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Emulsion stabilizing properties of various chitosans in the presence of whey protein isolate

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Abstract

The emulsion stabilizing potential of chitosans (CN) with various molecular weights and degrees of deacetylation (CNI = 1494 kDa, 78.1%DD; CNHI = 694 kDa, 78.5%DD; CNHI2 = 319 kDa, 78.5%DD; CNHK = 749 kDa, 67.7%DD) was compared in the presence of whey protein isolate. Phase separation evolutions revealed minimal stabilizing concentrations against syneresis from 0.1 to 0.125% in most CN preparations, except CNHI2 where it was higher (0.225%). Those stabilizing concentrations are the result of interfacial coadsorption saturation of CN with proteins, favouring interfacial electrostatic and steric stabilizing mechanisms. The emulsion characteristics (droplet size, limiting low-shear viscosity, and surface net charge), mostly distinctive at 0.1%CN, revealed the following order of stabilizing potentials: CNI ≈ CNHI > CNHK > CNHI2, which is in agreement with respective phase separation evolutions. The lower stabilizing potential of CNHI2 is explained by lower interfacial coadsorption efficiency with protein. In spite of a higher interfacial load of CNHK vs. CNI and CNHI, its lower stabilizing potential is essentially explained by a lower surface net charge.

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

1. Introduction

Protein (P) and polysaccharide (PS) mixtures are found in a great variety of food emulsions ranging from mayonnaise to ice cream (Dickinson, 1992, 1995a). In such systems, P are essentially used as emulsifiers. For food polysaccharides (mainly anionic PS), many kinds are known to have beneficial stabilizing properties by their thickening or gelling effect on the continuous phase (xanthan, carrageenan, alginate, carboxymethylcellulose, pectin) (Cao, Dickinson, & Wedlock, 1990; Dickinson, 1996; Dickinson and Galazka, 1991; Samant, Singhal, Kulkarni, & Rege, 1993). Other PS (arabic, guar, locust bean, and fenugreek gums) are known to stabilize emulsions through interfacial adsorption (Benichou, Aserin, & Garti, 2002; Dickinson, Galazka, & Anderson, 1991). However, depending on solvent conditions (e.g. pH, ionic strength), the stability and textural control from emulsions containing P–PS mixtures could be considerably affected by the strength and nature of the interfacial P–PS interactions.

When solvent conditions favour thermodynamic incompatibility between P and PS, repulsive interactions between adsorbed P and PS will predominate in emulsions, leading to a depletion flocculation and serum-phase separation (Tolstoguzov, 2000). Otherwise, when they favour P–PS interactions (complexation), emulsion formation will lead to interfacial P–PS coadsorption (Braudo, Plaschina, & Schwenke, 2001; Dickinson & Galazka, 1991, 1992; Kato, Tsutomo, & Kobayashi, 1989; Lippi & Taranto, 1981; Mishra, Mann, & Joshi, 2001). This could then lead to emulsion stabilization by increasing the surface net charge (electrostatic repulsion) and adsorption concentration (steric repulsion) between lipid droplets (Dickinson, 1995a, 1996; McClements, 1999; Syrbe, Bauer, & Klostermeyer, 1998. However, at high P/PS mixture ratios (low PS concentrations), insoluble P–PS complexes having poor emulsifying or stabilizing properties would be produced (Braudo et al., 2001; Xie & Hettiarachchy, 1997), leading to charge

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neutralization and bridging flocculation effects (Dickinson, 1995a, 1998; McClements, 1999; Moreau, Hyun-Jung, Decker, & McClements, 2003; Russel, 1993; Ward-Smith, Hey, & Mitchell, 1994).

The most intensively studied P–PS complexes are electrostatic complexes, formed by favourable electrostatic interactions between P and anionic PS, mainly at pH < pI of P and low ionic strengths (Dickinson, 1995a). The emulsifying and stabilizing properties of electrostatic complexes from various P–PS mixtures were highly improved as compared to P alone. Many examples have been reported: serum albumin-dextran sulphate, lysozyme-dextran sulphate, serum albumin-chondroitin sulphate, and α-lactalbumin-chondroitin sulphate (Kato et al., 1989), whey protein concentrate-pectin (Mishra et al., 2001), legumin-chitosan (Braudo et al., 2001; Plashchina et al., 2001), and soy protein isolate with xanthan gum or alginate (Lippi & Taranto, 1981; Xie & Hettiarachchy, 1997). Such improvement is related to increased protein hydration at the interface. With serum albumin-dextran sulphate or lysozyme-dextran sulphate mixtures (in 1:3 ratio), a high molecular weight of dextran sulphate (500 kDa) showed better emulsifying properties of complexes than a low molecular weight (5 kDa) because of increased steric stabilization (Kato et al., 1989). Moreover, a higher charge density from PS in P–PS complexes favours emulsion stability, as reported with ι-carrageenan (highly sulphated) vs. κ-carrageenan (less sulphated) (Wang, Gao, & Dublin, 1996), or with dextran sulphate (anionic) vs. dextran (non-ionic) (Dickinson & Galazka, 1992), caused by increased interfacial electrostatic stabilization.

