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Comparison of Emulsion-stabilizing Property between Sodium Caseinate and Whey Protein Concentrate: Susceptibility to Changes in Protein Concentration and pH

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Abstract The stability of corn oil-in-water emulsions coated by milk proteins, sodium caseinate (CAS), or whey protein concentrate (WPC), was compared under the environmental stress of pH change. Emulsions were prepared at 0.1 of protein:oil because the majority of droplets were relatively small (d_{32}=0.34 and 0.35 μm, d_{43}=0.65 and 0.37 μm for CAS- and WPC-emulsions, respectively) and there was no evidence of depletion flocculation. As the pH of the emulsions was gradually dropped from 7 to 3, there was no significant difference in the electrical charges of the emulsion droplets between the 2 types of emulsions. However, laser diffraction measurements, microscopy measurements, and creaming stability test indicated that WPC-emulsions were more stable to droplet aggregation than CAS-emulsions under the same circumstance of pH change. It implies that factors other than electrostatic repulsion should contribute to the different magnitude of response to pH change.

Keywords: oil-in-water emulsion, sodium caseinate, whey protein, milk protein, pH

Introduction

Milk proteins have been used as emulsifiers in a wide variety of emulsion-based food products, including beverage, frozen desserts, ice creams, and salad dressing (1,2). They can be conveniently divided into 2 major categories, caseins (ca. 80%) and whey proteins (ca. 20%). In nature, both are complex mixtures of different individual proteins. That is, caseins consist of α_{s1}-, α_{s2}-, β-, and κ-caseins, and exist as micelles that are typically 50-250 nm in diameter with being partly held together by mineral ions (2). Whey proteins consist of β-lactoglobulin, α-lactalbumin, serum albumin, and immunoglobulins, and are characterized by well-defined 3-dimensional structures held together by disulfide bridges (2). Currently, milk protein ingredients come in a variety of different forms depending on their composition and the preparation procedures used to manufacture (3,4). Among them, sodium caseinate (CAS) and whey protein concentrates (WPC) have gained much interest because they are easy to manufacture and less expensive than individual purified proteins. This study focused on the two milk protein ingredients.

CAS, a form of casein, has been widely used as an emulsion stabilizer because of its good stability, surface activity, and heat stability (5). The good surface activity of CAS is due to its flexible structure and localized distribution of hydrophobic and hydrophilic residue (6-8). Higher heat stability of CAS is derived from the relatively flexible casein structure which do not undergo appreciable heat-induced conformational changes like globular proteins do (9,10). Compared with whey proteins that form relatively thin (1-2 nm) interfacial membranes, CAS forms a thick interfacial membrane of up to 10 nm around dispersed oil droplets (7,11). All of these characteristics of CAS should lead to the rapid establishment of a thick sterically stabilizing layer that protects newly formed droplets against droplet flocculation and coalescence.

Unlike CAS-emulsions, whey protein-stabilized emulsions are relatively susceptible to conformational changes and aggregation under thermal processing (2). There were a number of studies that compared caseins and whey proteins under temperatures, from which the caseins and whey proteins came to be conveniently regarded as heat-stable and heat-sensitive milk proteins, respectively (2,7,9-16). However, studies dedicating to the direct comparison between caseins and whey proteins in relation to emulsions stability under the same circumstance of pH are limited. If any, they mainly focused on the specific individual proteins (e.g., α_{s1}-casein, β-casein, or β-lactoglobulin) not on the mixture-form of milk protein ingredients (e.g., CAS or WPC), because the formers were considered as those responsible for the functional characteristics of caseins or whey proteins due to their relatively high concentration and unique physicochemical properties. (2,11,14,15). However, WPC contains a variety of non-proteins that might affect its emulsifying property, e.g., phospholipids, lipids, minerals, and sugars. Therefore, for the application of CAS or WPC, it is necessary to give attention to the mixture-form of milk proteins that completely reflect the influence of these various factors on emulsion stability.

This study compared the stability of emulsions coated by 2 types of milk proteins, CAS or WPC, by focusing specifically on the effect of pH change. From the practical point of view, I particularly wanted to compare which milk protein ingredient was more stable to droplet aggregation and whose physicochemical characteristics assisted better in stabilizing the emulsions under the environmental stress of pH drop.

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Materials and Methods

Materials Sodium caseinate (ALANATE 180) and whey protein concentrate were kindly provided from New Zealand Milk Products (NZMP, Lot # 0034-W5166, Lemoyne, PA, USA) and Glanbia Nutritionals (Twin Falls, ID, USA), respectively. The proximate compositions of the 2 milk proteins were presented in Table 1. Analytical grade sodium hydroxide (NaOH), hydrochloric acid (HCl), imidazole, and sodium azide (NaN_3) were purchased from the Sigma-Aldrich (St. Louis, MO, USA). Acetic acid was purchased from Fisher Scientific (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 Protein solutions were prepared by dispersing the desired amount (0.05-2.0 wt%) of sodium caseinate (CAS) or whey protein concentrate (WPC) into buffer solution (5 mM imidazole/acetate buffer, pH 7) and stirring overnight at room temperature to ensure complete hydration. The pH of the CAS or WPC solutions was adjusted back to pH 7.0 if required.

