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Influence of Salt Concentrations on the Stabilities and Properties of Sodium Caseinate Stabilized Oil-in-Water Emulsions

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Abstract The influence of salt concentration on the stability of sodium caseinate (CAS)-stabilized emulsions (20 wt% corn oil, 3.2 wt% CAS, 5 mM imidazole/acetate buffer, pH 7) was examined. In the absence of salt, laser diffraction measurements and optical microscopy measurements indicated there were some large oil droplets (d>10 μm) in the emulsions stabilized by 0.8 to 3.2 wt% of CAS. The droplet aggregation (mostly droplet coalescence) observed in the emulsions containing ≤2.8 wt% CAS tended to decrease as the CAS concentration increased, however, after which concentration (at 3.2 wt% CAS) depletion flocculation occurred. The addition of $CaCl_2$ (5-20 mM) into the emulsions stabilized by 3.2 wt% CAS prevented the depletion flocculation although there was a small fraction of relatively large individual droplets in the emulsions, which was attributed to electrostatic screening effect and bridging effect of calcium ion. This study has shown that calcium ion that has been reputed to promote droplet aggregation could improve emulsion stability against droplet aggregation in CAS-stabilized emulsions.

Keywords: oil-in-water emulsion, sodium caseinate, salt, depletion flocculation, droplet aggregation

Introduction

Sodium caseinate (CAS), a form of casein, has been commonly used as an emulsifier in the food industry because it could lead to the rapid establishment of a thick sterically stabilizing layer that protects newly formed droplets against droplet flocculation and coalescence (1). The advantage of CAS is due to the fact that compared with many other food proteins such as soy, whey, fish, meat, and plant proteins, caseins are particularly disordered and substantially hydrophobic, which assists their rapid adsorption to oil droplet surfaces during emulsification (2, 3), and that they form a thick interfacial layer of up to 10 nm around dispersed oil droplets, while other proteins form relatively thin interfacial layers, e.g., 1-2 nm for whey proteins (2,4-7).

CAS-stabilized emulsions tend to be more stable to heating than whey protein-stabilized emulsions, presumably because the relatively flexible casein molecules do not undergo appreciable heat-induced conformational changes like globular proteins do (8,9). The emulsions system is also highly effective at protecting emulsified oils from oxidation, which has been attributed to their unique iron chelating properties and their ability to produce thick interfacial layers around the droplets (10). At neutral pH, above isoelectric point (pI) of CAS (pI = 4.6) (10), caseins provide good protection against droplet aggregation due to a combination of electrostatic and steric stabilization mechanisms (11).

In previous study, we showed that CAS-coated emulsions stabilized with 1 wt% of CAS had a small fraction of individual particles with diameters greater than 10 μm at pH 7 (12). We assume that the amount of CAS used in the study may not have been sufficient to form thick enough membranes to completely prevent coalescence although casein is an effective emulsifier at pH 7, because CAS is more effective at preventing coalescence when it forms a relatively thick interfacial membrane around the droplets (13,14). Therefore, here one of the objectives of this study is to determine if stable CAS-stabilized emulsions that do not have large coalesced individual emulsion droplets can be created by increasing the protein concentration.

While we increased the CAS emulsifier concentration depletion flocculation occurred before we could prepare stable emulsions that do not have relatively large individual droplets (see Results and Discussion). That sufficiently high concentrations of non-adsorbed CAS can promote emulsion instability through a depletion flocculation mechanism has been reported in previous studies (9,15). Here, there is the second objective of this study. We wanted to determine under which conditions stable CAS-stabilized emulsions that have no evidence of both coalescence and depletion flocculation could be formed. In this study we focus on the utilization of salt ($CaCl_2$) as a bridge for the formation of thick membrane and as a quencher of depletion flocculation by chelating non-adsorbed CAS molecules that trigger the flocculation. Salt ions may influence emulsion stability in a variety of different ways depending on their valence, size, and concentration (1). They increase the ionic strength of the aqueous phase, which reduces the electrostatic repulsion between droplets through electrostatic screening (16,17). They also bind to oppositely charged groups on the surface of emulsion droplets, decreasing the magnitude of their ζ-potential and thereby reducing the electrostatic repulsion between droplets and/or between droplets and free charged emulsifier molecules, where they act as ion bridges (16). We expect that all of these effects may induce adsorption of additional emulsifier molecules

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without causing depletion flocculation under a certain concentration of salt although previous research has shown that salt ions promote droplet aggregation that adversely impacts emulsion stability in protein-stabilized emulsions at neutral pH above a critical concentration by the same mechanisms (8,18-20). Therefore, in this study we investigated the influence of salt ($CaCl_2$) on the stability of CAS emulsions stabilized by 3.2 wt% CAS where depletion flocculation took place because we wanted to determine if thick enough membrane that could prevent droplet coalescence could be created without depletion flocculation by the addition of salts.