Chitosan (CN) is a unique PS of a cationic nature with hydrophilic zones rich in glucosamine and hydrophobic zones rich in *N*-acetyl-glucosamine, that enable it to adsorb on o/w interfaces (Schulz, Rodriguez, Del Blanco, Pistonesi, & Agulló, 1998) and interact with P emulsifiers at pH > pI (Braudo et al., 2001; Laplante, Turgeon, & Paquin, 2002). Soluble electrostatic P–CN complexes obtained at pH ≥ pro-protein pI were shown to favour emulsifying and stabilizing properties of P at low P/CN ratios up to 0.2–0.3 (Braudo et al., 2001). Literature suggests that one limiting factor for the application of CN as an emulsion stabilizer is the huge variability of the characteristics available such as molecular weight and degree of deacetylation, which would affect emulsion stability (Cho, No, & Meyers, 1998; Del Blanco, Rodriguez, Schulz, & Agulló, 1999; Schulz et al., 1998). With a model emulsion system containing CN in the presence of P emulsifier, no previous study has been done on the relationship between the structure of CN and its stabilizing property, nor on its responsible stabilizing mechanisms. The objective of this study was to find out which range of structural characteristics of CN are the most suitable for emulsion stability, and to better understand its stabilizing mechanisms. From a previous study (Laplante et al., 2002), which enabled us to evince the stabilizing effect of adding CN to WPI, an optimal emulsion stability was observed at pH 6.0 in the presence of 0.5%WPI. Similar conditions were used to investigate the effect of various molecular weights and degrees of deacetylation of CN on its stabilizing potential. As an experimental design, the emulsion stabilities were compared at various concentrations of various CN. The most discriminative concentration of CN on emulsion stability was then studied in greater detail in order to find out and explain the relationship between CN structure and the stabilizing potential.

2. Material and methods

2.1. Material

Chitin and the chitosan preparation (called 'CNI') produced from shrimp shells (*Pandalus borealis*) were kindly supplied by Marinard Biotech Ltd. (Rivière-au-Renard, Qc, Canada). The whey protein isolate (WPI) was provided by Davisco International (Le Sueur, MN). The composition (moist weight basis) was as follows: 80.32% β-lactoglobulin, 15.80% (-lactalbumin, 3.89% bovine serum albumin, 1.95% ash, 0.57% lactose and 3.82% moisture. Canola oil was purchased from a local supermarket. A lipophilic dye (Red Oil O, Sigma Co., St. Louis, MO) was added (0.06% (w/v)) to the oil. Reagents were from Fisher (Pittsburgh, PA) and Sigma-Aldrich (St. Louis, MO). Distilled water was used in all preparations.

2.2. Production and characteristics of chitosan preparations

The CNHI and CNHI2 preparations were produced from CNI by acidolysis in a solution containing 1% CNI in 1% AcOH + 0.05 M HCl (pH = 2.0). The reaction was run at 60 °C until the Brookfield viscosity reached 0.900 and 0.062 Pa s, respectively. To produce CNHK, a batch of chitin was first deacetylated by alkaline treatment during 30 min in NaOH 50 wt% at 100 °C (chitin/NaOH ratio = 1:10). From this deacetylated product, CNHK was obtained by acidolysis, as for CNHI. The main characteristics (viscosity average molecular weight (kDa); degree of deacetylation (DD); Brookfield viscosity (Cps)) of the four chitosan preparations used in this study were as follows: CNI = (1494 kDa; 78.1%DD; 4413 Cps); CNHI = (694 kDa; 78.5%DD; 490 Cps); CNHI2 = (319 kDa; 78.5%DD; 62 Cps), and CNHK = (749 kDa; 67.7%DD; 1030 Cps). The viscosity average molecular weight was determined by capillary viscosimetry, using the AMV rolling-ball microviscometer (Anton Paar, Graz, Austria). The internal capillary and ball diameters were, respectively, 1.6 and 1.5 mm, and the solvent system was 0.5 M AcOH/0.2 M AcONa (pH 4.5, 25 °C) (Terbojevitch & Cosani, 1997). The degree of deacetylation was obtained by using the acid–base titration method (Tan, Khor, Tan, & Wong, 1998).

The Brookfield viscosity was measured with model LV-DVII, spindle #4, 60 rpm, 25 °C).

2.3. Emulsion preparation

Each emulsion was made at 21 °C using 180 ml of a solution containing the required concentrations of CN 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. pH adjustments were made with NaOH. The concn. of CN was the variable, whereas WPI concn. (0.5 wt%), pH (6.0), and ionic strength ($\mu = 0.095$ M ($[Na^+] + [Cl^-]$)/2) were kept constant. 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 homogenisation was done with an Emulsiflex-C5 homogeniser (Avestin Co., Canada) in 2 passes (41.4 and 20.7 MPa) at 21 °C.

2.4. Stability of emulsions during storage

Emulsion stability evolutions in tubes were determined by daily measurements of thickness (centimeter units), of a distinctive clear or semi-transparent lower serum phase layer (syneresis) (Cao et al., 1990; Dickinson, 1996; Galazka, Dickinson, & Ledward, 2000), or a red gradient at the upper creamed phase layer (gradient creaming) (Laplante et al., 2002). Treatments were compared on the basis of phase separation heights obtained after 21 days, either by syneresis, expressed in % of total emulsion height in tubes (9.6 cm), or by gradient creaming, expressed in % of the total free oil layer height in tubes (1.0 cm).