O/W emulsions were prepared by blending 10 wt% corn oil and 90 wt% emulsifier 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 (protein:oil=0.04-0.18 for CAS and 0.0045-0.18 for WPC) were then stored at ambient temperature for 24 hr before being analyzed.

The influence of pH on the emulsions stabilized by CAS or WPC was examined on the emulsions where the ratio of the protein to oil was 0.1, i.e., 10 wt% corn oil and 0.9 wt% CAS or WPC. The emulsions were diluted with the imidazole/acetate buffer to a final droplet concentration of 5.0 wt%. The pH of the diluted emulsions was then adjusted to 3, 4, 5, 6, and 7 using HCl solutions.

Particle size determination To avoid multiple scattering effects, CAS-stabilized emulsions (CAS-emulsions) or WPC-stabilized emulsions (WPC-emulsions) were diluted to a droplet concentration of approximately 0.005 wt% using buffer solution at the pH of the sample, 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. All measurements were made on 2 freshly prepared samples and results are reported as averages.

It should be noted that dilution and stirring 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.

ζ-Potential measurements Prior to analysis, emulsions were diluted to a droplet concentration of approximately 0.006 wt% using buffer solution at the pH of the sample. 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. The ζ-potentials provides an estimate of the net charge on a particle measured at the 'shear plane', which depends on the charge on the actual particle plus the charge associated with any ions that move along with the particle in the 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 carge coupled device (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. In this measurement, aggregated droplets are clearly visible whereas fine oil droplets are visible only as a homogeneous texture. This microscopic measurement provides information about the tendency for droplets to aggregate in non-diluted emulsions and about whether droplet aggregation is due to coalescence or flocculation. Therefore, this technique should always be used to complement the particle size measurement. It should be noted that it may be difficult to determine whether droplets are just in close proximity or aggregated in a concentrated emulsion.

Creaming stability measurements Ten g of emulsion were transferred into a test tube (i.d. 15 mm, height 125 mm), tightly sealed with a plastic cap, and then stored for 1 week at room temperature. Emulsions tend to be separated into an optically opaque 'cream' layer at the top and a transparent (or turbid) 'serum' layer at the bottom with time. The serum layer was defined as the sum of the turbid and transparent layers. The total height of the emulsion (H_E) and the height of the serum layer (H_S) were measured. The extent of creaming was characterized by serum (%)=(H_S/H_E)×100. The % serum provided indirect information about the extent of droplet aggregation in an emulsion. All measurements were made on at least 2 freshly prepared samples.

Statistical analysis Experiments were performed twice

using freshly prepared samples. Averages and standard deviations were calculated from these duplicate measurements.

Results and Discussion

Composition of CAS and WPC The proximate composition of the milk proteins was presented in Table 1. CAS had much higher protein content and lower fat and lactose contents than those of WPC. In addition, there was a noticeable difference between the mineral compositions of the 2 proteins. While CAS contained mainly sodium, WPC consisted of calcium, potassium, chloride, and phosphorous, and which contents were relatively even. These differences in composition could partly account for differences in the observed properties and stability of O/W emulsions produced by these proteins (see later).

Determination of the optimum ratio of protein to oil for the preparation of stable CAS- or WPC-emulsions The purpose of this experiment was to determine the minimum amount of protein concentration to form stable emulsions. A series of corn oil-in-water emulsions were prepared with different ratios of protein to oil (protein:oil= 0.04-0.18 for CAS and 0.0045-0.18 for WPC) and protein types (CAS and WPC), and their mean droplet diameters (Fig. 1) and microstructures (Fig. 2) were measured.

For CAS protein, there was no significant change in the surface-weighted mean droplet diameters (d_{32}, which is more sensitive to the presence of small droplets) of the emulsions depending on CAS content (CAS:oil=0.04-0.18), with the average over all CAS:oil ratios being d_{32}= 0.34±0.01 μm (i.e., less than 5% of relative standard deviation) (Fig. 1A). On the other hands, the volume-weighted mean droplet diameters (d_{43}, which is more sensitive to the presence of large droplets) of the emulsions slowly decreased and eventually reached 0.39 μm at 0.18 of CAS:oil (Fig. 1B). For WPC protein, no noticeable difference was observed in both d_{32} (0.37±0.04 μm) and d_{43} (0.59±0.26 μm) of the emulsions when the ratio of WPC to oil increased from 0.04 to 0.18. Thus, 3 WPC-emulsions were additionally prepared at lower ratios of WPC to oil (WPC:oil=0.027, 0.009, and 0.0045). At the ranges of WPC:oil, there were fairly steep decrease in d_{32} and d_{43} depending on WPC content (Fig. 1A and 1B). Optical microscopy measurement confirmed that a population of large droplets observed at lower ratio of protein:oil tended to decrease with increasing protein content (Fig. 2). This phenomenon has been typically observed in protein-stabilized emulsions, and thus its possible reasons have been well established in the literatures (2,11,15,17-19).