Materials and Methods

Material Spray-dried sodium caseinate (ALANATE 180) with 1.2% sodium content and <0.1% calcium content was kindly provided from New Zealand Milk Products (NZMP, Lot #0034-W5166; Lemoyne, PA, USA). As stated by the supplier, this product contained 92.7% protein, 4.2% moisture, 3.5% ash, 0.8% fat, and 0.1% lactose. Analytical grade calcium chloride ($CaCl_2$), sodium hydroxide (NaOH), imidazole, and sodium azide (NaN_3) were purchased from the Sigma Chemical Company (St. Louis, MO, USA). Acetic acid was purchased from Fisher Science (Chicago, IL, USA). Corn oil was purchased from a local supermarket and used without further purification. Distilled and deionized water was used for the preparation of all solutions.

Emulsions preparation A buffer solution was prepared by dispersing 5 mM imidazole and acetic acid into water and then adjusting the pH to 7.0 using 1 M NaOH. Protein solutions were prepared by dispersing the desired amount (1.0-4.0 wt%) of sodium caseinate powder into buffer solution (5 mM imidazole/acetate buffer, pH 7) and stirring overnight at room temperature to ensure complete hydration. The pH of the casein solutions was adjusted back to pH 7.0 if required.

Oil-in-water emulsions were prepared by blending 20 wt% corn oil and 80 wt% casein solutions together using a high-speed blender (M133/1281-0; Biospec Products, Inc., Bartlesville, OK, USA) for 2 min. These coarse emulsions were then passed through a 2-stage high-pressure valve homogenizer (LAB 1000; APV-Gaulin, Wilmington, MA, USA) 5 times: 4,500 psi the first stage, 500 psi the second stage. Sodium azide (NaN_3, 0.04%) was added to the emulsions as an antimicrobial agent. The emulsions [20 wt% corn oil, 0.8-3.2 (0.8, 1.6, 2.0, 2.4, and 3.2) wt% CAS, pH 7.0] were then stored at ambient temperature for 24 hr before being analyzed.

Particle size determination To avoid multiple scattering effects, CAS-stabilized emulsions (CAS emulsions) were diluted to a droplet concentration of approximately 0.005 wt% using the same buffer solution used for the preparation of the CAS emulsions, and stirred continuously throughout the measurements to ensure the samples were homogenous. The particle size distribution of the emulsions was then measured using a laser light scattering instrument (Mastersizer MSS; Malvern Instruments, Worcestershire, UK). This instrument measures the angular dependence of the intensity of laser light (λ=632.8 nm) scattered by a dilute emulsion, and then finds the particle size distribution that gives the best fit between experimental measurements and predictions based on light scattering theory. The mean particle size was reported as the surface-weighted mean diameter, d_{32} ($=\Sigma n_i d_i^3/\Sigma n_i d_i^2$) or the volume-weighted mean diameter, d_{43} ($=\Sigma n_i d_i^4/\Sigma n_i d_i^3$), where n_i is the number of particles with diameter d_i. The d_{43} value is more sensitive to the presence of large particles than the d_{32} value, and therefore it can give a good indication of droplet aggregation. All measurements were made on 2 freshly prepared samples and results are reported as averages.

ζ-Potential measurements Prior to analysis, emulsions were diluted to a droplet concentration of approximately 0.006 wt% using the same buffer solution used for the preparation of the CAS emulsions. The ζ-potential of the droplets was then determined using a particle electrophoresis instrument (ZEM5002; Zetamaster, Malvern Instruments) that measures the direction and velocity of droplet movement in the applied electric field. An individual ζ-potential measurement was determined from the average of 5 readings taken on the same sample.

Optical microscopy Emulsions were gently agitated in a glass test tube before analysis to ensure that they were homogenous. A drop of emulsion was placed on a microscope slide and then covered with a cover slip. The microstructure of the emulsion was then observed using conventional optical microscopy (Nikon Microscope Eclipse E400; Nikon Corporation, Tokyo, Japan). The images were acquired using a CCD camera (CCD-300T-RC; DAGE-MTI, Michigan City, IN, USA) connected to Digital Image Processing Software (Micro Video Instruments Inc., Avon, MA, USA) installed on a computer.