2.5. Particle size determination

Immediately after emulsification and 21 days later, the volume 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, USA). 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 cP, refractive index = 1.401, 20 °C, sample dilution = 1:50 (v/v)). The appropriate final dilution was adjusted to obtain a photo-pulse rate of about 300 kHz. An acquisition run time of 15 min was done three times for each sample, so as to obtain a good Gaussian distribution fitting (chi-square < 2.0) and reproducibility. Other details on the method are provided by Robin & Paquin (1991).

2.6. Flow behaviour (limiting low-shear viscosity)

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

2.7. Electrophoretic mobility of lipid droplets

The surface net charge around the lipid droplets was 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 (0.2 M AcOH, pH 6.0, $\mu = 0.095$ M) as the respective aqueous phase. The effect of CN concn. was only measured with CNI in those experiments. All measurements were done in duplicate for each sample.

2.8. Protein and chitosan concentrations in creamed layer and respective loads at the oil–water interface

From each freshly made emulsion, a weighted quantity was centrifuged at $40{,}000 \times g$ 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, using the AcOH buffer as control. By subtracting the amount of protein and chitosan in the serum from the total amount in formulation, respective concns. in the creamed layer ($[P]_L$ and $[CN]_L$) were determined in wt% units. For treatments containing 0.1% of each CN preparation and controls, the values were divided by respective interfacial area, estimated from initial droplet diameters, which provided an evaluation of P and CN loads (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.

2.9. Experimental design and statistical analysis

We produced a completely randomized experimental design using 4 types of CN with 10 levels of CN concn. (0.0125–0.025–0.05–0.075–0.1–0.125–0.15–0.225–0.3–0.45 wt%), including controls with WPI alone (0.5%), and CNI alone (0.1%). Each treatment was repeated twice. SAS software was used for statistical analysis. For each measured parameter, determinations of the standard error of the mean

for the effect of CN concn. were obtained from ANOVA, and statistical comparisons for the effect of type of CN at 0.1% were done from LSD multiple comparison test at $p = 0.05$.

3. Results and discussion

3.1. Comparison of the stabilizing potential of CN preparations from phase separation tests in tubes

The comparison of the evolution of phase separation in tubes for various concentrations of each CN preparation enabled us to observe 2 types of phase separation phenomena: syneresis (Fig. 1a) and gradient creaming (Fig. 1b). Microscopic observations of emulsions have previously shown that syneresis was caused by a droplet flocculation, whereas gradient creaming resulted from a slight coalescence (Laplante et al., 2002). The phase separation by syneresis after 21 days (Fig. 1a) was maximal from 0% CN (control with WPI alone) to 0.05%. It decreased and eventually disappeared from 0.075–0.1% CNI, 0.1% CNHI, 0.1–0.125% CNHK, and 0.15–0.225% CNHI2. Those respective ranges of minimal stabilizing concns. against syneresis were used to establish this following decreasing order of stabilizing potential between CN preparations: CNI ≥ CNHI > CNHK > CNHI2. This provided a good estimation of respective maximal WPI/CN mixture ratios above which syneresis occurs: CNI and CNHI = 6.67–5.0; CNHK = 5.0–4.0; CNHI2 = 3.33–2.22.

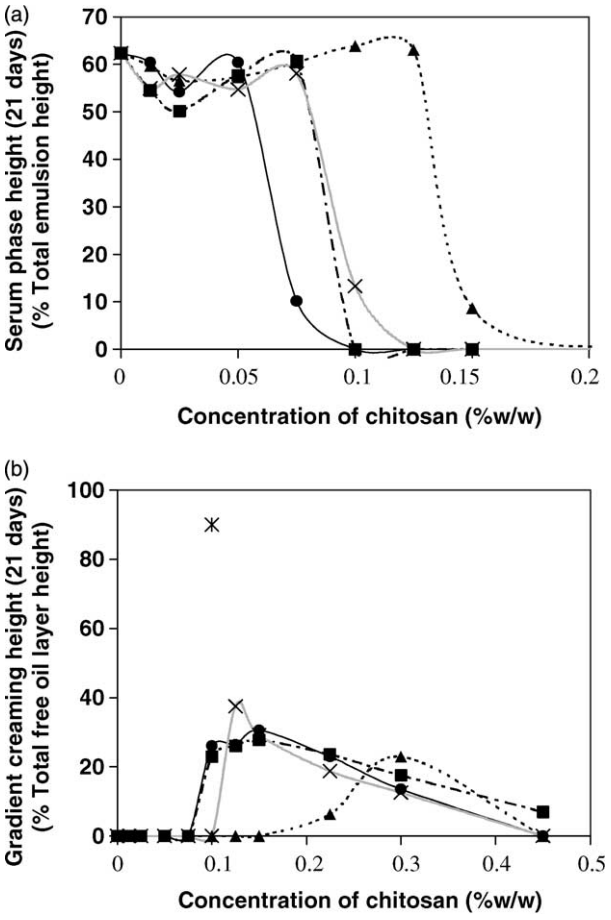

Fig. 1. Results of phase separation evolution in tubes where (a) represents the final height of syneresis after 21 days and (b) the final height of gradient creaming after 21 days. Symbols: —●— CNI; ···■··· CNHI; - -▲- - CNHI2; —×— CNHK; ✳ CNI alone.

At concns. ≥ 0.1%CN (Fig. 1b), low gradient creaming was observed with the various CN preparations, although CNHI2 exhibited this phenomenon at higher concns. A control containing 0.1% CNI alone produced the highest gradient creaming as a result of higher droplet coalescence.

