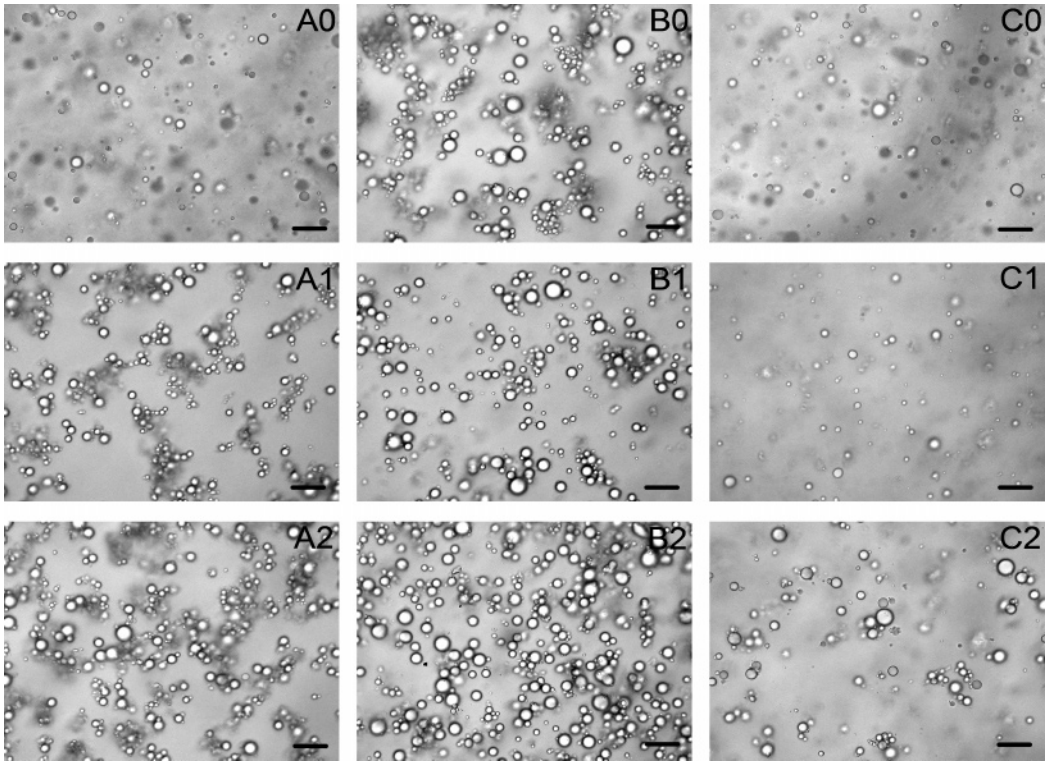

Figure 6. Photomicrographs of o/w emulsions prepared with egg white and containing 0 (**A**), 0.05 (**B**), and 0.4% (**C**) xanthan, following aging for 0 (**0**), 50 (**1**), or 100 (**2**) days. Photographs were taken after extensive emulsion dilution with 0%, 0.05%, or 0.4% xanthan buffer solutions. Bar length is 30 μm.

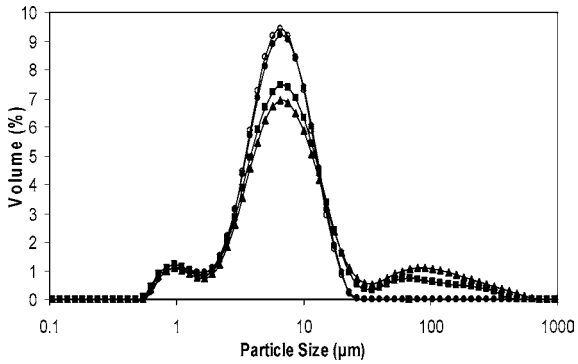

Figure 7. Influence of aging and pretreatment on the particle size distribution of egg white protein 30% o/w emulsions. Fresh (●) and aged for 100 days (○) emulsions, pretreated with SDS and DTT; aged for 50 (■) and 100 (▲) days emulsions, pretreated with SDS.