Influence of salt concentration on the stability of CAS emulsions We examined the influence of $CaCl_2$ on the mean particle diameters, ζ-potential, and microstructure of the emulsions (20 wt% corn oil, pH 7) stabilized by 3.2 wt% of CAS. This CAS concentration was selected because the emulsions stabilized with 3.2 wt% of CAS have shown the phenomena that are typical of depletion flocculation under the laser diffraction instrument and microscopy. O/W emulsion samples (20 wt% corn oil, 3.2 wt% CAS) were prepared as described in emulsions preparation section, and the emulsions were added with different amount of $CaCl_2$, with the final concentration of $CaCl_2$ being 0 to 20 mM (0, 5, 10, 15, and 20 mM) and stirred for 30 min. The pH of the all emulsions were adjusted back to pH 7 to exclude the influence of pH reduction ensued by the addition of $CaCl_2$ (19). The emulsion samples (10 g) were transferred into glass test tubes (i.d. 15 mm, height 125 mm) and stored at room temperature for 24 hr before being analyzed.

Statistical analysis Experiments were performed twice using freshly prepared samples. Averages and standard deviations were calculated from these duplicate measurements. All results were presented with average values because standard deviations were less than 5% for the emulsions containing non-flocculated droplets.

Results and Discussion

Influence of initial protein concentration on emulsion formation and stability We examined the influence of CAS concentration (0.8 to 3.2 wt%) on the formation and stability of corn oil-in-water emulsions produced by high pressure valve homogenization. The mean particle diameter (Fig. 1), particle size distribution (Fig. 2), electrical charge, and microstructure (Fig. 3) of the emulsions were measured. The ζ-potential of the droplets in the CAS emulsions was negative at pH 7.0 because the net electrical charge of adsorbed casein on the surface of the oil droplets was negative at the pH above protein's isoelectric point (pI= 4.6) (10). There was no significant change in ζ-potential with CAS concentration (0.8-3.2 wt%), with the average over all CAS concentrations being ζ=−41.0±1.8 mV for the CAS-stabilized emulsions. The fact that the emulsion droplets were coated by a biopolymer with an appreciable electrical charge suggests that electrostatic repulsion may play an important role in stabilizing them against droplet aggregation (1). Nevertheless, our studies on the influence of salt concentration suggest that steric interactions may play an important role.

Our previous study showed that CAS-coated emulsions stabilized with 1 wt% of CAS had a small fraction of particles with diameters greater than 10 μm at pH 7 although the vast majority of droplets had submicron diameters (12). Sharma *et al.* (13, 14) have shown that caseinate is more effective at preventing coalescence when it forms a relatively thick interfacial membrane around the droplets, e.g., when solution conditions favor multilayer formation or adsorption of casein micelles rather than individual casein molecules. These studies suggested that casein was a fairly effective emulsifier at pH 7 and the amount of caseinate used in the study (1 wt%) should have been sufficient to cover all of the oil-water interface formed during homogenization, but it may not have been sufficient to form thick enough membranes to completely prevent coalescence. Thus here we prepared various oil-in-water emulsions stabilized with increasing concentrations of CAS (0.8-3.2 wt% CAS). The volume-surface mean particle diameter (d_{32}) of the emulsions was relatively small and independent of CAS concentration: 0.34±0.01 μm. On the other hand, there was a fairly steep decrease in the volume-weighted mean particle diameter (d_{43}) when the CAS concentration was increased from 0.8 to 2.0 wt%, after which the mean particle diameter slowly decreased and eventually reached a relatively constant value: 0.39 μm (Fig. 1). The reason for the discrepancy of the mean particle diameters (d_{32} and d_{43}) is because they are sensitive to different aspects of the particle size distribution (1): d_{32} is more sensitive to the presence of small particles, whereas d_{43} is more sensitive to the presence of large particles. This can be seen when the full particle size distributions of the emulsions are examined (Fig. 2). At relatively low CAS concentrations (≤2.4 wt%), the particle size distributions were either bimodal or multimodal, consisting of a major peak corresponding to a large fraction of relatively small droplets and minor peaks corresponding to small fractions of relatively large particles (Fig. 2). The small population of large droplets observed in the emulsions (stabilized by 0.8-2.4 wt% CAS) might be due to the insufficient caseinate present to completely cover the entire oil-water interface created during homogenization, or because some droplet coalescence occurred during or after homogenization. On the other hand, the particle size distributions (PSD) at

