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The effect of chitosan on the properties of emulsions stabilized by whey proteins

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Abstract

The influence of the cationic amino polysaccharide chitosan content (0–0.5%) on particle size distribution, creaming stability, apparent viscosity, and microstructure of oil-in-water emulsions (40% of rapeseed oil) containing whey protein isolate (WPI) (4%) at pH 3 was investigated. The emulsifying properties, apparent viscosity and phase separation behaviour of aqueous WPI/chitosan mixture at pH 3 were also studied. The interface tension data showed that WPI/chitosan mixture had a slightly higher emulsifying activity than had whey protein alone. An increase in chitosan content resulted in a decreased average particle size, higher viscosity and increased creaming stability of emulsions. The microstructure analysis indicated that increasing concentration of chitosan resulted in the formation of a flocculated droplet network. This behaviour of acidic model emulsions containing WPI and chitosan was explained by a flocculation phenomenon.

Keywords: Chitosan; Whey protein isolate; Emulsions; Creaming stability; Flocculation

1. Introduction

Among the emulsifiers used in the food industry, whey proteins are most commonly applied as emulsions stabilizers. Proteins facilitate emulsion formation and improve their stability by reducing the interfacial tension and by forming a protective membrane around fat droplets (Dalgeish, 1996; McClements, 1999). Polysaccharides are often added to the oil-in-water emulsions to create a desirable texture and mouthfeel and to stabilize emulsion droplets against gravitational separation. In most cases this is achieved, either by enhancing the viscosity, or forming a gel network in a continuous phase, while some of them can show surface activity as well (Dickinson, 1995, 2003). The impact of protein and polysaccharide, both present in a continuous phase, on emulsion stability is dependent on the colloidal properties of protein/polysaccharide systems. These properties are related, not only to the individual functionality of protein and polysaccharide, but also to the nature and strength of the interactions between them (Dickinson, 1995; Syrbe, Bauer, & Klostermeyer, 1998).

Chitosan, a linear copolymer of glucosamine and *N*-acetyl glucosamine connected through β-(1-4) glucosidic linkages, is a polysaccharide, which has received a considerable attention due to its potential in a broad range of applications (Tharanathan & Kittur, 2003). Chitosan is a natural, nontoxic and biodegradable biopolymer, widely produced from crab and shrimp waste shells. Among abundant naturally occurring polysaccharides, which are neutral or acidic, chitosan is distinguished for its cationic nature ($pK_a \approx 6.5$). The use of chitosan in the food industry is related to its functional properties and nutritional and physiological activities (Muzzarelli, 1996; Shahidi, Arachi, & Jeon, 1999). Chitosan exhibits water-, fat- and dye-binding capacity (Knorr, 1982, 1983; No, Lee, & Meyers, 2000), emulsifying properties

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(Rodriguez, Albertengo, & Agullo, 2002; Schulz, Rodriguez, Del Blanko, Pistonesi, & Agullo, 1998), antimicrobial (Helander, Nurmiaho-Lassila, Ahvenaien, Rhoades, & Roller, 2001; Liu, Du, Wang, & Sun, 2004; Zheng & Zhu, 2003), hypolipidemic and hypocholesterolemic activity (Muzzarelli, 1996; Winterowd & Sanford, 1995).

Chitosan was shown to be useful in the preparation of stable emulsions without any other surfactant (Del Blanco, Rodriguez, Schulz, & Agullo, 1999; Rodriguez et al., 2002; Schulz et al., 1998), together with anionic or nonionic surfactants (Jumaa, Furkert, & Müller, 2002; Jumaa & Müller, 1999), in conjugates with different proteins, prepared by Maillard-type reaction (Babiker, 2002; Song, Babiker, Usui, Saito, & Kato, 2002; Usui et al., 2004), or in multiple layers with anionic components (Aoki, Decker, & McClements, 2005; Ogawa, Decker, & McClements, 2003; Ogawa, Decker, & McClements, 2004). Chitosan/whey protein systems and their influence on emulsions properties have been studied, so far, only by very few researchers. Guzey and McClements (2006) investigated the interaction of chitosan with β-lactoglobulin in aqueous solutions, and showed that those biopolymers could interact to form either soluble or insoluble complexes, depending on the pH. Laplante, Turgeon, and Paquin (2002, 2005a, 2005b) studied the effect of various factors, such as pH, ionic strength, concentration of whey protein isolate and chitosan with different characteristics (molecular weights and degrees of deacetylation) on the stability of emulsions. They found that the emulsion stability was mostly dependent on the electrostatic interaction between whey proteins and chitosan. Nevertheless, a significant gap still exists in understanding the influence of behaviour, in a continuous phase, on the formation of stable emulsions, in particular at low pH.