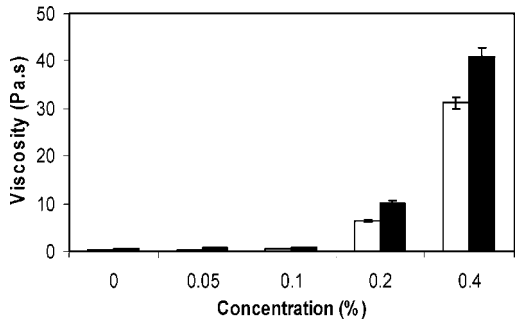

Figure 8. Effect of xanthan content on the viscosity at shear rate 1.5 s^{-1} of a fresh egg white protein 30% o/w emulsion (open bars) and following aging for 100 days (solid bars).

those of whey adsorb to the droplet surfaces of an o/w emulsion, they suffer surface denaturation and the process of conformational rearrangement may take several hours to be completed. The adsorbed molecules then expose reactive amino acid groups originally located in the interior of the native protein structure, leading to increased hydrophobic interactions between surface-adsorbed protein molecules. Intermolecular disulfide bond formation may also take place due to exposure of sulfur amino acids. In the absence of strong electrostatic repulsion between the emulsion droplets, for example, at pH close to the isoelectric point of the protein and/or at high ionic strength, the van der Waals attraction between the droplets is expected to dominate the electrostatic repulsion forces and the droplets will flocculate at a distance corresponding to the deep primary minimum of the overall potential interaction curve leading to the formation of strong droplet aggregates. Hydrophobic interactions between protein molecules adsorbed on neighboring droplets are expected to contribute to the strength of interdroplet interactions, while disulfide bridge formation should also play a role (*15*).

As shown in **Figure 2**, in the absence of xanthan, the emulsions prepared with egg white were more strongly flocculated than those of yolk. Additionally, the flocs of the former emulsions broke down into single droplets only when the emulsions were treated with SDS plus DTT, indicating that both hydrophobic interactions and disulfide bridges contributed to the formation of droplet aggregates. In the case of yolk emulsions, agitation in SDS solutions sufficed to disperse the flocs.

Ovalbumin appears to be the dominant egg white protein adsorbed at the emulsion droplet surface as its percentage at the oil−water interface is significantly higher compared to the one found in the white fraction of egg (**Figure 3**). Thus, the relative content of ovotransferrin, ovalbumin, ovomucoid, and lysozyme of egg white was 8.6%, 74.6%, 10.2%, and 6.6%, respectively, while the relative percentage of the first three proteins in the adsorbed fraction to the droplet surfaces was 6.7%, 86.8%, and 6.5% and lysozyme was completely absent

from the interface. The ovalbumin molecule has four cysteine residues and one disulfide bridge. Following adsorption to air−water interfaces, ovalbumin molecules unfold and rearrange, exposing hydrophobic and sulfur amino acids, previously buried in the interior of the molecule. As a result, hydrophobic interactions and disulfide bonds may develop between unfolded molecules, leading to the formation of a strong interfacial membrane (*4*). In the case of egg white-based emulsions, it may be hypothesized that, by analogy with the other globular proteins, under the environmental conditions prevailing in the continuous phase of the system, for example, pH close to the isoelectric point of ovalbumin and high ionic strength, the oil droplets will come very close to each other and their adsorbed protein layers will interact through hydrophobic and disulfide bonds, leading to strong droplet aggregate formation. On the other hand, yolk lipoproteins, the main emulsifiers of egg yolk, due to their flexible and surface-penetrating molecular structure, may rearrange at the oil droplet surfaces in such a way as to direct most of their hydrophobic groups toward the oil phase (*16*). As a result, the possibility of droplet−droplet interactions through hydrophobic effects is minimized. Additionally, extensive disulfide bridge formation between proteins adsorbed on neighboring droplets is not expected to take place. Yolk lipoprotein molecules are known to contain sulfur amino acids that may become involved in intermolecular covalent bond formation, following denaturation by heat treatment, and lead to the development of a gel network structure. Hydrophobic interactions appear to dominate the molecular interactions that result in the development of the network structure, while the disulfide bridges formed between unfolded protein molecules play a complementary role (*17*), in straight contrast to egg white-based gels that owe their high elasticity and cohesiveness to intermolecular disulfide bridges as well as to hydrophobic interactions (*18*).

