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Modulation and Stabilization of Silk Fibroin-Coated Oil-in-Water Emulsions

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Summary

The purpose of this study is to prepare and characterize stable oil-in-water emulsions containing droplets coated with silk fibroin. Silk fibroin, a native edible fibrous protein originating from silkworm cocoons, was used to prepare 10 % (by mass) corn oil-in-water emulsions at ambient temperature (pH=7.0, 10 mM phosphate buffer). Emulsions with relatively small mean particle diameter (d_{32}=0.47 μm) and extremely good creaming stability (>7 days) could be produced at silk fibroin concentration of 1 % (by mass). The influence of pH (2–8), thermal processing (60–90 °C, 20 min), and concentration of salt (c(NaCl)=0–250 mM) on the properties and stability of the emulsions was analyzed using ζ-potential, particle size, and creaming stability measurements. The isoelectric point of droplets stabilized with silk fibroin was pH~4. The emulsions were stable to droplet flocculation and creaming at any pH except intermediate value (pH=4.0) when stored at room temperature, which was attributed to their relatively low ζ-potential. Their ζ-potential went from around 25 to –35 mV as the pH was increased from 2 to 8. The emulsions were also stable to thermal treatment (60 and 90 °C for 20 min, pH=3 and 7), with a slight decrease in the magnitude of ζ-potential at temperatures exceeding 60 °C. The emulsions were unstable to aggregation and creaming even at relatively low salt concentrations (c(NaCl)=0–250 mM, pH=3 and 7) as a result of electrostatic screening effects. These results suggest that bulk oil stabilized with silk fibroin has improved physical stability and may provide a new way of creating functional oil products and delivery systems.

Key words: silk fibroin, corn oil, emulsion, stability, pH

Introduction

Emulsions are thermodynamically unstable systems and have a tendency to separate into two layers over time through a number of mechanisms. In order to make emulsions kinetically stable for a reasonable period of time, emulsifiers must be added. Among these emulsifiers, globular proteins play an important role in the formation and stabilization of oil-in-water (O/W) emulsions and thus have been extensively described in the literature (*1,2*). Globular protein emulsifiers can facilitate the production of small droplets to improve the long-term stability of emulsions against droplet aggregation by lowering the interfacial tension during homogenization. They can also generate repulsive forces (electrostatic) between droplets and/or form strong and cohesive interfacial membranes around the droplets that are resistant to rupture (*3*). Therefore, proteins extracted from a variety of natural sources (*e.g.* soy, whey, casein proteins) have been used as emulsifiers in food industry to confer desirable physicochemical properties to oil-in-water (O/W) emulsions.

One potential drawback of using globular proteins to stabilize O/W emulsions is their tendency to undergo conformational changes during storage after adsorption

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to the droplet surfaces because these changes can lead to emulsion instability (*4*). Thus, some researchers are now interested in finding some other types of protein emulsifiers that show the same or better emulsification ability as well as keep their conformation during storage.

Silk fibroin proteins, which are mainly from silkworm cocoons, have been widely recognized as potential sources of advanced materials for human beings and have been subject of numerous investigations. In the past decade, the use of silk fibroin as a biomaterial and carrier for controlled drug delivery expanded due to the unique combination of biodegradable, non-antigenic, relatively easy to prepare and biocompatible properties exhibited by this protein (*5–7*). Apart from these, silk fibroin proteins were also used as additive or absorbent in food science. It has been found that silk fibroin is a native edible fibrous protein and consists of one heavy chain (H) and one light chain (L) connected by a disulphide linkage. The primary structure of silk fibroin consists chiefly of the repeated polypeptide sequence of Gly-Ala-Gly-Ala-Gly-Ser linked together by intermolecular hydrogen bonds (*8*). The H chain of silk fibroin is composed of a considerable amount of hydrophobic amino acid residues; nevertheless, the presence of hydroxyl residues of serine and tyrosine along the chain gives affinity to water.

It is well known that silk fibroin can be processed into various forms, *e.g.* gel, powder, nanofibre, which gives silk fibroin a wide range of applications (*9*). However, there has not been any report on the utilization of silk fibroin as food emulsifier. One purpose of this study is to investigate whether physically stable O/W emulsions could be produced by using silk fibroin. The other purpose is to determine the influence of environmental stress (pH, salt and thermal processing) on their stability. Silk fibroin has been selected because it can be extracted from low-value by-products of cocoons that are currently discarded and may pollute the environment. Thus, the economic value of these by-products could be increased by finding new application and market for them.

Materials and Methods

Materials

Cocoons of *Bombyx mori* silkworm were kindly provided by College of Textiles and Garments, Southwest University, Chongqing, PR China. Corn oil was purchased from a local supermarket and used without further purification. Analytical-grade sodium chloride (NaCl), hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium azide (NaN_3), lithium bromide (LiBr), sodium carbonate (Na_2CO_3) and Oil Red O were purchased from Sigma Chemical Company (St. Louis, MO, USA). Distilled and deionized water was used for the preparation of all solutions.