The objective of this study was to evaluate the effect of chitosan concentration on the properties of acidic concentrated (40% (w/w)) emulsions containing whey protein isolate.

2. Materials and Methods

2.1. Materials

Acidic whey protein isolate (WPI) (Lacprodan DI-9213), containing 90% of protein, 5.0% of moisture, 0.5% of ash and 4.2% of lactose, was obtained from MD Foods Ingredients Amba, Denmark. Chitosan produced from shrimp shells had a deaceylation degree of 86%, 1.4% of moisture, and 1.37% of ash and was purchased from Marine Chemicals, India. Rapeseed oil was purchased from a local supermarket to prepare emulsions.

2.2. Methods

2.2.1. Emulsion preparation

WPI was dissolved in distilled water at room temperature under moderate magnetic agitation, for 1–2 h, and chitosan was dissolved in 1% of acetic acid solution under agitation for 5–6 h. The pH of these solutions was adjusted to 3.0 using 50% and 1% of acetic acid of analytical grade (Merck, Darmstadt, Germany). The continuous phases used to prepare emulsions consisted of 6.67% (w/w) of WPI and 0.17–0.83% (w/w) of chitosan. The solutions of appropriate concentrations of WPI and chitosan were preheated to 65 °C and then mixed. The oil was preheated to 65 °C and slowly added to a continuous phase while emulsifying it with a high shear dispersing system model Ultra Turrax (IKA Werke, Staufen, Germany) equipped with an 18 mm diameter dispersing tool (model S25KR–18G) at 13,500 rpm for 5 min. Oil-in-water emulsions contained 40% (w/w) of rapeseed oil, 4% (w/w) of WPI, and 0–0.5% (w/w) of chitosan. After preparation, the emulsions were kept at 4 °C. All emulsions were prepared at least in duplicate.

2.2.2. Phase diagrams

The phase diagrams of a WPI/chitosan system were obtained by preparing concentrated WPI and chitosan solutions at pH 3 and mixing them in a ratio of 1:1 to get WPI/chitosan solutions with concentrations of 3–11% (w/w) of WPI and 0.2–0.8% (w/w) of chitosan. The samples were kept for 72 h at a room temperature and the phase separation boundary was detected by visual observation.

2.2.3. Interfacial tension measurements

An automated drop volume tensiometer TVT1 (Lauda, Germany) was used in a dynamic mode for measuring interfacial tensions at the oil–water interface at 20 °C. In those measurements, the aqueous phases were chitosan solutions, WPI solutions and WPI/chitosan solution mixtures of the same concentrations as in continuous phases of emulsions at pH 3. The oil phase was rapeseed oil.

2.2.4. Creaming stability

Freshly prepared emulsions were transferred into glass test tubes and then stored at 30 °C. The extent of a transparent (or turbid) serum phase separation at the bottom of the tubes during 150 h was assessed by a Turbiscan MA1000 unit (Formulaction, L'Union, France), which scanned the product height by means of a laser beam. If creaming occurred, the reflection changed along the sample height. Creaming stability results were presented as the height of a sedimented serum phase expressed as a percentage of the total height of emulsion in the tube.

2.2.5. Particle size measurements

The particle size distribution and the average droplet size (diameter, d_{43}) were determined by means of a laser diffraction spectrometer LS 230 (Beckman-Coulter, Krefeld, Germany). Prior to measurements, the emulsions were dispersed in distilled water. Having made the emulsions, measurements were performed in 24 h.