Interestingly, microscopy observation clearly showed that the CAS rather promoted droplet aggregation at high ratio of protein:oil (here, at 0.18) where no aggregation

Table 1. Proximate composition (%) and mineral contents (mg/100 g) of sodium caseinate (CAS) and whey protein concentrate (WPC)[1)]

Constituent (%)	CAS	WPC
Protein	92.7	76.7
Fat	0.8	5.7
Moist	4.2	3.3
Lactose	0.1	7.7
Ash	3.5	3.0
Mineral (mg/100 g)		
Calcium	60	487
Magnesium	-	53
Sodium	1,380	182
Potassium	-	570
Chloride	-	280
Phosphorus	-	380

[1)]Provided by suppliers, New Zealand Milk Products for CAS and Glanbia Nutritionals for WPC.

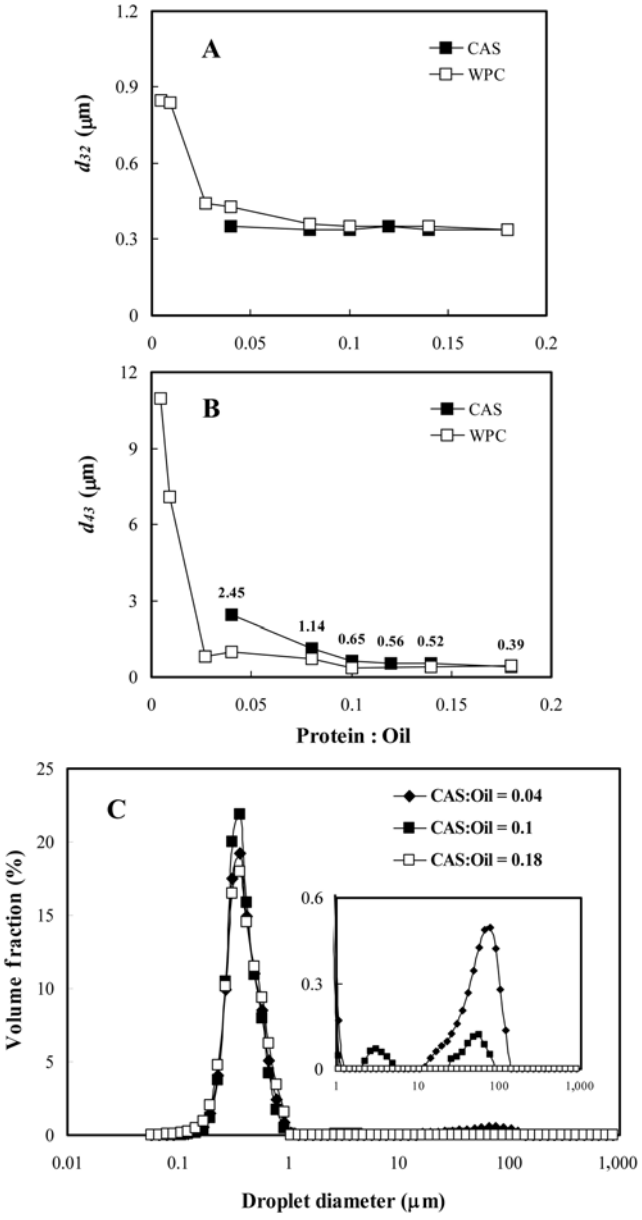

Fig. 1. Influence of protein:oil ratio on the mean droplet diameters (A: d_{32} and B: d_{43}) and droplet size distributions (C) of corn oil-in-water emulsions stabilized with CAS or WPC (5 mM imidazole/acetate buffer, pH 7). The results were presented with the averages of duplicated measurements and relative standard deviations were less than 5% for the emulsions containing non-flocculated droplets.