Silk fibroin stock solution preparation

Silk fibroin aqueous stock solutions were prepared as described by Wang *et al*. (*10*). White raw *Bombyx mori* silkworm cocoons were boiled for 20 min in an aqueous solution of 0.02 M Na_2CO_3 and rinsed with cold deionized water to remove the glue-like sericin proteins. The silk fibroin fibres were then soaked in toluene/methanol=2:1 (by mass) solution to remove the wax from the fibres. The silk fibroin fibres were dissolved in 9 M LiBr solution at 60 °C for 4 h. After complete dissolution, the mass fraction of the silk solution was about 20 %. This solution was dialyzed against distilled water using Slide-a-Lyzer dialysis cassettes (MWCO 3500, Thermo Scientific, Pierce Protein Research Products, USA) for 3 days to remove the salt. The solution was optically clear after dialysis and was centrifuged to remove the small amount of silk aggregates that formed during the process, usually from environment contaminants that are present on the cocoons. The final mass fraction of silk fibroin stock solution was about 8 %. This concentration was determined by weighing the residual solid of a known volume of solution after drying. All solutions were stored at 7 °C to avoid premature gelation.

Emulsion preparation

A buffer solution was prepared by dispersing 10 mM sodium phosphate into water and then the pH was adjusted to 7.0 using 1 M HCl or 1 M NaOH. Silk fibroin solutions were prepared by dispersing the desired amount (0.2–3 %, by mass) of silk fibroin stock solution into the buffer solution and stirring overnight at room temperature to ensure complete dissolution. The pH of the silk fibroin solutions was adjusted to pH=7.0 using 1 M HCl or 1 M NaOH if required.

Oil-in-water emulsions were prepared by blending 10 % (by mass) of corn oil and 90 % (by mass) of silk fibroin together using a high-speed blender (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland) for 2 min. These coarse emulsions were then passed through a two-stage high-pressure valve homogenizer (LAB 1000, APV Gaulin, Wilmington, MA, USA) five times: 6500 psi in the first stage and 650 psi in the second stage. Sodium azide (0.02 %) was added to the emulsions as an antimicrobial agent.

Surface tension measurement

A digital tensiometer with a Wilhelmy plate (K10ST, Krüss, Charlotte, NC, USA) was used to measure the surface tensions of 10 mM phosphate buffer solutions and silk fibroin solutions at 30 °C. The protein solutions were prepared by dispersing different mass fraction (0.2–3 %) of silk fibroin into the buffer (50 mL, pH=7.0) and stirring overnight at room temperature to ensure that they were homogeneous. After that, these solutions were placed in the tensiometer measurement cell for 40 min to obtain the correct temperature before measurements were performed. Surface tension measurements were represented as the average of duplicate measurements made on two freshly prepared samples, with standard deviation.

Influence of silk fibroin concentration on emulsion properties

The influence of silk fibroin concentration on the properties of emulsion was examined. Corn oil was diluted with different ratios of buffer and silk fibroin solutions to make samples with the same corn oil mass

fraction (10 %) but different silk fibroin mass fraction (0.2–3 %). The corn oil emulsion was adjusted to pH=7.0 and then stored at room temperature for 24 h prior to ζ-potential and light scattering analyses, and for 7 days prior to creaming stability analysis.

Influence of environmental stress on emulsion properties

The influence of pH, ionic strength, and thermal processing variations on the properties of silk fibroin-coated corn oil emulsion was examined. Final emulsions were prepared by diluting corn oil with different ratios of silk fibroin solution and salt solution at pH=7.0. The pH was then adjusted by the addition of HCl to give a series of samples with similar composition: 10 % (by mass) of corn oil, 10 mM sodium phosphate, pH=2–8, 0–500 mM NaCl, incubated in a water bath for 20 min at either 60 or 90 °C. The emulsions were then stored at room temperature for 24 h prior to ζ-potential and light scattering analysis and for 7 days prior to creaming stability analysis.

Destabilized oil measurements

The amounts of destabilized oil present in the emulsions were determined using the dye dilution technique described in detail by Palanuwech *et al*. (*11*). Before analysis, emulsions were agitated in a glass test tube to ensure that they were homogenous. A mass of 3 g of corn oil containing a known amount of oil-soluble red dye (0.001 % Oil Red O, by mass) was added to the top of 5 g of the emulsion to be tested. Samples were mixed by vortexing for 30 s and then centrifuged at 20 000 rpm for 20 min at 25 °C (Sorvall Centrifuges T-1270, Du Pont Company, Wilmington, DE, USA). The dyed free oil on top of the emulsion was removed with a pipette and placed into a 1.5-mL cuvette where its absorbance was measured at 517 nm using UV–VIS spectrophotometer (UV-2101 PC, Shimadzu Corporation, Japan). The percentage of destabilized oil (w_d) was calculated using the following equation:

$$w_d=(m_o(a-1)/m_e w)\cdot 100 \qquad /1/$$

where m_o is the mass of added dye solution, m_e is the mass of emulsion, w is the mass fraction of oil in the emulsion, and a is the ratio of the absorbance of the standard dye solution to that of the dye solution extracted from the top of the emulsion sample.