2.2.6. Flow behaviour

The emulsion viscosity was measured by means of a controlled stress AR-1000 rheometer (TA Instruments,

Alzenau, Germany) equipped with a cone–plate geometry. The cone diameter was 60 mm and the cone angle was 4°. Measurements were performed at a temperature of 20 °C for 3 days after the emulsions were made by increasing the share rate from 0 to 1200 s^{-1} for 2 min, keeping it at 1200 s^{-1} for 2 min and then decreasing it to 0 s^{-1} in 2 min. Determination of flow behaviour (n) and consistency index (K) were calculated on the shear stress (τ) while increasing shear rate (γ) using the power law model:

$$\tau = K \cdot \gamma^n.$$

The viscosity measurements of continuous phases of emulsions and chitosan solutions were performed by means of a controlled stress CSL 50 rheometer (Carri-Med Ltd, Dorking, UK) equipped with a cone–plate geometry. The cone diameter was 60 mm and the cone angle was 4°. Measurements were performed at a temperature of 20 °C for 2 days after solution preparation by increasing the shear rate from 0 to 500 s^{-1} in 1 min.

2.2.7. Light microscopy

The microstructure of emulsions was assessed by means of an AXIOSKOP microscope (Carl Zeiss AG, Jena, Germany). Prior to the measurements, the emulsions were diluted (1:1) using acetic acid solution at pH 3 and gently stirred in a glass test tube to ensure their homogeneity. A drop of emulsion was placed on a microscope slide, covered with a cover slip and then observed at 100- and 400-fold magnifications. The photomicrographs were taken 2 days after the emulsions were prepared.

3. Results and discussion

3.1. WPI and chitosan solutions

When WPI/chitosan mixtures were stored at a room temperature for 72 h, phase separation occurred in some of the samples. In these phase-separated systems a highly turbid lower layer, rich in chitosan, and a relatively transparent upper layer, rich in WPI, were visually observed. The height of the chitosan layer increased with increase in chitosan concentration. It can be assumed that, at an acidic pH, when both WPI and chitosan carry positive charges, thermodynamic incompatibility is likely to be the mechanism, which favours the phase separation. From the phase diagram of the WPI/chitosan systems, various combinations of concentrations can be obtained; e.g. it can be seen that phase separation occurs when the system consists of 0.7% (w/w) of chitosan and 5% (w/w) of WPI (Fig. 1). In the phase diagram, the ionic strength of the system was not taken into consideration. However, it was predetermined by the ash content in WPI (0.5 %) and chitosan (1.37%). Increasing content of the biopolymers in the system caused increase of the ionic strength of the system. As the ionic strength owns the screening of electric charges, the intensification of biopolymers self-association and, incompatibility of whey proteins with chitosan can be enhanced.

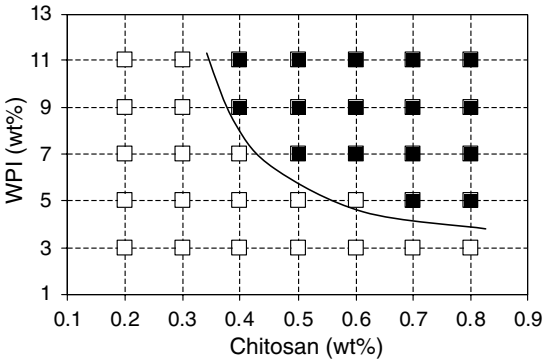

Fig. 1. Phase diagram of WPI/chitosan system at pH 3. ■ – two phases system, □ – one phase system.

The interfacial tension measurements of WPI, chitosan and WPI/chitosan solutions are presented in Fig. 2. It can be observed that the interfacial tension between oil and pure water slightly decreases throughout the whole range of measurement time, thus indicating that the oil contains polar impurities, which can adsorb at the interface. That oil, however, is used for the measurements in order to obtain accurate information about what happens with the WPI/chitosan system used to make emulsions. According to the interfacial tension measurements, chitosan alone gives lower interfacial activity than do whey proteins (Fig. 2). The mixtures of those two polymers have a slightly reduced surface tension compared to whey protein alone. It is shown that, under the condition of an incompatible biopolymer added to the protein solution, the proteins behave as if they were in a solution of higher concentration. Thus, the composition of biopolymers mixture solution determines the protein thermodynamic activity, its partition between the bulk of a dispersion medium and an interface, and its surface activity (Tolstoguzov, 1994). It can be assumed that chitosan has contributed to a better interfacial activity of the whey proteins by enhancing thermodynamic activity and, consequently, the protein adsorption. It was shown by other authors (Laplante et al.,