Tube tests have thus clearly shown a stabilization effect against syneresis at sufficient concns. of CN in presence of WPI. From those CN concns., the gradient creaming phenomenon corresponded to much better lipid droplet dispersion stability than controls containing CNI or WPI alone, respectively, showing high coalescence or flocculation, as previously reported (Laplante et al., 2002). Among the various concns. of CN tested, 0.1% seemed the most transitory point between syneresis (Fig. 1a) and gradient creaming (Fig. 1b) for most of the CN preparations. It also provided the best discrimination between their stabilizing effect (CNI = CNHI > CNHK > CNHI2). This is in close agreement with the previous order of stabilizing potentials based on minimal stabilizing concentrations against syneresis.

3.2. Effect of treatments on lipid droplet size ($d\bar{v}$)

Greater stability discrimation between treatments can be obtained by comparing respective droplet size evolution, which is very sensible to changes in the state of droplet dispersion (e.g. coalescence). The effect of the concentration of various CN on the volume average droplet diameter ($d\bar{v}$) was shown immediately after homogenisation (Fig. 2a) and 21 days later (Fig. 2b). The non-appearing experimental points (mainly at low CN concns. from 0 to 0.075%) represent unstable emulsions conditions that produced highly flocculated lipid droplets, which were not efficiently dissociated (as observed by microscopy). As a result, inaccurate droplet sizing was obtained. Initially (Fig. 2a), low droplet sizes (0.7–1.0 μm) were observed with most CN preparations from a minimal concn. of 0.1%CN. Above this concentration, higher values were mainly obtained with CNHI2, as explained by a higher tendency towards coalescence. After 21 days (Fig. 2b), $d\bar{v}$ values for CNI, CNHI, and CNHK could still be determined and changed insignificantly over time from a minimal concn. of 0.125%, clearly showing an emulsion stabilization against flocculation and coalescence. However, from 0.15 to 0.3%, CNHI2 produced the greatest increases in $d\bar{v}$, showing higher destabilization towards coalescence. The higher increases in $d\bar{v}$ with CNHI2 in Fig. 2a,b would originate from a higher tendency towards flocculation, as indicated by

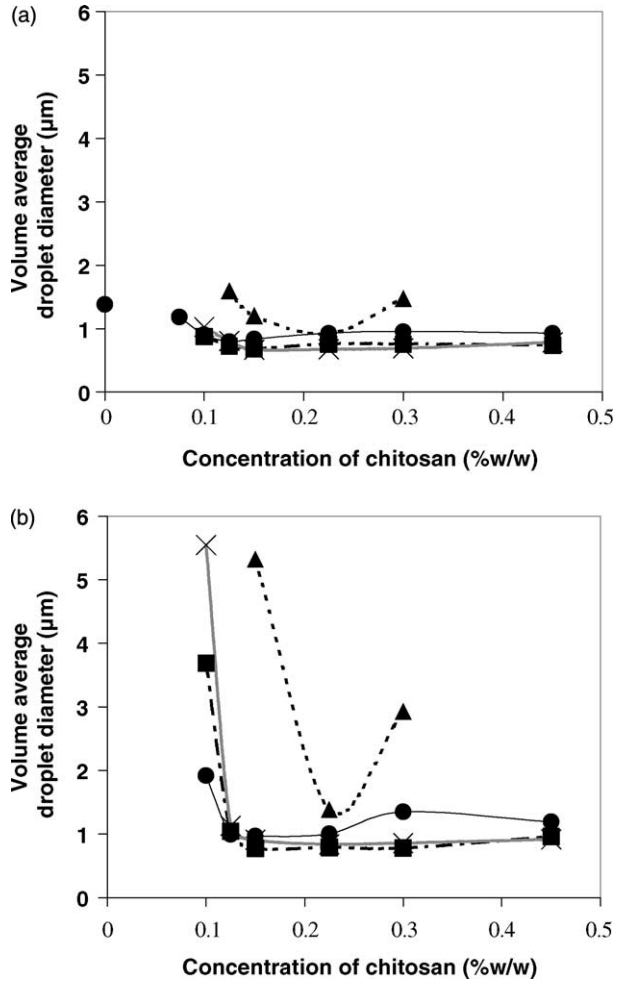

Fig. 2. Effect of concn. and type of chitosan on lipid droplet size (a) initially, and (b) after 21 days. SEM: ±0.232 (a); ±0.491 (b). Symbols: ●— CNI; ■··· CNHI; ▲-- CNHI2; ×— CNHK.

