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Emulsion-stabilizing properties of chitosan in the presence of whey protein isolate: Effect of the mixture ratio, ionic strength and pH

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Abstract

The emulsion-stabilizing properties of a chitosan preparation were compared as a function of the whey protein isolate/chitosan mixture ratio (WPI/CNI) and the ionic strength (μ), at pH 5.5 and 6.0. At both pH conditions, general decreases in emulsion stability towards charge neutralization flocculation and syneresis were observed at WPI/CNI > 5. This was particularly evident at pH 6.0, due to a lower surface net charge (lower electrostatic stabilization). In counterpart, when μ was increased, the higher load of chitosan at pH 6.0 produced higher stabilities (higher steric stabilization), in spite of comparable decreases of surface net charge at both pH conditions. The transition from soluble to insoluble protein–chitosan complex formation in mixtures at pH 6.0 and WPI/CNI > 5.0 was due to an emulsion destabilization towards syneresis, whereas soluble complex formation at pH 5.5 also produced syneresis. It showed that soluble protein–chitosan adsorbing complex formation prior homogenization is not essentially linked to emulsion stabilization.

Keywords: Chitosan; Whey protein isolate; Emulsion; Stability; Interface; Adsorption; Complexes

1. Introduction

In many model emulsion systems containing globular proteins (P) and anionic polysaccharides (PS), many studies have shown the destabilizing effect of increasing μ (Chanamai & McClements, 2002; Demetriades & McClements, 1999; Galazka, Dickinson, & Ledward, 2000; Keowmaneechai & McClements, 2002), or increasing the protein/polysaccharide (P/PS) ratio in mixtures or complexes (Braudo, Plaschina, & Schwenke, 2001; Dickinson & Galazka, 1991b; Galazka et al., 2000; Larichev, Gurov, & Tolstoguzov, 1983; Lippi & Taranto, 1981; Xie & Hettiarachchy, 1997). Depending on PS addition after or before emulsification with P emulsifier, the interfacial P/PS coadsorption was shown to be, respectively, performed by PS complexation on the primary adsorbed P layer (Dickinson, 1995, 1996, 1998a, 1998b), or by P/PS complex adsorption (Braudo et al., 2001; Dickinson & Galazka, 1991b, 1992; Kato, Tsutomo, & Kobayashi, 1989; Lippi & Taranto, 1981; Mishra, Mann, & Joshi, 2001). In the former case, high P/PS mixture ratios (low PS concentrations) usually induced a bridging flocculation. In the latter case, high P/PS mixture ratios usually produced insoluble complexes having poor emulsifying or stabilizing properties (Braudo et al., 2001; Xie & Hettiarachchy, 1997) mainly leading to a charge neutralization flocculation effect (McClements, 1999, chap. 3; Moreau, Hyun-Jung, Decker, & McClements, 2003; Russel, 1993, chap. 1). However, very few studies have been done on P/PS complexes containing chitosan (Braudo et al., 2001; Hattori, Numamoto, Kobayashi, & Takahashi, 2000).

In this paper, the effects of the P/PS mixture ratio and ionic strength (μ) on the stabilizing properties of chitosan were presented in a model emulsion system containing whey protein isolate (WPI) as emulsifier. A chitosan preparation called CNI was used in this study because of its good stabilizing potential shown in a previous study where a central

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composite experimental design (pH × % CNI × % WPI) revealed its favourable stabilizing effect at pH 5.5 and 6.0 (Laplante, Turgeon, & Paquin, 2002, 2004). However, the comparison of emulsion stability between those pH conditions was inappropriate because of unrelated factorial combinations of CNI and WPI concentrations. Those pH were thus compared here under various conditions of whey protein isolate/chitosan mixture ratios (WPI/CNI) and ionic strengths (μ), where a low concentration of CNI (0.1%) was used in order to avoid any viscosifying effect. We also achieved an experiment on WPI/CNI complex formation by studying the effect of WPI/CNI on yields of soluble vs. insoluble complex formation, and their impact on emulsion stability. Consequently, the results of this study would permit a more accurate knowledge of the limiting range of pH, mixture ratios, and μ conditions for emulsion-stabilizing properties of chitosan in view of potential applications, with particular attention on its stabilizing mechanisms (surface net charge, interfacial adsorption concentration).

2. Materials and methods

2.1. Materials

The chitosan preparation, CNI (viscosity average Mw = 1494 kDa; 78.1% DD), was kindly supplied by Marinard Biotech Ltd. (Rivière-au-Renard, QC, Canada). This chitosan preparation, the whey protein isolate (Davisco International, Le Sueur, MN), the canola oil and the lipophilic dye Red Oil O (Sigma, St. Louis, MO) (0.06% (w/v) in oil) were the same as reported earlier (Laplante et al., 2002, 2004). Reagents were purchased from Fisher (Pittsburgh, PA) and Sigma–Aldrich (St. Louis, MO). Distilled water was used in all preparations.