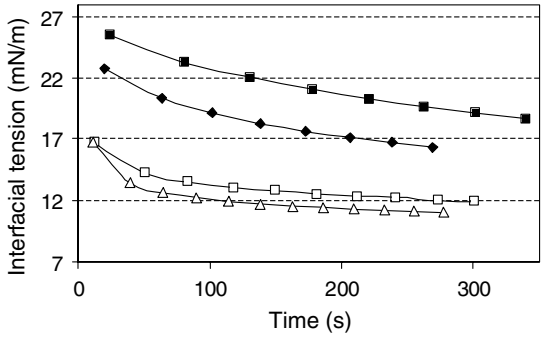

Fig. 2. Interfacial tensions at oil–water interface as a function of time (pH 3, 20 °C): water (■), 0.5% (w/w) chitosan (◆), 6.67% (w/w) WPI (□), mixture of 6.67% (w/w) WPI and 0.5% (w/w) chitosan (△).

2002, 2005a, 2005b) that only at pH >5, when WPI–chitosan interactions are favourable, is interfacial co-adsorption of those two biopolymers made possible.

The viscosities of WPI solutions and WPI/chitosan mixtures, used as a continuous phase for the emulsions, were compared with those of chitosan solutions (Fig. 3). The apparent viscosities of mixtures increased with increasing chitosan concentration. The solutions of chitosan up to 0.5% (w/w) showed higher viscosity than did those of the WPI/chitosan mixtures with appropriate concentrations of chitosan. Chitosan is an associative polymer (Nystrom, Kjoniksen, & Iversen, 1999) and it can be assumed that the proteins disrupt the network of chitosan molecules, which leads to a decreased viscosity of the solution. The difference between the viscosities of WPI/chitosan mixtures and pure chitosan solutions was less pronounced when the concentration of chitosan increased. The viscosity of WPI/chitosan mixture with 0.83% (w/w) of chitosan was slightly higher than that of chitosan solution of the same concentration. The increase in viscosity of a WPI/chitosan system can be explained by the increased self-association of biopolymers favoured by thermodynamic incompatibility in these systems and formation of a strong network.

3.2. Emulsions

The emulsions were characterised by particle size distribution, creaming stability, apparent viscosity and microscopic images. The particle size distribution, presented in Fig. 4, indicates that the addition of chitosan caused a gradual reduction in a particle size of emulsions. These measurements showed that average particle size, d_{43}, decreased from 10.95 ± 0.62 μm in emulsions stabilized with 4% (w/w) WPI alone to 5.97 ± 0.28, 4.06 ± 0.28 and 3.54 ± 0.19 μm in emulsions with 0.1, 0.3 and 0.5% (w/w) of chitosan addition, respectively. Such an effect can be attributed to an increase in surface activity of a continuous phase caused by the addition of chitosan, as shown in Fig. 2.

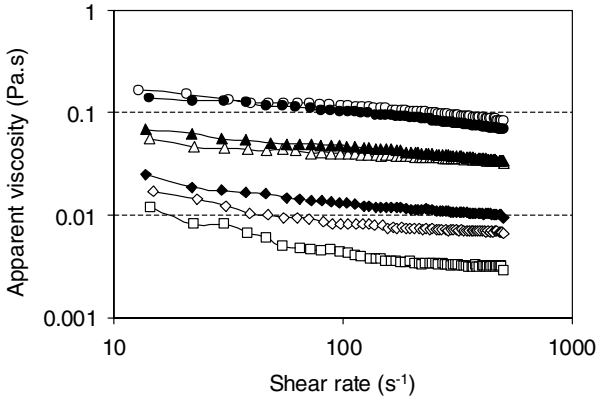

Fig. 3. Apparent viscosity of chitosan solutions (closed symbols) and WPI/chitosan mixtures with 6.67% (w/w) WPI (open symbols) at different concentrations of chitosan: 0% (□), 0.17% (◆◇), 0.50% (△▲) and 0.83% (w/w) (○●); 20 °C.

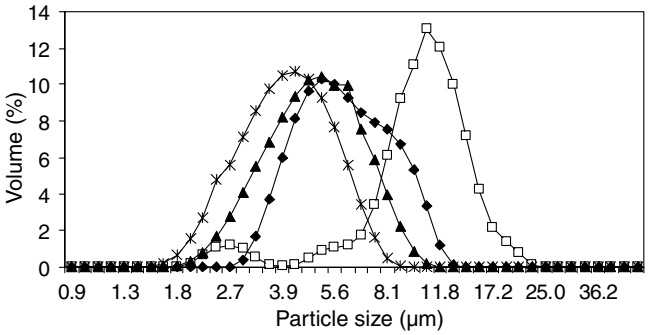

Fig. 4. Size distribution of emulsions (40% (w/w) oil, 4% (w/w) WPI) with different concentrations of chitosan: 0% (□), 0.1% (◆), 0.3% (▲) and 0.5% (w/w) (✱).