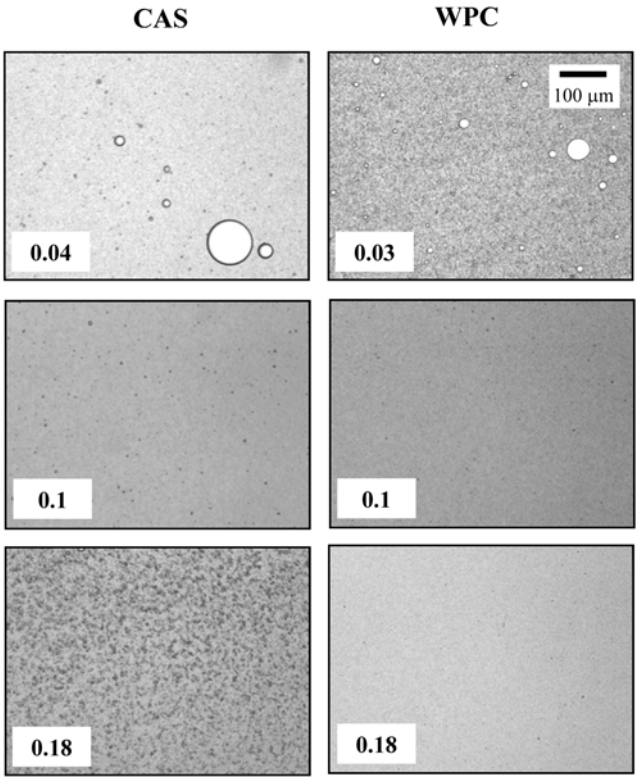

Fig. 2. Influence of protein:oil ratio on the microstructures of corn oil-in-water emulsions stabilized with CAS or WPC (5 mM imidazole/acetate buffer, pH 7). More than 6 pictures were taken per each emulsion and a representative one was presented.

was observed in WPC-emulsions (Fig. 2). However, at 0.18 of CAS:oil, the droplet size distribution of CAS-emulsion was mono-modal with a peak corresponding to relatively small droplets (0.1 to 1 μm), and thus did not indicate any presence of large droplet aggregates (Fig. 1C). The discrepancy was attributed to the depletion flocculation that could be induced by non-adsorbed CAS protein molecules in the emulsions. That is, when the CAS emulsion that was undergoing depletion flocculation was diluted for droplet sizing measurement, the aggregates broke down since the concentration of non-adsorbed CAS protein molecules fell below the critical flocculation concentration (CFC), thereby any peak corresponding to large droplets was not observed in the emulsion. Typically, non-adsorbed molecules are excluded from a narrow region surrounding the emulsion droplets (depletion zone), which causes osmotic potential difference between the depletion zone ([molecules]=0) and surrounding continuous phase. For that reason, depletion flocculation occurs at above CFC to dilute non-adsorbed molecules and thus to reduce the concentration gradient. Physically, emulsion droplets are weakly aggregated and thereby the volume of surrounding continuous phase is increased as much as that of depletion zone is reduced (2).

Both caseinate and whey proteins have been known to be capable of inducing depletion flocculation when added in sufficiently high concentration (2,10,20). However, typical phenomenon of depletion flocculation was not observed in WPC-emulsion at the same ratio of 0.18 (Fig. 2). It indicated that the lowest concentration required to cause depletion flocculation (CFC) was lower in CAS than WPC, which difference was presumably due to the intrinsic physical characteristic of CAS. Compared with whey proteins, non-adsorbed sodium caseinates exist as small self-assembled protein particles called casein sub-micelles in the aqueous phase (5). Previous studies have reported that the sub-micelles have the potential for generating an appreciable depletion force, unlike individual casein molecules that are too small to induce a depletion attraction between droplets. (2,5,10,20). It has been well known that the strength of the depletion attraction between emulsion droplets increases (i) as the effective size of non-adsorbed molecules increases (compare casein sub-micelles with individual whey proteins); (ii) as the size of emulsion droplets increases (however, at 0.18 of protein: oil, the sizes of CAS- and WPC-emulsion droplets were not different. see Fig. 1 and 2) (2).

The mean droplet size was slightly smaller in the WPC-emulsions than in the CAS-emulsions at the same ratio of protein to oil (Fig. 1). Initially, this result was surprising because (i) CAS could adsorb more readily than whey proteins because of its higher flexibility and higher hydrophobicity (19) and (ii) CAS could provide rather extended membranes up to about 10 nm thick while whey proteins give membranes that are only about 2 nm thick (11). There are a couple of possible reasons to account for the unexpected result. Firstly, WPC contained, unlike individual whey protein or whey protein isolates, higher amounts of fat as well as whey protein itself (Table 1). Fats such as phospholipids adsorbs more rapidly than proteins to the surfaces of the droplets formed during homogenization, which would facilitate the production of small droplets and increase the repulsive interactions between the droplets once formed. Indeed, even 4% increase of fat in WPC (4 g fat increase/100 g WPC) significantly improved emulsifying property and emulsion-stabilizing property of WPC (2). Secondly, fats and proteins interacts at the oil-water interface during the WPC-emulsions preparation, which would increase the thickness of the interfacial membrane around the emulsion droplets and thus provide good stability against droplet aggregation (21). Lastly, the amount of CAS used in this study was not sufficient to form 10 nm thick membranes although it was enough to cover all oil-water interfaces formed during homogenization. There are 2 types of CAS conformations depending on CAS:oil; (i) CAS molecules are spread over an entire surface area at relatively low ratio of CAS:oil and (ii) CAS molecules are packed in the presence of excess CAS (11). One might expect that the latter provides higher steric repulsion and coalescence stability than the former. The fact of that a small population of relatively large droplets were observed in the CAS-emulsions even at 0.1 of CAS:oil (Fig. 2) suggested that the CAS content was not sufficient to be packed over the oil-water interfaces, thus the adsorbed CAS membrane might not be as thick as 10 nm in this preparation condition.