2.2. Emulsion preparation

Each emulsion was made at 21 °C from 180 ml of solution containing required concentrations of CNI and WPI in 0.2 M AcOH and 0.02% (w/v) sodium azide, with 20 ml of canola oil, in order to get the complete emulsion containing 10% (v/v) oil. The pH adjustments were made with NaOH. Adjustments of pH at 6.0 and 5.5 have, respectively, produced ionic strengths of 0.095 and 0.088 M. The ionic strength (($[Na^+] + [Cl^-]$)/2) was adjusted by adding NaCl. Once the oil was added, a pre-mixing was done with Ultra-Turrax (Janke-Kunkel, GmbH) for 30 s, with an electrical input of 30 V. The homogenization was done with an Emulsiflex-C5 homogenizer (Avestin, Canada) in two passes (41.4 and 20.7 MPa) at 21 °C.

2.3. Stability of emulsions during storage

Comparison of the evolution of phase separation between each treatment was done over a period of 21 days by daily measurements of the thickness of serum layer (syneresis) (Cao, Dickinson, & Wedlock, 1990; Dickinson, 1996, chap. 16; Galazka et al., 2000), or creamed phase (gradient creaming). As reported earlier (Laplante et al., 2002, 2004), the gradient creaming is defined as the distinctive reddish creamed oil layer formed on the top of emulsions as a result of droplet coalescence, whereas syneresis is defined as the distinctive clear or semi-transparent lower serum phase resulting from droplet flocculation. Results were compared as percentages of total emulsion height in tubes (9.6 cm) for syneresis as well as for gradient creaming.

2.4. Particle size determination

Immediately after emulsification and 21 days later, the volume-weighted average diameter of droplets in emulsions ($d\bar{v}$) was determined by photon correlation spectroscopy (PCS), using a Nicomp 370 Submicron Particle Sizer (Pacific Scientific, Hiac-Royce Instruments Division, Menlo Park, CA). The parameter ($d\bar{v}$) is defined as the average diameter of a sphere having the same volume as the particle (Everett, 1985). It was used in this study because it is the best droplet size estimation compatible with PCS techniques, it is well suited to describe large particles such as those found in milk or dairy emulsions and it can also be used directly with any stability model using particle size (Stokes law, DVLO theory) (Robin & Paquin, 1991). Prior to analysis, each emulsion sample was agitated 5 min in dissociating buffer (8 M urea, 50 mM EDTA, 7 mM β-mercaptoethanol, pH 6.0, viscosity = 1.70 Cps, refractive index = 1.401, 20 °C, and sample dilution = 1:50 (v/v)). The appropriate final dilution was adjusted to obtain a photo-pulse rate around 300 kHz. An acquisition run time of 15 min was done three times for each sample, permitting good Gaussian distribution fitting ($\chi^2 < 2.0$) and reproducibility. Other details on the method are provided by Robin and Paquin (1991). The droplet diameters were determined from non-flocculated dispersions, as verified by microscopic observations of each sample.

2.5. Flow behaviour (determination of low-shear viscosity)

From each emulsion and corresponding solution mixture, the shear rate dependency of the steady-state viscosity was measured 2 days after emulsification, using an ARES rheometer (Rheometric Scientific, Piscataway, NJ), with a Couette geometry, at a shear rate range of 0.02–500.0 s^{-1} and 25 °C. From these curves, the low-shear viscosity (η) was obtained from the reading of the viscosity at a fixed low-shear rate of 0.04 s^{-1}, where the maximal viscosity was mainly observed. All measurements were done in triplicate for each sample.

2.6. Surface net charge measurements

Measurements of surface net charge around lipid droplets were evaluated by the electrophoretic mobility (Hunter, 1981) with a Malvern Zetasizer 4 system (Malvern

Instruments Ltd, Worcester, UK), immediately after emulsification. Each emulsion sample was diluted 1/2000 in the same buffer conditions as the respective aqueous phase. All measurements were done in duplicate for each sample.

2.7. Protein and chitosan load at the oil–water interface

From each freshly made emulsion, a weighted quantity was centrifuged at 40,000*g* for 60 min at 20 °C. Protein assay (BCA assay method, Pierce Co., Rockford, IL) and chitosan assay (indole method, Dishe & Borenfreund, 1950) were done with serum, taking the AcOH buffer as control. Due to the low protein content of CNI (0.254 wt%) and the low range of WPI and CNI concentrations in formulations, no interference between proteins and chitosan occurred for both determination methods. By subtracting the amount of protein and chitosan in serum from the total amount in formulation, respective concentrations in creamed layer were determined in wt% units. Those values were then divided by respective interfacial area estimated from initial droplet diameters, permitting evaluation of loads of protein and chitosan (mg/m^2 surface) (Euston, Singh, Munro, & Dalgleish, 1996; Tomas, Paquet, Courthaudon, & Lorient, 1994; Tornberg, 1978). All measurements were done in duplicate for each sample.