The differences in droplet−droplet interactions between the egg white and yolk emulsions are reflected in their rheological properties as influenced by xanthan concentration (**Figure 4**). Thus, for the same xanthan content the white-based emulsions exhibited significantly higher ($p < 0.05$) viscosity values, irrespective of the shear rate conditions applied, indicating that the residual emulsion structure upon application of shear was made up of larger and/or more strongly flocculated droplet aggregates than the yolk emulsions.

Under the microscope, egg white emulsions, in the absence of xanthan or at relatively low polysaccharide content, appeared as randomly flocculated, phase-separated systems, while above 0.20% xanthan, phase separation into droplet-rich and droplet-depleted areas disappeared and the droplets were evenly distributed against a continuous phase background (**Figure 5**). Following dilution of emulsion samples with their respective continuous phase, single droplets and a limited number of small-sized flocs were detected in the absence of xanthan or above 0.10% xanthan, while at relatively low polysaccharide concentrations large droplet aggregates were detected (**Figure 6**). Depletion effects originating from the presence of xanthan could have contributed to the persistence of droplet−droplet interactions during dilution and to the appearance of large flocs at low polysaccharide contents. Such effects probably resulted in the deepening of the primary minimum of the potential interaction curve, originating from the combination of van der Waals, electrostatic, and hydrophobic interdroplet forces. At higher xanthan contents, immobilization of dispersed oil droplets in a weak gel-like network structure, formed by the polysaccharide molecules, which exhibited high low-shear viscosity, slowed down the flocculation and droplet rearrangement processes (*19*, *20*). According to Moschakis et al. (*21*), the rate of microstructure evolution of casein-based emulsions containing xanthan depended on polysaccharide content. At a relatively high xanthan concentration, the relaxation rate of xanthan-rich regions was relatively low and these regions became spherical, due to interfacial tension effects, after long aging time periods, since the local viscoelasticity in the region was very high. Additionally, droplet interaction and rearrangement processes within the xanthan-depleted region were also expected to be greatly retarded, leading to an extended droplet network with an open structure that could be dispersed into single droplets and small-sized aggregates upon dilution and stirring.

Following storage over a period of 50 days, both the control egg white emulsion as well as the emulsion with 0.20% xanthan flocculated and the flocs were not dispersed upon agitation, while the sample containing 0.40% xanthan required longer aging times to produce strong flocs (**Figure 6**). The slow process of xanthan- and droplet-rich phase structure relaxation, both dependent on the local xanthan concentration, should have been the reason for the low rate of droplet−droplet interaction and rearrangement processes and the formation of strong droplet aggregates at high xanthan concentrations. Irrespective, however, of the presence or absence of xanthan from the emulsion, droplet interaction during storage appeared to involve both hydrophobic bonds and disulfide bridges since a combination of SDS plus DTT was needed to fully disperse a system aged for 100 days (**Figure 7**). Depending on xanthan concentration, the final droplet−network structure may collapse, leading to extensive emulsion creaming, or alternatively retain its open structure since droplet rearrangement is greatly slowed down by the presence of xanthan and, additionally, by the establishment of stronger droplet−droplet interactions that result in significantly higher ($p < 0.05$) emulsion viscosity values at relatively increased xanthan contents (**Figure 8**). The emulsion, therefore, on aging may gradually become more stable until structure consolidation takes place to an extent that a practically stable system is produced with aging time.

In conclusion, egg white protein appears to function very satisfactorily as emulsifier, producing salad dressing emulsions that exhibit a remarkable stability against oil droplet coalescence. The major egg white protein, ovalbumin, dominates the adsorption process as it is found at relative concentrations as high as 87% of the adsorbed protein fraction. Although, however, the egg white-based emulsions exhibited higher stability against creaming than those prepared with yolk, xanthan gum addition at a concentration higher than 0.10% was necessary to obtain fairly stable emulsions. At a lower xanthan content, droplet−droplet interactions, attributed to the depression of repulsive electrostatic forces between neighboring droplets under the conditions prevailing in dressings (low pH, relatively high ionic strength), lead to strong floc formation involving hydrophobic interdroplet interactions and disulfide bridges. Above 0.10% xanthan, floc formation is a very slow process since, following phase separation into droplet-rich and xanthan-rich regions, structural relaxation phenomena are greatly slowed down by the high local viscosity of the xanthan solution, leading to practically stable emulsions.

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Received for review May 9, 2006. Revised manuscript received October 10, 2006. Accepted October 21, 2006.

JF061306D