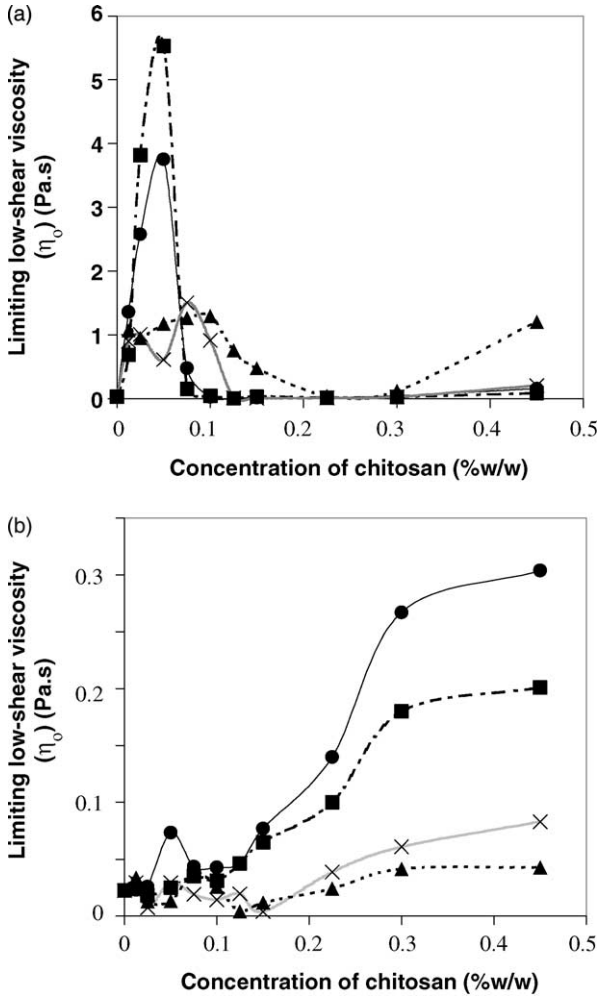

Fig. 3. Effect of concn. and type of citosan on the limiting low-shear viscosity of (a) emulsions and (b) corresponding solutions. SEM: ±0.349 in (a) ±0.029 in (b). Symbols: ●— CNI; ■··· CNHI; ▲-- CNHI2; ×— CNHK.

the fewer conditions where droplets were sufficiently dissociated for measurements.

Those results indicate that a minimal concentration of 0.125% could prevent any droplet size increase after 21 days from flocculation and coalescence with CNI, CNHI, and CNHK. This concentration is slightly above the minimal stabilizing concentration against syneresis for CNI and CNHI (0.1%), suggesting insufficiently strong flocculation destabilization at these concentrations to produce a continuous network forming syneresis (Dickinson, 1995a, 1996). In agreement with tube observations, it is worth noting that 0.1%CN seemed the most transitory point between stability and instability, and the most discriminative concentration for comparing the stabilizing potential of CN. This concentration was therefore considered later for statistical comparison of the stabilizing potential of CN preparations.

3.3. Effect of treatments on the rheological behaviour (η_o)

The effect of various concns. of each CN on the limiting low-shear viscosity (η_o) was compared in emulsions (Fig. 3a) and corresponding solutions (Fig. 3b). With each CN, values of η_o from emulsions decreased to a minimum (close to corresponding solutions) around respective minimal stabilizing concns. against syneresis. This seems to confirm that droplet flocculation phenomenon is responsible of syneresis. The highest increases in η_o were obtained with emulsions containing CNI and CNHI at 0.05% (around half their minimal stabilizing concn.). This suggests that those enough long and highly charged PS chains would favour bridging flocculation of droplets through stronger electrostatic associations and longer chain extensions in aqueous phase (Dickinson, 1995b). The increase in η_o at 0.45%CNHI2 could be explained by a depletion flocculation attributable to its low viscosity-stabilizing properties at high concentrations, as reported for other types of PS (Dickinson, 1995a, 1996).

Concerning the rheological behaviour of solutions, it worth mentioning the very low η_o values obtained from 0 to 0.125%CN (Fig. 3b). However, noticeable η_o increases were mainly observed at higher concns. This reveals

the negligible rheological-stabilizing role of CN preparations (no increase in continuous phase viscosity) from 0 to 0.125%CN. This suggests that interfacial stabilization mechanisms of CN would exclusively explain the emulsion stabilization in this range of concentrations.

3.4. Electrophoretic mobility of lipid droplets in presence of WPI and CNI

The increase in surface net charge (electrostatic stabilization) is a very important interfacial stabilization mechanism against charge neutralization flocculation and coalescence and, as such, must be considered. To study the relationship between this mechanism and the emulsion stabilization with CN, we compared the electrophoretic mobility (Em), a measure of lipid droplet surface net charge, at various CN concns. by using CNI (Fig. 4) because of its good stabilizing potential from previous results. The Em was shown to increase vs. CNI concn., from negative to positive values at 0.025%. At 0.1%CNI (minimal stabilizing concn. against syneresis), the Em value ($+1.36\times 10^{-8}$ m^2 s^{-1} V^{-1}) almost reached the maximum obtained at 0.125%CN ($+1.45\times10^{-8}$ m^2 s^{-1} V^{-1}). The stabilizing effect of CNI could thus be explained by a saturation of positive surface net charge at 0.1% from interfacial coadsorption with WPI. Such result related with maximal increases of η_o around half this saturating CN concn. (Fig. 3) clearly shows the charge neutralization and bridging flocculation as the destabilizing mechanisms bellow 0.1%CN (WPI/CN ratio above 5/1). Other emulsions made from coadsorbing P–PS mixtures have been reported to destabilize as a result of charge neutralization flocculation and bridging flocculation (Dickinson, 1995a; Dickinson & Galazka, 1992; McClements, 1999; Ward-Smith et al., 1994).

At 0% CNI (WPI alone), it is worth noting that the highly negative Em (-1.21×10^{-8} m^2 s^{-1} V^1) is comparable, in absolute value, to the one obtained in the presence of 0.075%CNI ($+1.17\times10^{-8}$ m^2 s^{-1} V^1). However, the latter produced higher emulsion stability against droplet flocculation and syneresis (see Fig. 1a). This would indicate that both electrostatic and steric stabilization from CN are at play. The interfacial concns. of CN and P vs. CN concn. were thus evaluated.