The formation of smaller droplets, due to addition of chitosan, was confirmed by photomicrographs of emulsions (Fig. 5). The flocculation of droplets in the presence of chitosan was also observed. Prior to the observation, the emulsions were diluted. Due to the high concentration of droplets, the images of concentrated emulsions were not very informative; it was difficult to detect whether droplets were flocculated or just in close proximity. Fig. 5b shows that 0.1% (w/w) of chitosan leads to a small flocculation of droplets in emulsions, despite the possibility that dilution can disturb the structure of some flocs. An increase in concentration of chitosan, up to 0.5% (w/w), provoked a more extensive flocculation of oil droplets (Fig. 5c and d). The nature of protein–polysaccharide interactions is very relevant to the mechanism of emulsion droplet flocculation (Cao, Dickinson, & Wedlock, 1990). Considering the thermodynamic incompatibility of whey proteins and chitosan in a continuous phase (Fig. 1), it can be noted that there is no adsorption of chitosan to the protein-coated droplets surfaces. Therefore it is not misleading to presume that, in the photomicrographs (Fig. 5b–d), observed droplet flocculation was due to a depletion attraction induced by non-adsorbing chitosan in the emulsions. At the chitosan concentrations used in this study, the free chitosan concentration in the continuous phase should be sufficiently high, beyond a critical concentration, to induce depletion flocculation, whose strength increased with the concentration of chitosan, resulting in more extensive flocculation of droplets. Our assumption is in accordance with the works of Laplante et al. (2002, 2005a). They found that, at acidic conditions (pH $\leqslant$5.0), when interactions between WPI and chitosan in emulsions were unfavourable, chitosan induced droplet flocculation by a depletion mechanism.

In Fig. 6 the creaming of emulsions stabilized with WPI alone, and those containing WPI and different concentrations of chitosan, are compared. The serum layer height is plotted against the storage time. The creaming measurements indicated that the emulsions prepared with WPI alone were relatively unstable. These emulsions showed the highest initial creaming (serum separation) rate. The addition of chitosan slowed the rate of emulsion creaming.