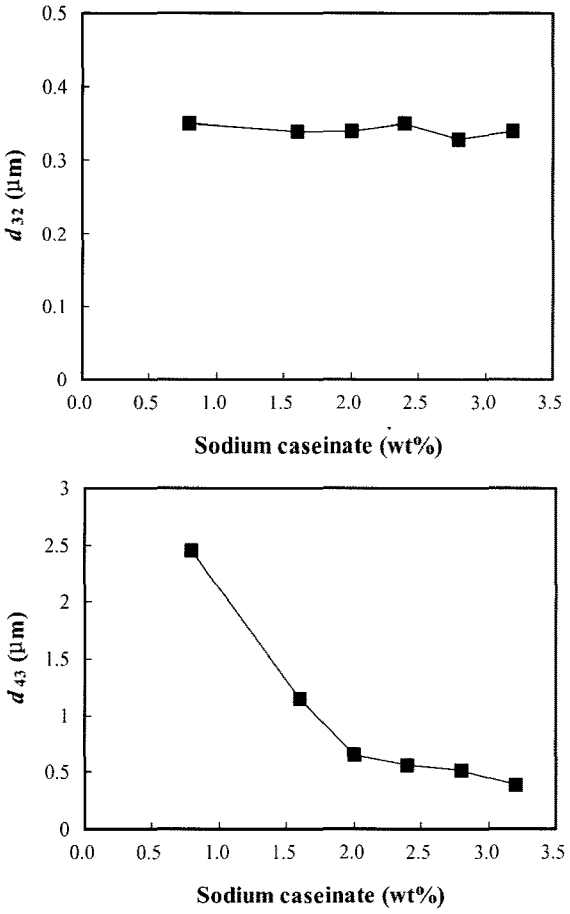

Fig. 1. Influence of protein concentration on mean particle diameters (d_{32} and d_{43}) of 20 wt% corn oil-in-water emulsion stabilized with CAS (5 mM imidazole/acetate buffer, pH 7). The results were presented as the averages of duplicated measurements and relative standard deviations were less than 5%.

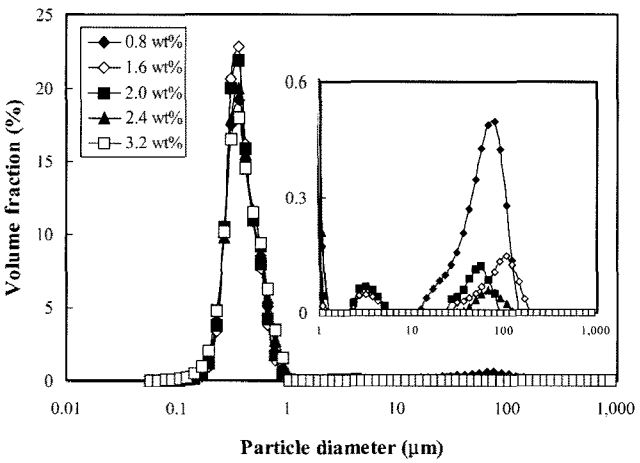

Fig. 2. Influence of CAS concentration on particle size distribution of 20 wt% corn oil-in-water emulsions. Inside box with magnified y scale shows clearly multi-modal distributions of emulsions stabilized with 0.8-2.4 wt% CAS.

higher CAS concentrations (2.8 and 3.2 wt%) appeared mono-modal (Fig. 2, PSD for 2.8 wt% was not shown but similar to that of 3.2 wt%). There are a number of possible reasons to account for the observed decrease in mean droplet size with increasing protein concentration: (i) the total droplet surface area that could be stabilized by the protein increased; (ii) the rate at which the droplet surfaces were covered with protein increased; (iii) the frequency of droplet collisions decreased due to the increase in aqueous phase viscosity. All of these factors should facilitate droplet disruption and prevent droplet coalescence within the homogenizer, thereby leading to the formation of smaller droplet sizes (1). Nevertheless, we believe this may have been an artifact of the sampling procedure for the laser diffraction measurements.

Optical microscopy indicated that there were some large droplets (d>10 μm) in all CAS stabilized emulsions regardless of protein concentration (Fig. 3). Nevertheless, the amount of large droplets observed in the emulsions at lower CAS concentrations tended to decrease as the CAS concentration increased (Fig. 3). Initially, these results