2.8. Determination of yields of complex formation in WPI-CN solutions

The determination of yields of complex formation and turbidity measurements were based on methods reported by Chavasit and Torres (1990) and Wang et al. (1996). The solutions were kept at 4 °C during 3 days prior to analysis in order to let sediment the insoluble complex fraction. From solution mixtures of CNI + WPI, the soluble complex (supernatant) was separated from insoluble complex (precipitate) by decantation and its turbidity was measured by the absorbance at 500 nm. Each complex fraction produced a pellet after centrifugation at 40,000*g* for 30 min at 4 °C. Those pellets were gently washed twice with deionized water, freeze-dried and weighted. The yield of soluble and insoluble complex formation was calculated on a total dry matter basis (g/g of total dry weight of WPI + CNI). Measurements were done in duplicate for each sample.

2.9. Experimental design and statistical analysis

In all the experiments, the CNI concentration has been kept constant at 0.1 wt%. In a first set of experiments, a randomized experimental design contained five levels of WPI concentrations (0–0.25–0.5–0.75–1.0 wt%). This, respectively, corresponded to those mixture ratios (WPI/CNI): 0:1 (0); 2.5:1 (2.5); 5:1 (5); 7.5:1 (7.5); 10:1 (10), which were done at pH 5.5 and 6.0. For the sake of simplicity, results were presented with corresponding values in parentheses. In a second set of experiments where WPI/CNI was kept at 5:1, a randomized experimental design contained four levels of μ (0.088–0.15–0.2–0.3 M) at pH 5.5 and four levels (0.095–0.15–0.2–0.3 M) at pH 6.0. Each treatment was repeated twice. Statistical analysis were realized by using SAS software. For each measured parameter, statistical comparison of the effects was done by using the least significant difference (LSD) multiple comparison test at $p = 0.05$.

3. Results

3.1. Comparison of phase separation evolution

The evolution of the phase separation for treatments containing various mixture ratios (WPI/CNI) at pH 6.0 and 5.5 is shown in Fig. 1a. The two types of phase separation phenomena observed (e.g., syneresis and gradient creaming) have previously shown to be caused by droplet flocculation and coalescence, respectively (Laplante et al., 2002, 2004). In the presence of CNI alone (WPI/CNI = 0), instability was observed due to a rapid droplet coalescence leading to quick gradient creaming, which seemed to be favoured at pH 5.5. This droplet coalescence was confirmed by microscopic observations of larger droplets without any presence of flocculation. The highest stabilities were observed from WPI/CNI = 2.5 to 5.0, as seen by a minimal gradient creaming at both pH conditions. At WPI/CNI = 7.5, a slight destabilization towards syneresis occurred mainly at pH 6.0, whereas at WPI/CNI = 10, such type of destabilization was the highest and comparable at both pH conditions.

The evolution of phase separation for treatments containing various ionic strengths (μ) at pH 6.0 and 5.5 is shown in Fig. 1b. At pH 6.0, the increase of μ resulted in a transition from low gradient creaming to low syneresis above $\mu = 0.095$ M. At pH 5.5, it resulted in a transition from low gradient creaming to high syneresis above $\mu = 0.088$ M, showing a lower stability against the increase of μ.

3.2. Comparison of droplet size ($d\bar{v}$)

The effect of WPI/CNI (Fig. 2a) and μ (Fig. 2b) on the volume average droplet diameter ($d\bar{v}$) at pH 6.0 and 5.5 is shown initially ($d\bar{v}_o$) and after 21 days ($d\bar{v}_{21}$). In some conditions, the effect of WPI/CNI on droplet sizes (Fig. 2a) generated important destabilizations, as shown by the non-appearing results from WPI/CNI = 7.5 to 10, which correspond to instable emulsions producing highly flocculated lipid droplets. In fact, those dispersions were not efficiently dissociated at the reading (as observed by microscopy) and produced inaccurate droplet sizing. For $d\bar{v}_o$ results, relatively low values were obtained from WPI/CNI = 2.5 to 7.5, ranging between 0.9 and 1.7 μm, whereas the increases at WPI/CNI = 0 and 10 were, respectively, explained by slight emulsion destabilizations towards droplet coalescence and flocculation. For $d\bar{v}_{21}$ results, the indeterminable value obtained at WPI/CNI = 0