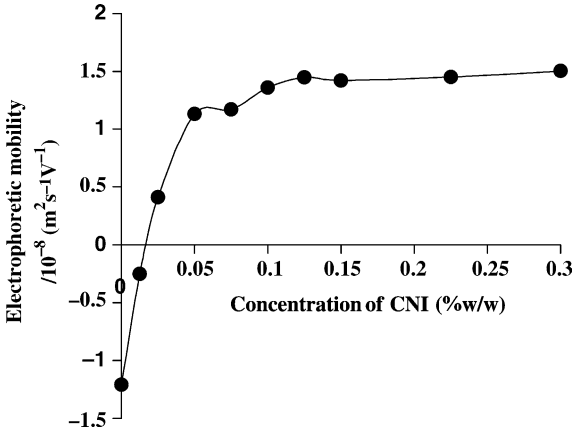

Fig. 4. Effect of CNI concentration on surface net charge around lipid droplets. SEM: ±0.0925.

3.5. Protein (P) and chitosan (CN) concentrations in creamed layer with corresponding P/CN ratios

Assuming comparable initial droplet sizes (same homogenisation treatment), the effect of %CN on the %P in the creamed layer ($[P]_L$) (Fig. 5a) and %CN in the creamed layer ($[CN]_L$) (Fig. 5b) would provide gross estimates for comparing interfacial adsorption concns. From $[P]_L$ and $[CN]_L$ results, the corresponding P/CN ratios in creamed layer (Fig. 5c) were also presented, providing a direct information on interfacial composition. The CN preparations produced slight increases in $[P]_L$ vs. %CN (Fig. 5a), reaching similar maximal values from 0.05 to 0.125%CN. This suggests a maximal interfacial P adsorption in this range of concentrations. Higher CN concentrations produced noticeable decreases in $[P]_L$, which were less pronounced with CNHI2. The results of $[CN]_L$ (Fig. 5b) show substantial increases to maximal values at 0.075–0.125%CNI, 0.125%CNHI, 0.125%CNHK, and 0.225–0.3%CNHI2. Above 0.075% of CNI, CNHI, and CNHK, the decreases in P/CN ratios (Fig. 5c) were also clearly slowing down. Those results from Fig. 5a,b agree with the achievement of interfacial adsorption saturation from each CN preparation around respective minimal stabilizing concn. against syneresis. This corroborates the combined role of electrostatic and steric stabilization of emulsions by CN in order to prevent droplet flocculation and coalescence. Under such emulsion conditions favouring P–PS coadsorption, proteins typically form a primary adsorbed layer. The surface net charge being linked to the adsorption concn. of a charged PS in the secondary layer, sufficiently increasing its adsorption will therefore combine not only electro-repulsive, but also the steric-repulsive stabilizing effects (Dickinson, 1995a, 1996; Syrbe et al., 1998; McClements, 1999; Ward-Smith et al., 1994). This is caused by an increased thickness in the polymer coating containing a complete secondary layer of hydrophilic charged PS segments exposed to the aqueous phase (Dickinson, 1994; Izgi & Dickinson 1995; McClements, 1999; Xie & Hettiarachchy, 1997).

Comparing CNHI2 with other CN, the lower increase in $[CN]_L$ or decrease in P/CN ratio vs. CNHI2 concn. suggest a lower coadsorption efficiency with P that would explain its higher minimal stabilizing concn. against syneresis. It also worth to mention the markedly different and irregular profiles of $[CN]_L$ and $[P]_L$ above 0.125%CN. This would be caused by the significant increases in continuous phase viscosity (see Fig. 3b), lowering the efficiency of creamed phase separation from serum during centrifugation. It would