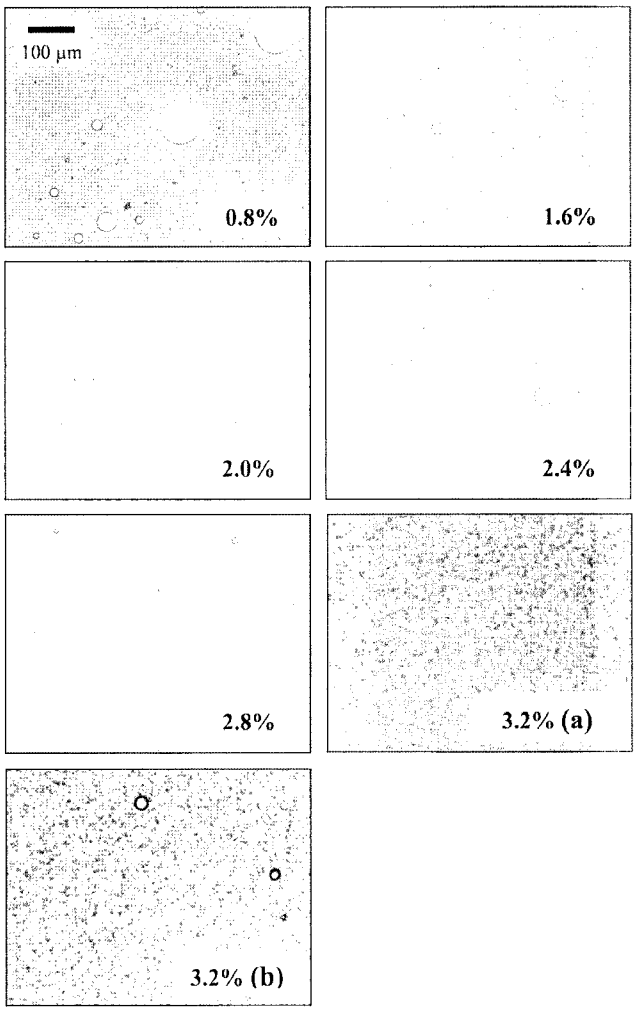

Fig. 3. Photomicrographs of 20 wt% corn oil-in-water emulsions stabilized with CAS (0.8-3.2 wt% CAS). More than 6 pictures were taken per each emulsion and a representative one was presented. For the emulsions stabilized with 3.2 wt% CAS, (a) was most representative and commonly observed under microscopy while (b) was rare.

seemed surprising because the laser diffraction measurements did not indicate the presence of any large droplets in the emulsions containing 3.2 wt% CAS (Fig. 2). We attribute the difference between the laser diffraction and optical microscopy to sampling errors associated with the laser diffraction instrument. It should be noted that the theory used to calculate the particle size distribution by the laser diffraction instrument assumes that the particles are spherical and homogeneous, but the particles in emulsions containing flocs are non-spherical and non-homogenous. In addition, dilution and stirring employed during laser diffraction instrument measurement are likely to disrupt weakly flocculated droplets, and stirring may form large oil droplets in emulsions that exhibit extensive oiling-off. Therefore, the particle size data on flocculated and highly coalesced emulsions should be interpreted with caution. Laser diffraction measurements can be used to measure the mean particle diameter (d_{32} and d_{43}) and the extent of droplet aggregation (% of d>1 μm) in diluted emulsions, while optical microscopy can provide information about the microstructures of original emulsions that are non-diluted. These facts underline the importance of using a range of different analytical techniques to accurately characterize the stability of emulsions prone to droplet aggregation.

Oppositely to the results from the laser diffraction instrument measurements (Fig. 1 and 2), optical microscopy measurements indicated that the increase in CAS concentration rather promoted flocculation and coalescence in the emulsions stabilized with the CAS concentration above a certain level (here, >2.8 wt%) (Fig. 3). We proposed that the droplet aggregation observed at 3.2 wt% of CAS might have been due to a depletion flocculation induced by non-adsorbing CAS particles in the emulsion (1,21). The full particle size distribution that obtained from the diluted emulsions supported the postulate because the dilution employed during laser diffraction measurement could decrease free CAS concentration in the emulsion sample thus prevent formation of flocs and/or disrupt the existent flocs induced by depletion flocculation, thereby any peak corresponding to large droplets was not observed in the emulsion (Fig. 2). The presence of free non-adsorbing colloidal particles causes an attractive interaction between the droplets that is often large enough to promote emulsion instability (1). That the sufficiently high concentrations of non-adsorbed caseinate can promote emulsion instability through a depletion flocculation mechanism has been also reported in previous studies (9,15). The appearance of large oil droplets in the emulsions containing 3.2 wt% CAS (Fig. 3) can be attributed to the fact that coalescence is more likely when flocculation occurs because the droplets are forced closer together for extended periods (1). Accelerated coalescence in oil-in-water emulsions due to an increased depletion attraction between flocculated droplets has been reported (22,23).

The above results indicate that at least 2.8 wt% CAS is required to make 20 wt% of corn oil-in-water emulsions where the majority of oil droplets are relatively small and there is little evidence of depletion flocculation. Nevertheless, we expect that stable CAS-stabilized emulsions might be prepared with 3.2 wt% of CAS, provided that salt ($CaCl_2$) is added into the emulsions to prevent the depletion