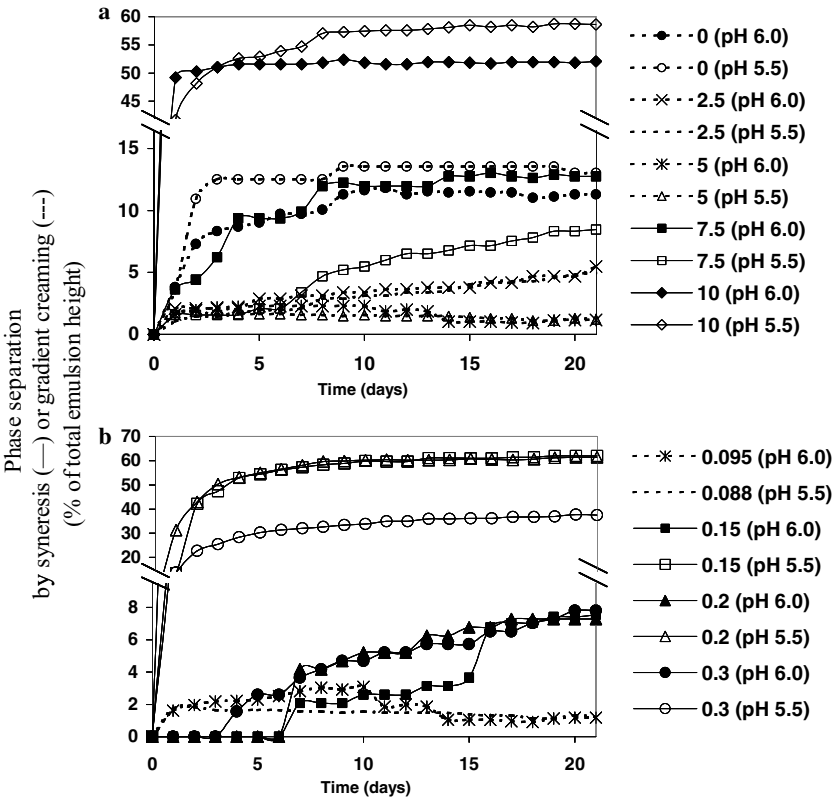

Fig. 1. (a) Effect of the mixture ratio (WPI/CNI) on phase separation evolution with 0.1% CNI at pH 5.5 and 6.0. (b) Effect of ionic strength on phase separation evolution with 0.1% CNI at pH 5.5 and pH 6.0 (WPI/CNI = 5).

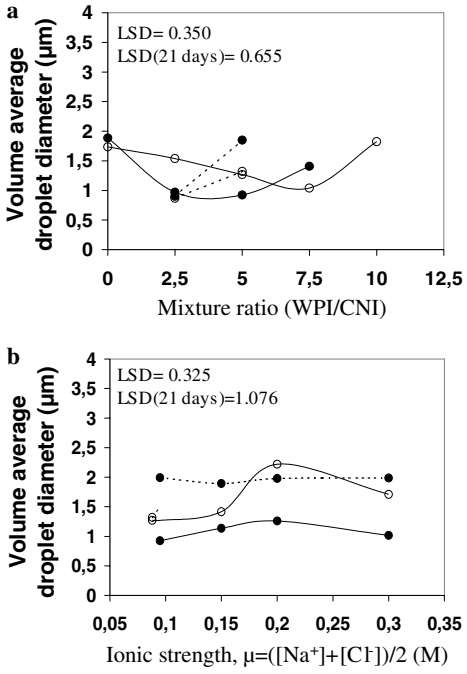

Fig. 2. Effect of the mixture ratio (WPI/CNI) (a) and μ (b) on droplet size with 0.1% CNI. Symbols: –●–, pH 6.0; --●--, pH 6.0 (21 days); –○–, pH 5.5; --○--, pH 5.5 (21 days).

was caused by an important evolution of droplet coalescence and creaming from most of the lipid phase. The $d\bar{v}_{21}$ values were only determinable at WPI/CNI = 2.5 and 5.0, clearly putting evidence of the higher stabilities against flocculation and coalescence in such conditions. No significant difference of $d\bar{v}_{21}$ values was obtained between both pH condition, even if at pH 6.0 it tended to be higher when WPI/CNI = 5.0.

Concerning the effect of μ on droplet sizes (Fig. 2b), the condition at pH 5.5 showed significant increases of $d\bar{v}_{o}$ at $\mu > 0.15$ M, whereas no significant change was observed at pH 6.0. The non-appearing $d\bar{v}_{21}$ values at pH 5.5 corresponded to instable emulsions producing highly flocculated lipid droplets and indeterminable droplet sizing, as explained earlier. At pH 6.0, $d\bar{v}_{21}$ values were increased vs. initially, but were unaffected by μ. This clearly indicates the higher emulsion stability at pH 6.0 against droplet flocculation induced by μ, in agreement with the previous lower evolutions towards syneresis (Fig. 1b).

3.3. Comparison of low-shear viscosity (η)

The effect of WPI/CNI on the low-shear viscosity (η) (Fig. 3a) revealed negligible η values from WPI/CNI = 0 to 5.0. The significant increases of η at WPI/CNI > 5.0 evince the increasing droplet flocculation destabilization, in agreement with increases of syneresis (Fig. 1a) and results of droplet sizes (Fig. 2a). Moreover, no significant difference of η with pH condition was observed. The effect of μ (Fig. 3b) only affected η values at pH 5.5, which significantly increased to a maximum at $\mu = 0.2$ M (≈2.25 Pa s).