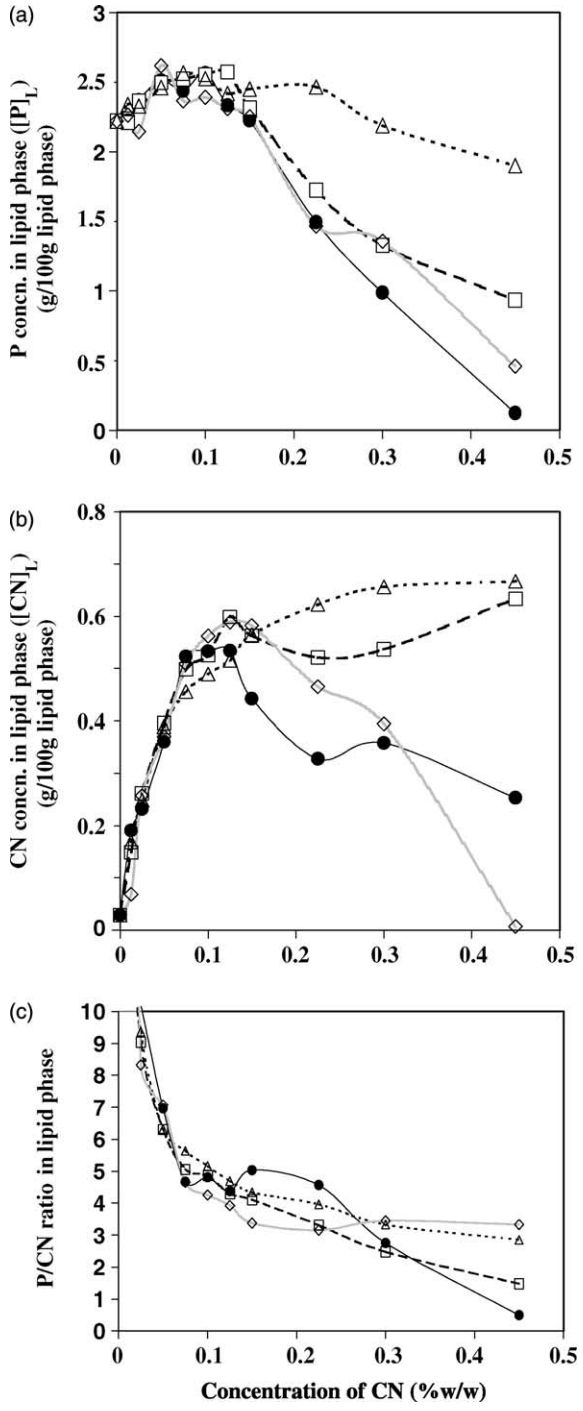

Fig. 5. Effect of concentration and type of chitosan on concn. of protein (a) and chitosan (b) in lipid phase, with corresponding P/CN ratios in lipid phase (c). Standard error of the mean: ±0.092 in (a), ±0.027 in (b). Symbols: —●— CNI; — -□- — CNHI; · · ·△· · · CNHI2; —◇— CNHK.

then overestimate P and CN concns. in the serum, containing residual droplets after centrifugation. An underestimation of respective concns. in the creamed layer would then be obtained. CNHI2 being the lower viscosity preparation, lipid-phase separation was better, explaining its higher $[P]_L$ and $[CN]_L$ values above 0.15%CN (Fig. 3a,b).

3.6. Comparison of the stabilizing potential of various chitosans at 0.1%

From phase separation evolutions in tubes and droplet size analysis, 0.1%CN was shown as the most discriminative concentration for comparing the stabilizing potential of CN preparations. Moreover, at this concentration, any stabilizing effect from the CN preparations against charge neutralization and bridging flocculation producing syneresis was shown to be independent of the continuous phase viscosity, but mainly dependant of the interfacial adsorption, which favoured both electrostatic and steric stabilizations. To validate the comparative analysis at 0.1%, a multiple comparison test (least significant difference at $p = 0.05$) was done for each emulsion characteristic studied previously (Table 1). The characteristics ($d\bar{v}_{21}$ and η_o) quantitatively known to increase when emulsion stability is decreased, respectively, followed those increasing orders: CNI ≤ CNHI ≤ CNHK and CNI ≤ CNHI < CNHK < CNHI2 (≤ indicates lower value without a significant difference). The electrophoretic mobility (absolute values of surface net charge) quantitatively related to the emulsion stability, followed this order: CNHI ≥ CNI > CNHK > CNHI2. Those results were in close agreement and confirmed the relative order of stabilizing potential of CN preparations against charge neutralization flocculation producing syneresis in tubes (Fig. 1a). The surface net charge (electrostatic stabilization) is thus a valuable mechanism explaining differences in emulsion stabilizing properties between CN preparations in the model system.

As for the effect of interfacial composition on the stabilizing properties of CN preparations, $[P]_L$ and $[CN]_L$ were used as gross estimates for comparing interfacial adsorption concns. Therefore, respective P and CN loads were calculated to confirm those differences. With $[P]_L$, only one significant difference (CNI > CNHK) was observed, whereas respective P loads did not reveal any significant difference, indicating no effect of CN preparations on P adsorption. With $[CN]_L$, the following order was obtained: CNHK > CNI ≥ CNHI > CNHI2, in agreement with the relative order of CN loads from CNI, CNHI, and CNHK. With CNHI2, load calculations could not be determined, because of the presence of flocculated droplets during droplet sizing. The decreases of $[CN]_L$ from CNI to CNHI2 is in close agreement with their decreasing order of stabilizing potential and surface net charge. This clearly shows that decreasing the molecular weight from CNHI (694 kDa) to CNHI2 (319 kDa) would disfavour coadsorption efficiency with P, leading to a significant decrease in both electrostatic and steric stabilizing mechanisms (decreased stabilizing potential against charge neutralization and bridging flocculation producing syneresis). Other studies suggest that adsorbed PS of low molecular weight produce weaker electrostatic attractions with P emulsifiers, and produce lower steric-stabilizing control due to lower chain extension towards the aqueous phase (Dickinson & Galazka, 1991;

Table 1
Results of comparative analysis of the effect of chitosans (0.1%) on emulsion characteristics including controls with WPI or CNI alone

Characteristics	CNI	CNHI	CNHI2	CNHK	WPI alone	CNI alone	LSD[a]
Droplet size[b] (initial)	0.903b	0.874b	(n.d.[1])	1.026b	1.261b	3.225a	1.528
Droplet size[b] (21 days)	1.927b	3.684b	(n.d.[1])	5.547a	(n.d.[1])	(n.d.[2])	1.811
(η_o) (emulsions)[c]	0.022c	0.062c	1.379a	0.922b	0.035c	0.013c	0.167
$[P]_L$[d]	2.565a	2.555ab	2.526ab	2.392b	2.213c	<0.001d	0.172
Protein load[e]	3.566b	3.416b	(n.d.[3])	3.759ab	4.273a	0.001c	0.544
$[CN]_L$[d]	0.533b	0.525b	0.489c	0.562a	0.025d	n.d.[d]	0.024
CN load[e]	0.741b	0.703b	(n.d.[3])	0.884a	0.050c	n.d.[d]	0.090
Electrophoretic mobility[f]	1.359ab	1.448a	0.797d	1.104c	−1.210bc	1.389ab	0.230
(Zeta potential)	(16.95)	(17.98)	(9.90)	(13.75)	(−15.05)	(17.30)	