Overall, the above results indicate that the ratio of protein:oil should be at least 0.1 to produce emulsions where the majority of droplets are relatively small, and should be also less than 0.18 to prevent depletion flocculation (to minimize the influence of protein surplus).

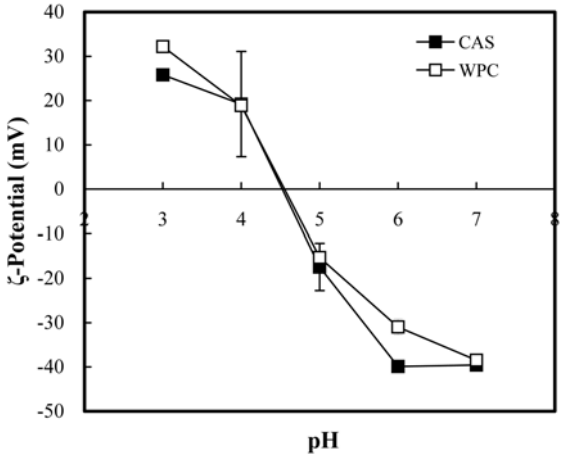

Fig. 3. Influence of pH drop on the ζ-potential of corn oil-in-water emulsions stabilized with CAS or WPC. Both emulsions were prepared at 0.1 of protein:oil. The pH of the emulsions was gradually reduced from 7 to 3.

Consequently, 0.1 of protein:oil was used in the remainder of this study.

Influence of pH on the net electrical charge of emulsion droplet For both CAS- and WPC-emulsions, the magnitude of the electrostatic repulsion between the droplets in the emulsions was strongly influenced by pH (Fig. 3). The ζ-potential of the droplets in the emulsions went from negative (–40 mV for CAS and –39 mV for WPC) to positive (+26 mV for CAS and +32 mV for WPC) as the pH was gradually dropped from 7 to 3. It was because the net electrical charge of the protein adsorbed on the surface of the droplets switched from negative to positive as the pH decreased from above to below the protein's isoelectric point (pI). For both emulsions, the ζ-potentials became 0 at around pH 4.5, which indicated that the number of positively charged groups on the adsorbed protein molecules was equal to the number of negatively charged groups and therefore the net surface charge of the droplets was neutral. The ζ-potential vs. pH measurements suggested that the pI of CAS or WPC should be somewhere between pH 4 and 5, which was in agreement with previously reported values (3,4,22).

The electrical charge of the droplets stabilized by WPC was slightly higher, albeit not significant, than that of the droplets stabilized by CAS at all pHs (Fig. 3). This might have been because of the differences in the number and pK_a of ionizable amino acid side groups of CAS and WPC proteins and the differences in the contents of ionic fats (e.g., phospholipids such as phosphatidylcholine and phosphatidylethanolamine) and divalent ions (e.g., Ca^{2+}). The type and content of molecules in the interfacial membrane surrounding the oil droplet affect on the droplet's surface charge. For example, all of Na^+, K^+, Ca^{2+}, and Cl^- ions are capable of binding to droplets surfaces that present opposite charges to the ions and thus decreasing electrostatic repulsion between droplets. In particular, divalent ions are far more effective in altering the droplet charge than monovalent ions (23). Indeed, the content of $CaCl_2$ required for destabilizing O/W emulsions stabilized by β-lactoglobulin was 2 orders of magnitude lower than that of NaCl (23,24). For that reason, the fact of that the amounts of divalent ions such as Ca^{2+} and Mg^{2+} were higher in WPC than CAS (Table 1) might partly account for the difference in the ζ-potentials. That is, positively charged divalent Ca^{2+} or Mg^{2+} ions were attracted to the negatively charged droplet surface of WPC-emulsion at pH above pI, by which the net negative charge of the WPC-emulsion droplet surface was much lowered than that of CAS-emulsion droplet surface where monovalent Na^+ ions were bound.

The major mechanism preventing droplet aggregation in protein-stabilized emulsions is electrostatic repulsion (23), for which the absolute magnitude of ζ-potential should be sufficiently high (typically, $|\zeta|>20$ mV) to overcome the various attractive interactions between droplets such as van der Waals, hydrophobic, or depletion. Therefore, the result from ζ-potential measurement implied that the emulsion droplets were likely to aggregate at pH 4 and 5 for both CAS- and WPC-emulsions.

Influence of pH on the size of emulsion droplet The mean droplet diameters and microstructures of the CAS- and WPC-emulsions were determined as the pH dropped gradually from 7 to 3 following the emulsions preparation at pH 7. The mean droplet diameters (d_{32} and d_{43}) were relatively small at pH 6 and 7 for CAS-emulsions and at pH 3, 6, and 7 for WPC-emulsions but were appreciably higher at intermediate pH values (pH 4 and 5), which indicated that considerable droplet aggregation occurred at pH values within one unit either side of the pI of the proteins (Fig. 4-6).