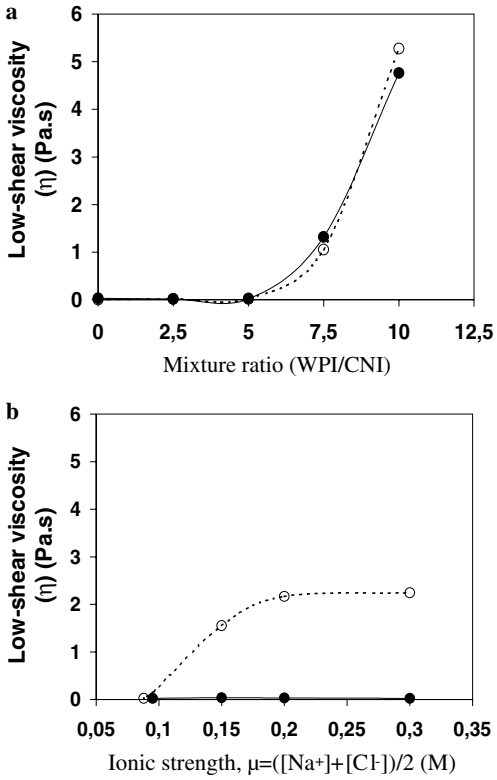

Fig. 3. Effect of the mixture ratio (WPI/CNI) (a) and μ (b) on low-shear viscosity with 0.1% CNI. Symbols: –●–, pH 6.0; --○--, pH 5.5.

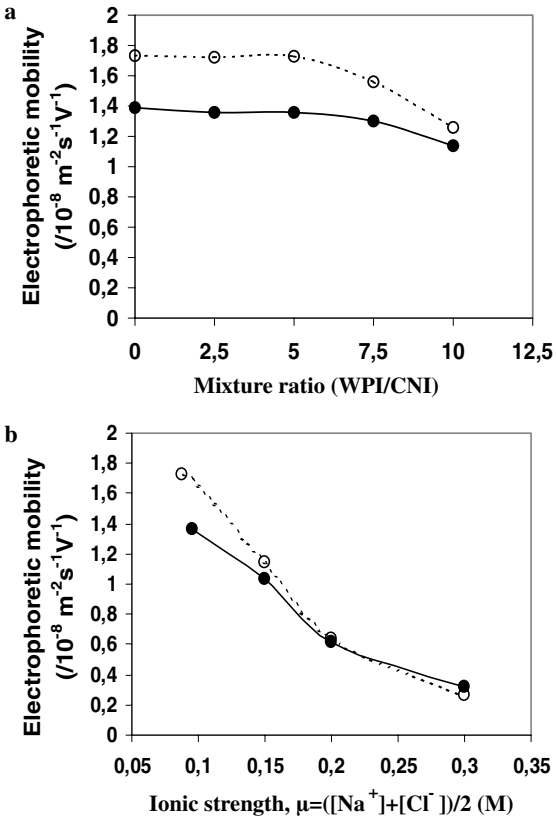

Fig. 4. Effect of the mixture ratio (WPI/CNI) (a) and μ (b) on the electrophoretic mobility (Em) with 0.1% CNI (Em × 12.45 = corresponding Zeta potential). Symbols: –●–, pH 6.0; --○--, pH 5.5.

This obviously shows the lower stability at pH 5.5 vs. 6.0 against the μ-induced droplet flocculation destabilization, in agreement with the higher syneresis (Fig. 1b), and droplet sizing results (Fig. 2b). Due to the low concentration of CNI (0.1%) in those treatments, only low η values ($\leqslant$0.04 Pa s) were obtained in corresponding mixture solutions (results not shown). Therefore, a negligible viscosity-stabilizing effect of CNI occurred in the continuous phase.

3.4. Comparison of electrophoretic mobility

The effect of WPI/CNI on the electrophoretic mobility (Em) (Fig. 4a) showed maximal Em from WPI/CNI = 0 to 5.0, where significantly higher values were observed at pH 5.5. At WPI/CNI > 5.0, a general tendency towards decreasing Em values vs. WPI/CNI was observed, particularly at pH 5.5, which became insignificantly different vs. pH 6.0 when WPI/CNI = 10. The effect of μ (Fig. 4b) showed maximal Em at low μ, being significantly higher at pH 5.5. Significant decreases of Em vs. μ were observed, showing comparable values at both pH from $\mu \geqslant 0.15$ M. Those results indicate that conditions at WPI/CNI > 5.0 or at $\mu \geqslant 0.15$ M, corresponding to emulsion destabilization towards syneresis (Figs. 1a and b) and droplet flocculation (Figs. 2a and b and 3a and b), could be explained by decreases of lipid droplet surface net charge, confirming the charge neutralization flocculation mechanism.