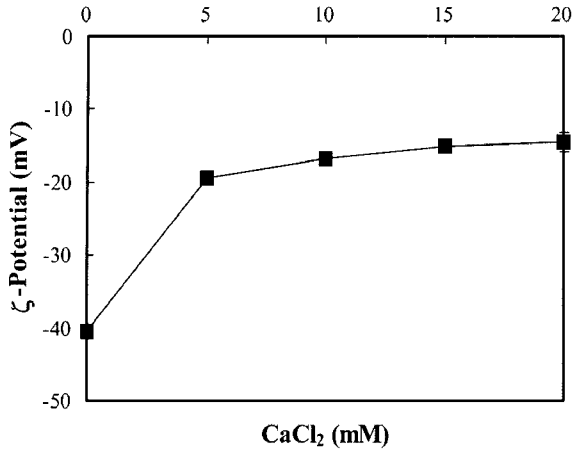

Fig. 4. Influence of $CaCl_2$ on the ζ-potential of 20 wt% corn oil-in-water emulsion stabilized with 3.2 wt% CAS.

flocculation observed at this CAS concentration because positively charged calcium ions can bind with negatively charged free non-adsorbing CAS that triggers droplet aggregation. Consequently, we used this 3.2 wt% of CAS protein concentration (i.e., a protein-to-oil ratio of 1 : 6.25 g/g) in the subsequent experiment examining the influence of salt concentration on the stability of CAS-stabilized emulsions.

Influence of $CaCl_2$ on emulsion stability The purpose of this experiment was to prepare stable CAS emulsions by adding calcium chloride (0-20 mM) into the CAS-stabilized emulsions thus preventing the depletion flocculation between emulsion droplets. The influence of salt concentration on the ζ-potential (Fig. 4), mean particle diameter (Fig. 5 and 6), and microstructure (Fig. 7) of 20 wt% corn oil-in-water emulsions stabilized by 3.2 wt% CAS at pH 7 was measured after they were stored at room temperature for 24 hr.

Measurements of the ζ-potential of the droplets in the emulsions containing various $CaCl_2$ concentrations are shown in Fig. 4. In the absence of $CaCl_2$, the ζ-potential of the CAS-coated droplets was around −40 mV. The magnitude of the ζ-potential on the droplet in the emulsions decreased (less negative) sharply with increasing $CaCl_2$ concentration, changing from −40 to −19 mV as the $CaCl_2$ concentration was increased from 0 to 5 mM, after which concentration the electrical charges also became less negative with increasing $CaCl_2$ concentration (5-20 mM) but the extent of the decrease was considerably less. This decrease can be attributed to electrostatic screening effects since $\zeta \propto 1/\sqrt{I}$ for a spherical particle with constant surface charge density, where I is the ionic strength of the aqueous solution surrounding the particle (16). It can be also attributed to the binding of Ca^{2+} ions with the CAS protein molecules attached to the droplets surfaces. The ζ-potential of the droplets in the emulsion decreased to 48% of its initial value when the $CaCl_2$ concentration was increased from 0 to 5 mM, but the ζ-potentials of the emulsions containing 10-20 mM $CaCl_2$ decreased only to 75-86% of the value at 5 mM $CaCl_2$. The lower sensitivity of the ζ-potential of the droplets in the emulsions (containing 10-20 mM $CaCl_2$) to $CaCl_2$ suggests that following charge

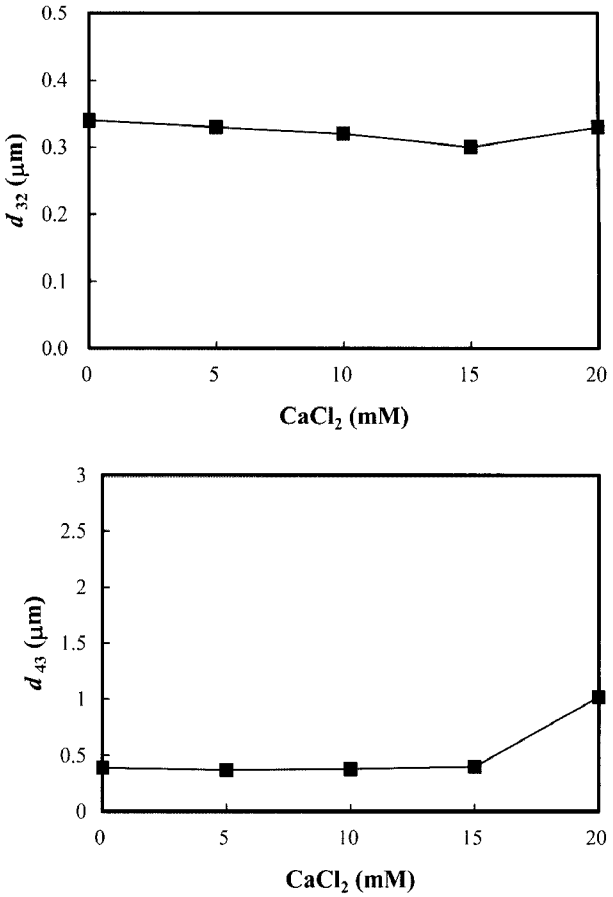

Fig. 5. Influence of $CaCl_2$ on mean particle diameters (d_{32} and d_{43}) of 20 wt% corn oil-in-water emulsion stabilized with 3.2 wt% CAS.