(n.d.[1]), not determinable value due to the presence of flocculated lipid droplets during measurements; (n.d.[2]), not determinable value due to extensive coalescence and creaming causing unrepresentative volume fraction of lipid droplets remaining in suspension during measurements; (n.d.[3]), not determinable value caused by indeterminable droplet sizing (see n.d.[1]); (n.d.[4]), not determinable value due to high coalescence from lipid droplets.

[a] Least significant difference between treatments at $p=0.05$. Treatments significantly different have different letters in their box, beginning with 'a' for the highest value.

[b] Volume average diameter, in (μm) units.

[c] Limiting low-shear viscosity (25 °C), in (Pa s) units.

[d] $[P]_L$ and $[CN]_L$, respectively, represents protein and CN concns. in creamed layer, in (g/100 g creamed layer) units.

[e] Expressed in (mg/m^2) units (Calculated from corresponding initial droplet diameters and load results).

[f] Expressed in ($\times 10^{-8}$ m^2 s^{-1} V^{-1}) units. The corresponding Zeta potentials are expressed in mV.

Hattori, Numamoto, Kobayashi, & Takahashi, 2000; Kato et al., 1989).

Results have also revealed a decrease in stabilizing potential caused by a lower degree of deacetylation from CNHI (78.5%DD) to CNHK (67.7%DD), with comparable molecular weights. This is mainly explained by a significant reduction in surface net charge (lower charge density from CNHK), in spite of significantly higher adsorption concn. with CNHK. This latter would be explained by stronger hydrophobic interactions with lipid droplets due to the more hydrophobic structure of CNHK. However, low deacetylated chitosans are known to have a higher tendency towards hydrophobic dependant self-aggregation in solutions (Amiji, 1995; Anthonsen, Vårum, Hermansson, Smidsrød, & Brant, 1994; Ottøy, Vårum, Christensen, Anthonsen, & Smidsrød, 1996). Therefore, adsorbed CNHK chains would be more tightly packed around droplets. They would then produce a thinner interfacial adsorbed layer with low hydration and steric stabilization, favouring droplet flocculation (McClements, 1999). Because the relative order of stabilizing potential of CN preparations was mainly linked to surface net charge (electrostatic stabilization), this would then be the most important stabilizing mechanism of the CN preparations compared in this study. It is worth mentioning that other experiments using 0.1% CN added after emulsion formation with P revealed no significant difference of physico-chemical characteristics and stability compared to mixed components added before homogenisation. This confirms the CN stabilizing mechanism by surface thickness and net charge increases. This also shows that a stabilizing effect of CN could either be performed by P–CN complex adsorption or by interfacial P–CN complexation.

A comparison of the controls containing WPI or CNI alone with WPI–CN mixtures shows that the significantly lower values of $[P]_L$ and $[CN]_L$ vs. mixtures are in agreement with the 'synergistic' interfacial adsorption of mixed components. Given the relatively low droplet dispersion stabilities of controls against flocculation and coalescence during droplet measurements, overestimations or indeterminable load results were in disagreement with $[P]_L$ and $[CN]_L$ values. The apparently higher P load obtained with the control containing WPI alone can be explained by its higher susceptibility towards interfacial P aggregation due to low protein hydration in the aqueous solvent system used in emulsions (0.2 M acetic acid, pH 6.0, $\mu=0.095$ M). Although comparable surface net charges (in absolute values) were observed between controls and most of the mixtures, the higher stability against droplet flocculation and coalescence with mixtures would be, respectively, brought from the combination of high viscoelastic properties from the P layer (Leman & Kinsella, 1989) with high electrostatic and steric properties provided from the charged PS layer (Dickinson, 1992, 1998; McClements, 1999).

4. Conclusion

In a model emulsion at pH 6.0 containing 0.5%WPI in the presence of various concns. and types of CN, we have shown that interfacial coadsorption saturation of CN with P favours the positive surface net charge and CN adsorption concn. Those effects, respectively, provide electrostatic and steric stabilization mechanisms of CN against charge neutralization and bridging flocculation producing syneresis. Based on minimal stabilizing concns. against syneresis after 21 days, the emulsion stabilizing potential of CN preparations compared in this study followed this decreasing order: CNI (0.1%) ≥ CNHI (0.1%) > CNHK (0.125%) > CNHI2 (0.225%). This indicates a reduced emulsion

stability with a low molecular weight of 319 kDa (CNHI2) or a low DD of 67.7% (CNHK). The most unfavourable effect from a low molecular weight preparation on stability is mainly explained by a loss of interfacial coadsorption efficiency, and, to a lesser extent, from a low DD this is mainly explained by a loss of interfacial net charge. Preliminary tests have shown soluble complex formation in WPI–CN mixtures producing emulsion stabilization. Future work will focus on the role of this complex in emulsion stabilization.

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