3.5. Comparison of interfacial concentrations (loads) of protein and chitosan

A significant increase of protein load vs. WPI/CNI was observed (Fig. 5a), with comparable values at both pH conditions. The chitosan loads were also significantly increasing with WPI/CNI (Fig. 5c). Even if values were lot much lower compared to protein loads, significantly higher values were obtained at pH 6.0. At WPI/CNI = 10 (pH 6.0) and 0 (CNI alone), load results were not determinable. This was caused by indeterminable droplet sizing at WPI/CNI = 10, and by a high variability from determinations of chitosan concentration in the creamed layer at WPI/CNI = 0, where protein load was considered as negligible. For the effect of μ, a significant decrease of protein load vs. μ was observed (Fig. 5b), with no effect from the pH. At a lot much lower extent, the chitosan loads were also significantly decreasing vs. μ (Fig. 5d), and significantly higher values were obtained at pH 6.0. From the load results, the effect of WPI/CNI and μ on the interfacial proportion of protein and chitosan was illustrated by comparing the corresponding protein–chitosan load ratios (Figs. 5e and f). Assuming a nil protein–chitosan load ratio at WPI/CNI = 0, it was increasing to maximal values at WPI/CNI = 5.0, followed by decreases at higher WPI/CNI, especially at pH 5.5, where protein–chitosan load ratios

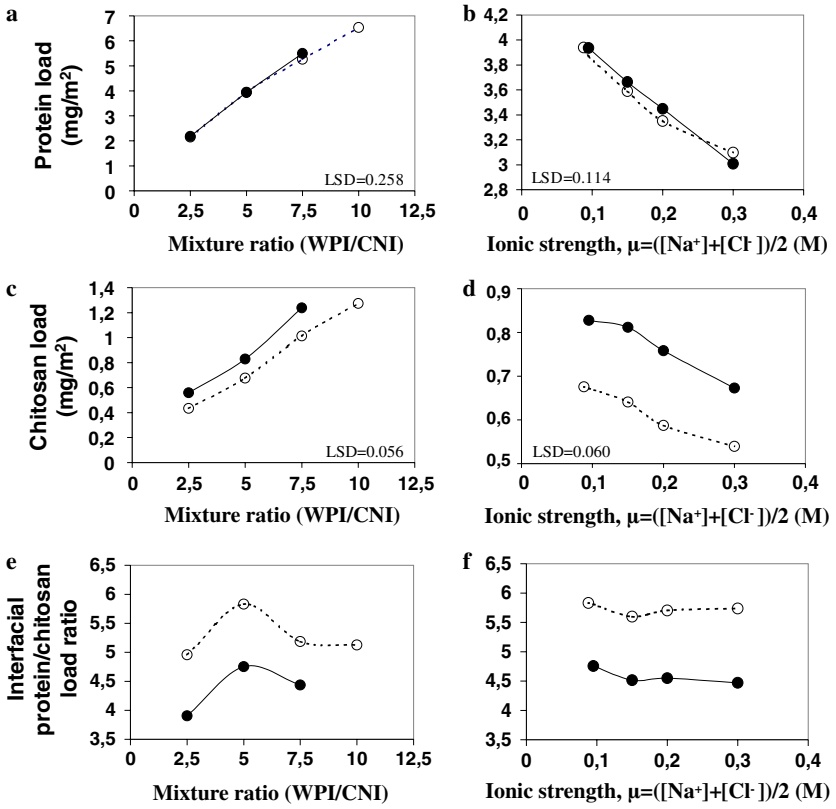

Fig. 5. Effect of the mixture ratio (WPI/CNI) (a) and μ (b) on protein load. Effect of WPI/CNI (c) and μ (d) on chitosan load. Effect of WPI/CNI (e) and μ (f) on interfacial protein–chitosan load ratio. Symbols: –●–, pH 6.0; --○--, pH 5.5.

were higher vs. pH 6.0. The effect of μ (Fig. 5f) showed no noticeable change of protein–chitosan load ratio vs. μ, which tended to remain higher at pH 6.0.