At pH 6 to 7, the droplet size distributions of CAS- and WPC-emulsions were monomodal with a peak consisting of small droplets (0.1 to 1 μm) (Fig. 4D), but there was a small fraction of droplets with diameters greater than 1 μm ($d>1$ μm≈12% at pH 6 and 3% at pH 7) (Fig. 4C). Optical microscopy measurements also indicated that the majority of oil droplets were small and there was a small population of relatively large individual droplets in CAS- or WPC-emulsions at the pHs (Fig. 5). At visual observation, no creaming was found in the emulsions (Fig. 6), which suggested that the majority of the droplets in the emulsions were small enough to resist creaming and were not aggregated. The good emulsion stability against droplet aggregation at the pH 6 and 7 was completely in accordance with the prediction made from the ζ-potential measurement (Fig. 3). That is, at the pH 6 and 7, the protein (CAS or WPC) molecules were negatively charged (Fig. 3), thus which induced electrostatic repulsion between the neighboring protein molecules inside the adsorption membranes as well as between the adsorption membranes on 2 neighboring emulsion droplets. The electrostatic repulsion was appreciably large enough to overcome a variety of attractive interactions between droplets, therefore, the droplets were not aggregated and creaming did not occurred within the experimental period.

At pH 4 and 5 where close to the pI of the adsorbed proteins, there was evidence of extensive droplet aggregation in both CAS- and WPC-emulsions (Fig. 4-6). The reason for the droplet aggregation probably lies in the decrease in the absolute magnitude of the electrical charge on the

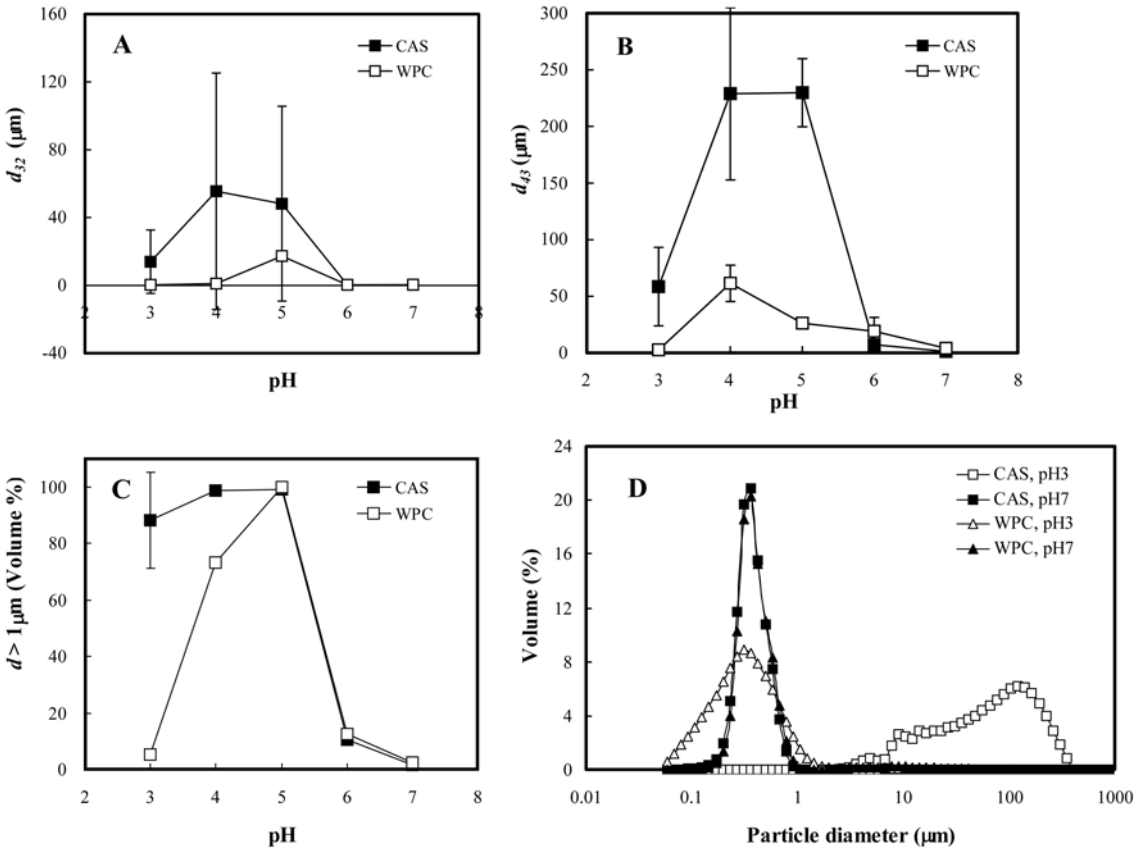

Fig. 4. Influence of pH drop on the mean droplet diameters (A: d_{32} and B: d_{43}), volume fractions (%) of d>1 μm (C), and droplet size distributions (D) of corn oil-in-water emulsions stabilized with CAS or WPC (protein:oil=0.1). The pH of the emulsions was gradually reduced from 7 to 3.