regulation mechanism operated on the droplet surface (16,24). Charge regulation may have occurred because salt screened the electrostatic repulsion between the anionic droplets and the anionic CAS molecules, which promoted adsorption of additional proteins to the droplet surfaces. Thus, the decrease in droplet ζ-potential caused by electrostatic screening effects was compensated for by an increase in the negative surface charge density of the droplets due to adsorption of additional CAS molecules through the bridging role of calcium ions. Nevertheless, the overall influence of $CaCl_2$ would be expected to be fairly complex since electrostatic screening effects would also change the conformation of the adsorbed CAS molecules, and alter the overall strength of the droplet-droplet interactions. Measurements of the mean particle diameters and particle size distributions of the emulsions containing various $CaCl_2$ concentrations are shown in Fig. 5 and 6. The surface-weighted mean particle diameter (d_{32}) of the emulsions was relatively small and independent of salt concentration from 0 to 20 mM $CaCl_2$: 0.32±0.02 mM. On the other hand, salt concentration had a little impact on the volume-weighted mean particle diameter (d_{43}) of the emulsions (Fig. 5). As the $CaCl_2$ concentration was increased from 0 to 15 mM the d_{43} did not change statistically (0.39±0.01 m), but there was a little increase in the d_{43} (ca. 1 μm) of CAS-stabilized emulsions when the $CaCl_2$ concentration reached 20 mM, which can be also seen when the full particle size

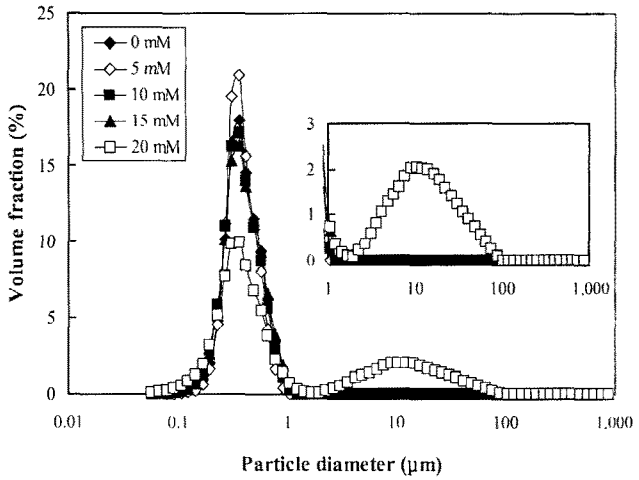

Fig. 6. Influence of $CaCl_2$ on particle size distribution of 20 wt% corn oil-in-water emulsions stabilized with 3.2 wt% CAS. Inside box with magnified y scale shows clearly bimodal distribution of CAS-stabilized emulsions containing 20 mM $CaCl_2$.

distributions of the emulsions are examined; i.e., monomodal and bimodal for the emulsions containing 5-15 and 20 mM $CaCl_2$, respectively (Fig. 6). These results suggest that the CAS stabilized emulsions were relatively stable to droplet aggregation even at high ionic strength where electrostatic interaction should be highly screened (Note that the absolute value of the ζ-potential over the range from 5 to 20 mM $CaCl_2$ remained relatively low: <20 mV (Fig. 5)). Hence, we can conclude that polymeric steric repulsion (rather than electrostatic repulsion) plays a major role in preventing the droplets from aggregating. Nevertheless optical microscopy indicated that there was a small population of large droplets in all non-diluted CAS-stabilized emulsions regardless of added salt concentration, however which phenomenon was not detected by laser diffraction instrument. To obtain an accurate measurement of the particle size distribution of an emulsion using laser diffraction, a representative sample must pass through the laser beam during the data acquisition period. If an emulsion contains large droplets, this fraction of the oil may cream rapidly and remain on the surface of the emulsion, thereby not passing through the laser beam and not being detected. The depletion flocculation observed in the emulsions stabilized by 3.2 wt% CAS without $CaCl_2$ was prevented with the addition of $CaCl_2$ over the ranges studied here (5-20 mM) although there were evidences of a small number of large individual oil droplets (Fig. 7), which could be attributed to the adsorption of additional CAS protein molecules to the droplets surface via calcium ion bridge and/or the chelating effect of bivalent calcium ion toward free non-adsorbed CAS protein molecules, both should decrease available CAS proteins for depletion attraction. Optical microscopy indicated that there was no evidence of droplet flocculation but rather there were droplet coalescences in the emulsions stabilized by 3.2 wt% CAS in the presence 5-20 mM $CaCl_2$. We postulate that some of the droplet coalescences in the emulsions might have occurred during or right after homogenization prior to $CaCl_2$ addition as showed in the microstructure picture at 0 mM $CaCl_2$ (Fig. 7) or that some coalescences