3.6. Comparison of yields in protein–chitosan complex formation vs. emulsion stability

Results of protein and chitosan loads (Figs. 5a and c) have clearly shown favourable adsorption of both components from WPI + CNI mixtures by increasing the WPI concentration from 0% to 1% in the presence of 0.1% CNI. This suggests the formation of a soluble stabilizing protein–chitosan complex which adsorbs at the oil–water interface. Therefore, to put evidence of the relationship between formation of a soluble protein–chitosan complex and its related stabilizing properties, a comparison of the yields in soluble complex formation was done at various mixture ratios (WPI/CNI) with 0.1% CNI at pH 6.0 (Fig. 6a) and pH 5.5 (Fig. 6b). A general observation of those results showed an agreement between differences in soluble complex formation and respective absorbances at 500 nm for both pH conditions. At pH 6.0, the formation of soluble complex from WPI/CNI = 2.5 to 6.0 reached a maximal yield at WPI/CNI = 5.0. Such conditions leaded to formation of emulsions with comparably good stabilities. At WPI/CNI = 6.0, an insoluble complex (amorphous precipitate) appeared in solutions without disturbing the emulsion stability. However, at WPI/CNI $\geqslant$ 7.0, the highly increased formation of insoluble complex and disappearance of soluble complex resulted in emulsions producing syneresis. This suggests an essential role of the soluble WPI/CNI complex for emulsion stabilization and the deleterious role of insoluble complex. At pH 5.5 (Fig. 6b) only an increasing formation of soluble complex vs. WPI/CNI was observed, with the yields becoming significantly higher vs. pH 6.0. However, it is worth to notice at pH 5.5 an emulsion destabilization by syneresis at WPI/CNI $\geqslant$ 7.0 which was obtained exclusively in the presence of a soluble complex. This disagrees with the previously suggested essential role of the soluble complex for emulsion stability.

4. Discussion

4.1. Effect of the mixture ratio (WPI/CNI): Emulsion stability vs. interfacial composition and net charge

The results of phase separation evolution in tubes (Figs. 1a and b), droplet sizes (Fig. 2a), and low-shear viscosity (Fig. 3a) were in good agreement with an optimal emulsion stability obtained from WPI/CNI = 2.5 to 5.0, followed by a gradual loss of stability at higher WPI/CNI, due to an increase of droplet flocculation leading to syneresis. Such destabilization was explained by a gradual loss of droplet surface net charge (charge neutralization flocculation) at

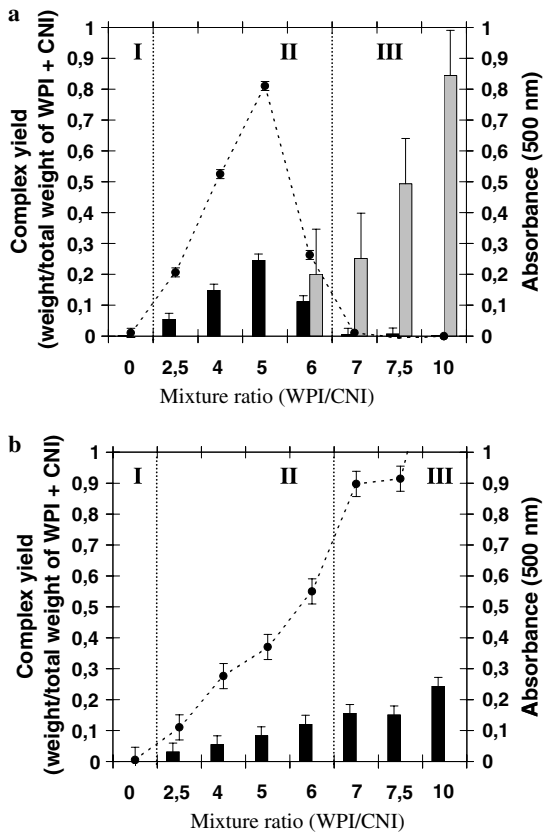

Fig. 6. Effect of the mixture ratio (WPI/CNI) on complex yields in the presence of 0.1% CNI at pH 6.0 (a) and pH 5.5 (b). The resulting phase separation phenomena of emulsions under those conditions are specified in zones, where we observed coalescence (I), stable emulsions (II), and flocculation leading to syneresis (III). Error bars refer to the corresponding LSD values. Symbols: ■, soluble complex; □, insoluble complex; --●--, Abs 500 nm.

WPI/CNI > 5.0 (Fig. 4a). Considering the effect of interfacial composition on emulsion stability, the continuous increases of protein and chitosan loads vs. WPI/CNI (Figs. 5a and c) are explained by favourable coadsorption of chitosan with protein emulsifier, governed by electrostatic interactions at pH > pI of protein (Laplante et al., 2002, 2004). Therefore, by increasing the WPI/CNI over 5.0 (Fig. 4a), the decreases of positive surface net charge and stability were expected to be explained by increasing proportions of protein in the droplet coating (protein–chitosan load ratio). Surprisingly, we rather observed an upper limit of protein–chitosan load ratio at WPI/CNI = 5.0 for both pH conditions tested. This would suggest a quick transition from low to high protein abundance in the protein–chitosan complex at WPI/CNI > 5.0, forming a primary adsorbed layer. Such layer would then favour the coadsorption of free chitosan molecules forming a secondary layer. More exactly, the higher availability of anionic groups from adsorbed proteins in a primary layer would permit stronger electrostatic interactions with chitosan in a secondary layer, resulting into higher abundance of adsorbed chitosan vs. protein from WPI/CNI = 5.0 to 7.5. However, at WPI/CNI = 10, due to a saturation of protein over chitosan in the mixture, a limitation of free chitosan molecules in the continuous phase would quickly limit this effect, as shown by stable protein–chitosan load ratios from WPI/CNI = 7.5 to 10 at pH 5.5.