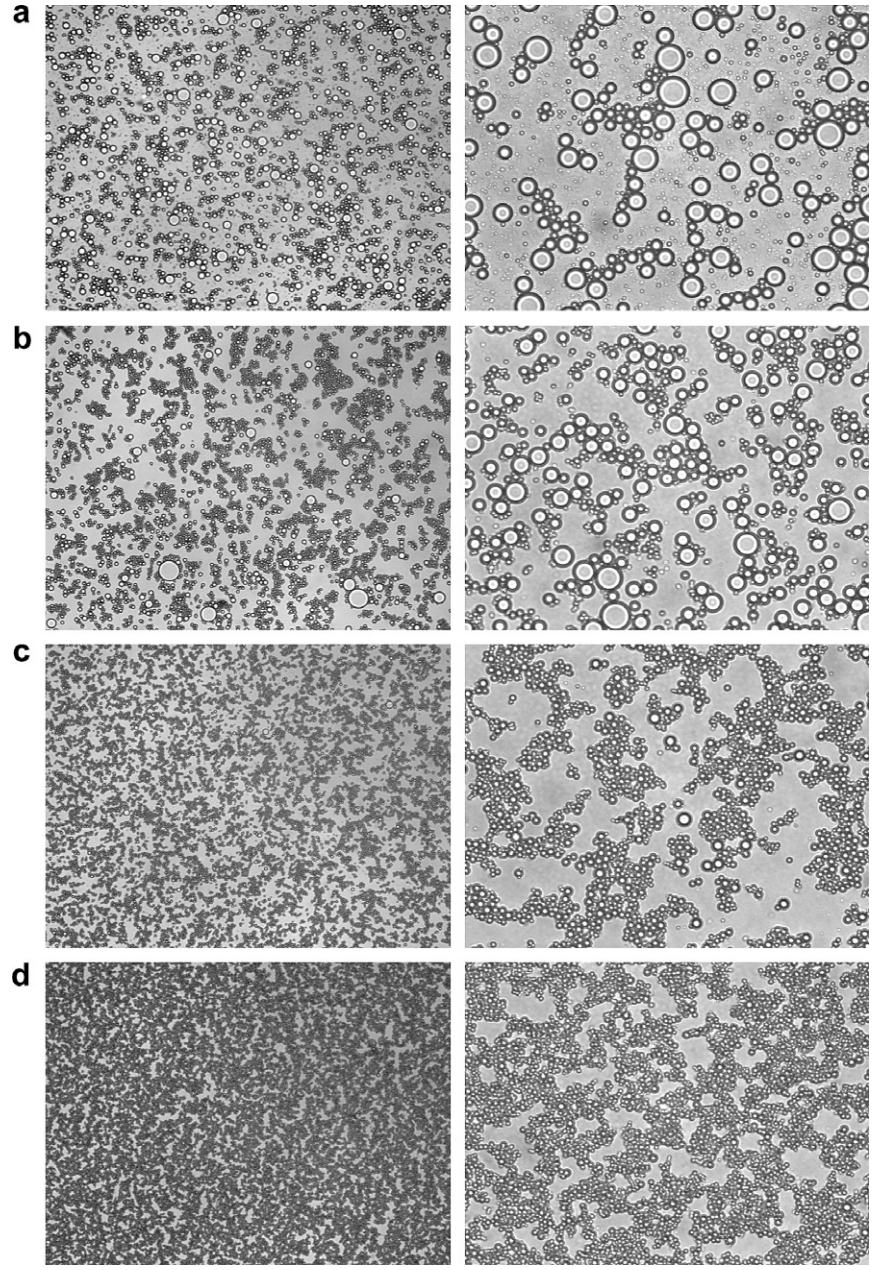

Fig. 5. Photomicrographs of emulsions (40% (w/w) of oil, 4% (w/w) of WPI) with different concentrations of chitosan: 0% (a), 0.1% (b), 0.3% (c) and 0.5% (w/w) (d). Photomicrographs on the right side – magnification ×400, on the left – ×100; emulsions were diluted (1:1).

The serum phase of emulsions stabilized by WPI and chitosan mixtures was clear compared to the turbid one of the emulsions stabilized without chitosan. This fact indicates the presence of individual polydisperse droplets in a serum phase of emulsions stabilized by WPI alone. In emulsions with chitosan, the droplets can be linked into flocs as seen in the photomicrographs (Fig. 5b–d). The increase in the chitosan content from 0.1% to 0.5% (w/w) led to a gradual reduction of a serum layer height, assessed after 150 h, thus indicating a decrease in the packing efficiency of droplets in a cream layer. The emulsions with 0.5% (w/w) of chitosan showed no serum separation at the bottom of tubes after 150 h. The creaming rate and the droplet packing efficiency were both dependent on the continuous phase viscosity and the droplet size distribution in emulsions. The increase in viscosity of a continuous phase with addition of chitosan (Fig. 3) can improve creaming stability by slowing down the diffusion of droplets and flocs. Owing to higher continuous phase viscosity, the flocs can be subjected to less restructurating, and possibly held in a more open structure, which could reduce the serum layer thickness in these emulsions.

Beside the smaller droplets in emulsions with chitosan, their flocs formation can determine the emulsion stability

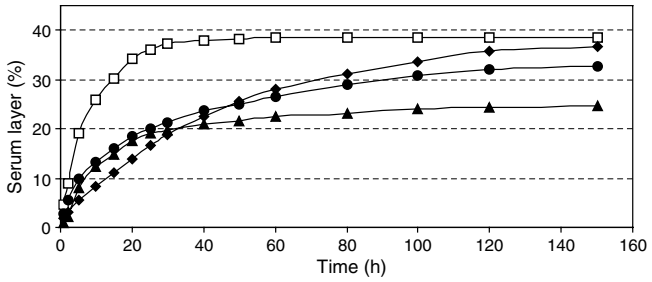

Fig. 6. Creaming stability of emulsions (40% (w/w) oil, 4% (w/w) WPI) containing various concentrations of chitosan: 0% (□), 0.1% (◆), 0.2% (●) and 0.3% (w/w) (▲); 30 °C.

to a greater extent. Whereas the droplet flocculation led typically to faster creaming in moderately dilute emulsions as flocs tended to separate at a faster rate than did individual dispersed droplets, flocculation in the concentrated emulsions had the opposite effect. Increased hydrodynamic interference, induced by the higher droplet packing fraction, and the formation of a coherent three-dimensional network structure in concentrated emulsions can have a stabilizing influence (Cao et al., 1990).