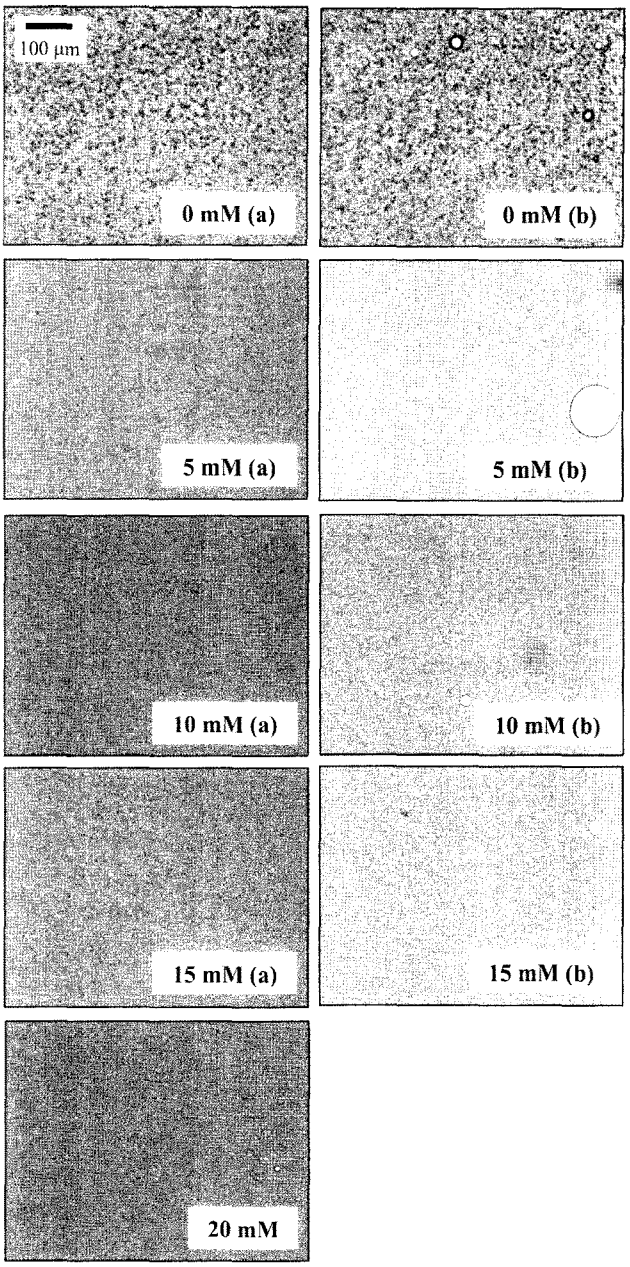

Fig. 7. Photomicrographs of 20 wt% corn oil-in-water emulsions stabilized with 3.2 wt% CAS at different $CaCl_2$ concentration (0-20 mM $CaCl_2$). More than 6 pictures were taken per each emulsion and a representative one was presented. (a) was most representative and commonly observed under microscopy while (b) was rare. For the emulsion containing 20 mM $CaCl_2$, all separate parts of the emulsion samples within microscopy frame were similar.

might have occurred as a result of droplet flocculation that rapidly advancing to the stage of coalescence although this seems unlikely because the interfacial membrane formed by proteins generates strong short-range repulsive forces and is resistant to rupture thus highly stable to droplet coalescence (25).

Therefore, the results indicate that the instability of 3.2 wt% CAS-coated emulsions due to the presence of a surplus of CAS protein emulsifiers could be improved with the addition of salt that has been reputed to promote droplet aggregation in protein-stabilized emulsions at neutral pH.

In conclusion, the aim of this study was to examine the influence of salt on the stability of 20 wt% corn oil-in-water emulsions stabilized by CAS. In the absence of salt there were some large droplets (d>10 μm) in all CAS-stabilized emulsions regardless of the protein concentration (0.8-3.2 wt% CAS). Laser diffraction measurements and microscopy measurements indicated that depletion flocculation occurred in the emulsions stabilized with CAS concentration above a certain level (>2.8 wt%, here 3.2 wt%). The depletion flocculation observed in the emulsions could be prevented by the addition of 5-20 mM $CaCl_2$, which was attributed to electrostatic screening effect and bridging effect of the calcium ions. This study has shown that calcium ions, the major multivalent cations found in most nutritional foods, could improve the stability against droplet aggregation in the emulsion-based foods that use proteins as emulsifiers.

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