About the effect of pH at WPI/CNI > 5.0, the slightly higher stability against syneresis at pH 5.5 vs. pH 6.0 could be explained by the higher positively ionized state of chitosan amino groups ($pK_a = 6.3$), allowing a higher droplet surface net charge (higher electro-repulsive stabilizing effect between droplets) (Fig. 4a). It also explains the higher surface net charges produced from lower loads of chitosan at pH 5.5. In fact, the higher availability of cationic charges per chitosan molecule would favour the formation of electrostatic protein–chitosan complexes with higher protein content, positive net charge, and solubility than complexes obtained at pH 6.0, as seen from the absence of insoluble complexation at pH 5.5 (Fig. 6b). The small beneficial effect of the slightly lower μ at pH 5.5 (0.088 M) compared to 0.095 M at pH 6.0 would also favour the surface net charge by reducing the charge screening effect around lipid droplets. Therefore, the higher electrostatic stabilizing control at pH 5.5 was the determining mechanism explaining its slightly higher stability, whereas the higher load of chitosan at pH 6.0 (higher steric-stabilizing control) could explain why stabilities observed at both pH conditions remained relatively close.

4.2. Effect of ionic strength on emulsion stability

Results of phase separation evolution in tubes (Figs. 1a and b), droplet sizes (Fig. 2b), and limiting low-shear viscosity (Fig. 3b) have clearly shown at pH 5.5 an important loss of emulsion stability vs. μ which favoured the charge neutralization flocculation and syneresis. At pH 6.0, a nearly negligible loss of stability was observed. The less charged state of chitosan at pH 6.0 performed a less important decrease of surface net charge vs. pH 5.5 when μ was increased from 0.088 to 0.15 M (Fig. 4b). This is due to a lower susceptibility of the less densely charged structures to the charge screening effect (McClements, 1999, chap. 3). Moreover, at pH 6.0, the higher chitosan loads and lower protein–chitosan load ratios vs. μ (Figs. 5d and f) showed a higher adsorption efficiency of chitosan due to more favourable hydrophobic interactions with lipid surface. This could then allow a better steric-stabilizing control. Therefore, the steric stabilization in this emulsion system is the main stabilizing mechanism against the increase of μ.

4.3. Role of the protein–chitosan complex formation in solution vs. emulsion stabilization

In this study, we confirmed the presence of protein–chitosan complex formation at pH 6.0 and 5.5 (Figs. 6a and b). In both cases, the significant positive correlation ($R^2 > 0.95$) between the total load of adsorbed polymer (protein + chitosan load) vs. the total protein–chitosan

complex yield in solution confirmed the interfacial adsorption of the complex. At pH 6.0, the emulsion stabilization against syneresis was dependent on the formation of a soluble adsorbing protein–chitosan complex. The beneficial effects of soluble complexes on surface net charge and hydration properties could explain their role in emulsion stabilization (Dickinson, 1995, chap. 15; Dickinson & Galazka, 1991a), whereas insoluble complexes have sometimes been proven to disfavour such essential requirements (Benichou, Aserin, & Garti, 2002; Dickinson, 1995, chap. 15). However, the presence of soluble protein–chitosan complex prior homogenization was not necessarily linked to emulsion stability in our model system. In fact, a complementary experiment at pH 6.0 (not shown) revealed comparable emulsion stability and characteristics between an emulsification produced from WPI + CNI mixture (presence of protein–chitosan complex) and a sequential emulsification process using protein in the first step (absence of protein–chitosan complex). This clearly confirms that emulsion stabilization could either be performed by interfacial protein–chitosan complex adsorption or by chitosan complexation on a primary adsorbed protein layer, those complex formations being mainly based on electro-attractive forces.

5. Conclusions

In a model emulsion system containing WPI as emulsifier in the presence of 0.1% chitosan (CNI) as stabilizer, close decreases of emulsion stability towards charge neutralization flocculation and syneresis were obtained at pH 6.0 and 5.5, when the mixture ratio (WPI/CNI) was higher than 5.0. The close stabilities obtained at both pH conditions were explained by a higher droplet surface net charge stabilization from chitosan adsorption at pH 5.5, which was counterbalanced at pH 6.0 by a higher steric stabilization due to higher chitosan adsorption. The higher steric stabilization at pH 6.0 vs. 5.5 condition also explains its higher resistance to μ-promoted flocculation and syneresis. Even if conditions permitting soluble protein–chitosan complex formation at pH 6.0 produced emulsion stability, it was not the case at pH 5.5. Therefore, complex formation before homogenization is not necessarily linked to emulsion stability. Those results would permit a better explanation of the influence of conditions on the stabilizing mechanisms of chitosan and would contribute to rationalize the potential applications of chitosan in view of food emulsion applications.

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