droplets at the pHs (Fig. 3). The lowered net charge (|ζ|< 20 mV) reduced the level of electrostatic repulsion between protein-coated membranes, thereby promoted emulsion aggregation. At the pHs, the extent of droplet aggregation appeared greater in CAS- than in WPC-emulsions (Fig. 4 and 5). Microscopy pictures clearly showed that there were much larger flocs observed with empty spaces appearing between the droplets in CAS-emulsions (Fig. 5). Normally, electrostatic interactions play a key role in stabilizing protein-coated droplets against aggregation. However, at pH≈pI, electrostatic repulsion between the emulsion droplets might be negligible due to their small net charge (|ζ|<20 mV), and in fact the ζ-potential values at pH 4 and 5 were similarly low for both CAS- and WPC-emulsions (Fig. 3). Therefore, the difference in the degree of droplet aggregation can not be explained by the electrostatic repulsion between adsorption membranes on the surfaces of 2 neighboring droplets. As discussed earlier, initially it was expected that CAS might provide thicker membrane than WPC because of its flexible and disordered structure. Thus the resulting emulsions coated by the protein molecules might be sterically stabilized with a greater membrane thickness with dangling tails, in contrast to the emulsions compactly coated by globular whey proteins. However, microscopy picture indicated that the CAS-emulsion droplets at 0.1 of CAS:oil might have not been surrounded by membranes as thick as they were expected (Fig. 2), and it was reported that the membrane thickness of CAS-emulsions tended to diminish as pH was close to pI because of the conformational change in pH-sensitive dangling tails that had the highest density of charged groups (15). Thus, firstly, the difference between CAS- and WPC-emulsions at pH 4 and 5 was attributed to the difference in the steric repulsion depending on the molecular weights of constituents in CAS or WPC, because (i) compared to CAS, WPC contained higher proportions of high molecular weight proteins, and (ii) the thickness of an adsorbed membrane increased with increasing protein molecular weight and thus the emulsions coated by higher molecular weight proteins showed better stability to droplet aggregation (25-27). However, this seems unlikely because the functionality of WPC is mostly induced by a large fraction of low molecular weight proteins such as β-lactoglobulin (18 kDa) and α-lactalbumin (14 kDa) not by a small fraction of high molecular weight proteins such as heavy chain immunoglobulins (>80 kDa), lactoferrin (ca. 80 kDa), and bovine serum albumin (69 kDa). Therefore, the difference might be rather due to the higher viscosity of WPC- than CAS-emulsions (6). It was reported that the surface membranes made by whey proteins were more viscous than those of casein, and which was attributed to the unfolding and interaction of globular whey proteins at the oil-water interface (6).

Creaming stability measurements at pH 4 and 5 (Fig. 6) confirmed the results from light scattering and microscopy.

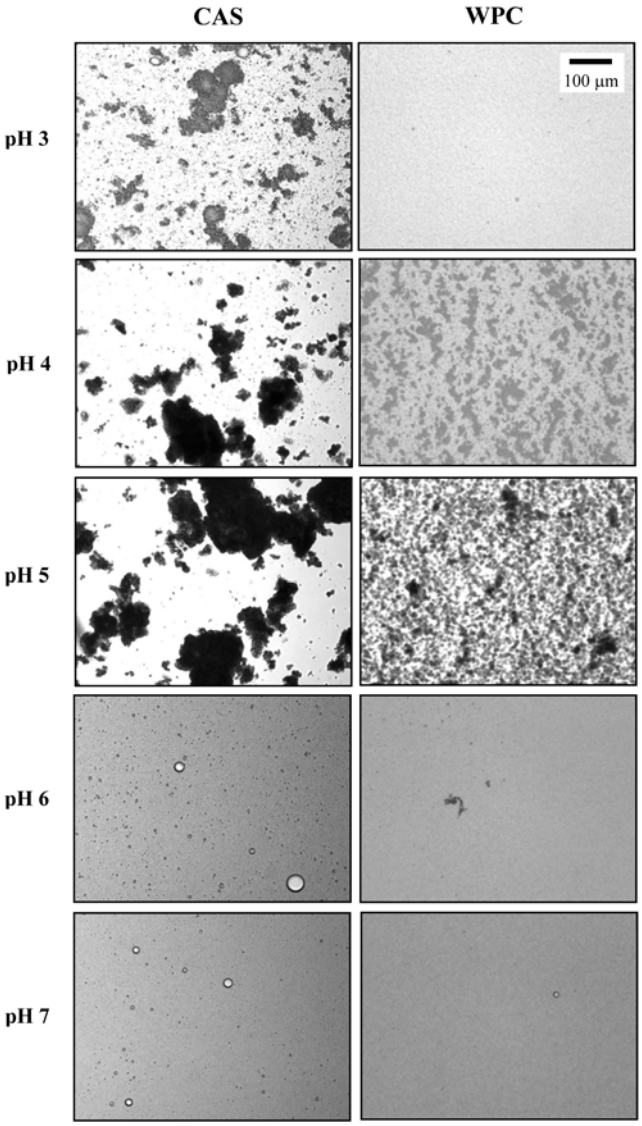

Fig. 5. Influence of pH drop on the microstructures of corn oil-in-water emulsions stabilized with CAS or WPC (protein:oil=0.1). The pH of the emulsions was gradually reduced from 7 to 3. More than 6 pictures were taken per each emulsion and a representative one was presented.