The rheological properties of emulsions at 20 °C have also been determined (Figs. 7, 8, Table 1). In the absence of chitosan, the emulsions exhibited close to Newtonian behaviour with relatively low apparent viscosities as a function of a shear rate. The flow behaviour indices of the emulsions showed that, at increasing chitosan concentrations, pseudoplasticity turns out to be apparent. The flow curves of emulsions at increasing and decreasing shear rates indicated that all emulsions were reversible. With increasing chitosan concentrations, from 0.1% to 0.5% (w/w), the apparent viscosity and thixotropy of emulsions increased progressively. The non-Newtonian behaviour of emulsions can be attributed to the formation of a structure of flocculated droplets that gradually breaks down with increasing shear rate. The substantial enhancement of creaming stability of emulsions containing chitosan is consistent with the higher viscosity of these emulsions.

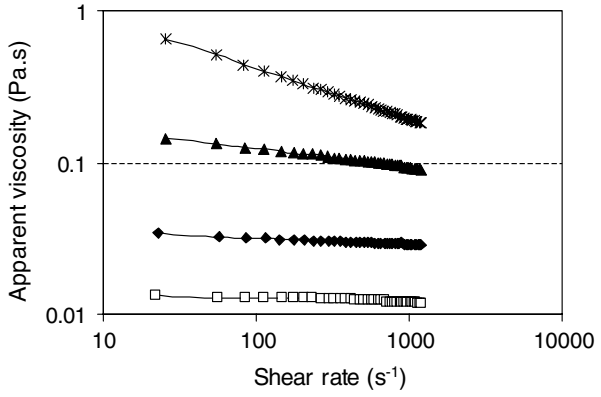

Fig. 7. Apparent viscosity of emulsions (40% (w/w) oil, 4% (w/w) WPI) with different concentrations of chitosan: 0% (□), 0.1% (◆), 0.3% (▲) and 0.5% (w/w) (∗); 20 °C.

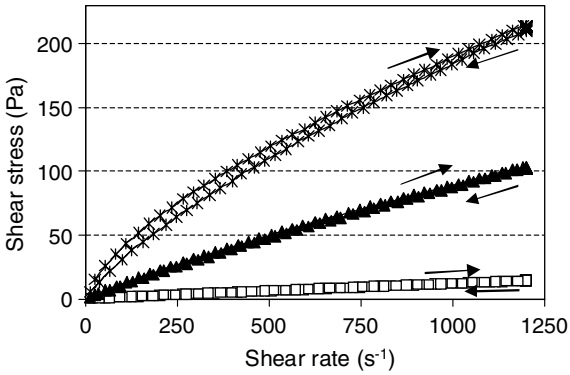

Fig. 8. Flow curves of emulsions (40% (w/w) oil, 4% (w/w) WPI) with different concentrations of chitosan: 0% (□), 0.3% (▲) and 0.5% (w/w) (∗), at increasing and decreasing shear rate; 20 °C.

Table 1
Flow behaviour indices (n), consistency coefficients (K) and thixotropy for emulsions (40% (w/w) oil, 4% (w/w) WPI) prepared with different concentrations of chitosan

Chitosan (% (w/w))	K (Pa s^n)	n	Thixotropy (Pa/s)
0	0.015	0.966	139
0.1	0.038	0.965	888
0.2	0.083	0.939	1435
0.3	0.220	0.880	3941
0.5	2.129	0.654	13,505

4. Conclusions

The presence of chitosan in a continuous phase during the formation of oil-in-water emulsions leads to an increase in stability of acidic (pH 3) oil-in-water emulsions containing whey protein isolates. This effect has been confirmed by the increased viscosity, the creaming stability, and the smaller droplet size formation in emulsions. The phenomenon of depletion flocculation can explain this behaviour of acidic model emulsions containing WPI and chitosan.

The results of this study suggest that chitosan, used under acidic conditions, has potential application as a functional ingredient in products with optimised and stable structure formulation. Taking into consideration that chitosan is a physiologically active natural material, it can be expected that it would improve the nutritional quality of these products.

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