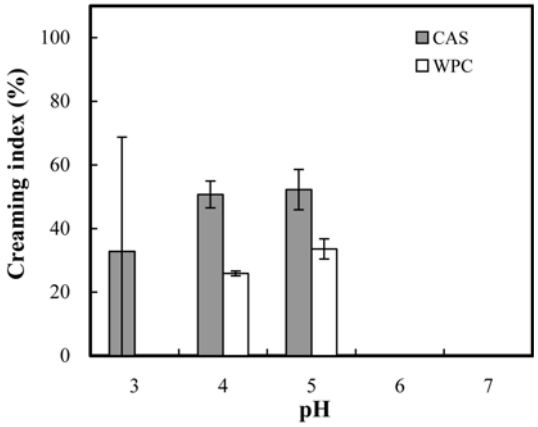

Fig. 6. Influence of pH drop on the emulsion creaming stability of corn oil-in-water emulsions stabilized with CAS or WPC (protein:oil=0.1). The values are creaming indexes determined at 1 day after emulsion preparation.

At pH 5, both emulsions were clearly separated into an opaque cream layer at the top and a transparent serum layer at the bottom (creaming index=52% for CAS- and 34% for WPC-emulsions) within 1 day. It suggested that all of the droplets were aggregated and rapidly moved upwards due to gravity. High creaming index (CI) was also obtained at pH 4 (51% for CAS- and 26% for WPC-emulsions), but the creaming rate of the emulsions was lower at the pH 4 than at pH 5. The emulsions at pH 4 were separated into a droplet-rich layer (presumably with large droplets) at the top and a turbid layer (with relatively small droplets that were evenly distributed) at the bottom at 1 day after the emulsion preparation. This suggested that there was a population of aggregated droplets that was unstable to creaming and a population of non-aggregated droplets that was relatively stable to creaming.

At pH 3, noticeable difference was observed in the stability of the emulsions depending on the milk protein types (Fig. 4-6). For WPC-emulsions at pH 3, the mean droplet diameters were relatively small (d_{32}=0.2 μm, d_{43}=2.6 μm) (Fig. 4A and 4B) and the droplets with diameters greater than 1 μm was less than 5% (Fig. 4C). There was no evidence of droplet aggregation, i.e., monomodal droplet size distribution with a peak consisting of submicron sizes of droplets (Fig. 4D), microscopically homogeneous morphology (Fig. 5), and no serum (Fig. 6). On the other hand, for CAS-emulsions at pH 3, light scattering measurements indicated that there was an appreciable droplet aggregation occurred (d>1 μm=88%) at the pH (Fig. 4C). The droplet size distribution was monomodal but very wide by ranging from 10 to 1,000 μm (Fig. 4D). Optical microscopy also indicated that there were some large individual droplets and a population of large flocs in the CAS-emulsions at pH 3 (Fig. 5). Presumably, the large degree of droplet aggregation led to the extensive creaming observed in the emulsions (CI=33%) (Fig. 6). The results were not expected because the ζ-potential measurements at pH 3 did not imply any significant difference in the emulsion stability between CAS- and WPC-emulsion, i.e., ζ-potentials were +26 and +32 mV for CAS- and WPC-emulsions, respectively (Fig. 3). It indicated again that the stability of the emulsions coated by these two milk proteins cannot be explained exclusively by electrostatic repulsion (see molecular weight effect and viscosity effect that were discussed earlier.). Alternatively, taking the more extensive aggregation of CAS- than WPC-emulsions at pH 4 and 5 into consideration, the flocculation of CAS-emulsions at pH 3 might have occurred at intermediate pH values while pH was dropped from 7 to 3. In order to reduce the emulsion pH from 7 to 3 it was necessary to pass through the pI of the adsorbed CAS.

In conclusion, the aim of this study was to compare the stability of emulsions coated by 2 milk proteins, CAS and WPC by investigating the effect of pH change. Laser diffraction measurements, microscopy measurements, and creaming stability test indicated that WPC-emulsions were more stable to droplet aggregation than CAS-emulsions under the same environmental stress of pH change. The

above results have valuable implications for the application of CAS or WPC as natural emulsifiers in the production of emulsion-based foods that inevitably go though pH changes during processing.

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