Foam capacity measurement

The method of Coffman and Garcia (*12*) was employed for foam capacity studies. Silk fibroin solutions containing different mass of silk fibroin dispersed in 10 mM buffer solution (100 mL, pH=7.0) were prepared. The solution was blended vigorously for 2 min using a high-speed blender (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland), then transferred to a measuring cylinder. Volumes were recorded before and after blending. The volume of foam formed in 30 s was recorded. The percentage of volume increase, which serves as the index of % volume change, is expressed as follows:

$$\Delta V=((v_2-v_1)/v_1)\cdot 100 \qquad /2/$$

where v_2 is the volume of silk fibroin solution after blending, and v_1 is the volume of silk fibroin solution before blending.

ζ-potential measurement

The emulsions were diluted to a mass fraction of approx. 0.005 % of oil using buffer solution to avoid multiple scattering effects. Diluted emulsions were injected directly into the measurement chamber of a particle electrophoresis instrument (ZEM 5003, Zetamaster, Malvern Instrument, Worcester, UK). The ζ-potential was then determined by measuring the direction and velocity of the droplet movement in an applied electrical field. The ζ-potential measurement was reported as the average and standard deviation calculated from the measurement of two freshly prepared samples, with five readings taken per sample. ζ-potential measurements were made after the emulsions were stored for 24 h at room temperature.

Particle size analysis

The emulsions were diluted to an oil mass fraction of approx. 0.006 % using buffer solution to avoid multiple scattering effects. Particle size distribution of the emulsions was measured using a laser light scattering instrument (Mastersizer X, Malvern Instruments Ltd., Malvern, UK). This instrument measures the angular dependence of the intensity of laser light scattered by dilute emulsions and then finds the particle size distribution that gives the best agreement between theoretical predictions and experimental measurements. A refractive index ratio of 1.08 was used by the instrument to calculate the particle size distributions. Measurements are reported as the surface weighed mean particle diameter:

$$d_{32}=\Sigma N_i d_i^3/\Sigma N_i d_i^2 \qquad /3/$$

where N_i is the number of droplets of diameter d_i. The particle size measurements are reported as the average and standard deviation of measurements calculated from a minimum of two freshly prepared samples, with two readings taken per sample. Particle size measurements were made after the emulsions were stored for 24 h at room temperature.

Optical microscopy

The microstructure of the selected emulsions was determined using optical microscopy (Nikon microscope Eclipse E400, Nikon Corporation, Japan). Emulsions were gently agitated in a glass test tube before measurement to make sure that they were homogeneous. A drop of the emulsions was then placed on a glass slide and observed under the microscope at a magnification of 400×. Images of emulsions were acquired using digital image processing software (Micro Video Instruments Inc., Avon, MA, USA). Optical microscopy measurements were made after the emulsions were stored for 24 h at room temperature.

Creaming stability measurement

A mass of 10 g of emulsions was transferred into a glass test tube (internal diameter 15 mm, height 125 mm), tightly sealed with a plastic cap, and then stored at room temperature for 7 days. After storage, some of the emulsions were separated into a number of layers: a 'cream' layer at the top, a 'suspension' layer in the middle, and a 'serum' layer at the bottom. These layers could be distinguished by their visual appearance: the cream layer was optically opaque and whiter than the original emulsion, the suspension layer had the same appearance as the original emulsion, and the serum layer was either slightly turbid or transparent. The total height of the emulsion (H_T), the height of the serum layer (H_{SL}), and the height of the cream layer (H_{CL}) were measured. The extent of creaming was characterized by creaming indices (CI), which were defined for the serum and cream layers respectively, as follows:

$$CI_{SL}=100\cdot(H_{SL}/H_T) \qquad /4/$$

$$CI_{CL}=100\cdot(H_{CL}/H_T) \qquad /5/$$

The creaming indices provided indirect information about the extent of droplet aggregation in an emulsion, for example, the higher the creaming indices, the greater the particle aggregation.

Statistical analysis

Experiments were performed at least twice using freshly prepared samples. Average and standard deviations were calculated from these duplicate measurements.

Results and Discussion

Optimum conditions to form silk fibroin-coated droplets

The purpose of these experiments was to establish the optimum silk fibroin concentration required to form stable emulsions. The electrical charge (ζ-potential), particle size distribution, creaming stability, microstructure, amount of destabilized oil and mean particle diameter (d_{32}) of emulsions (10 % corn oil, by mass; 10 mM phosphate buffer, pH=7.0) containing different silk fibroin mass fractions (0.2–3 %) were measured 24 h after preparation.

The electrical charge of the emulsion droplets was negative at pH=7.0 because this pH was above the pI of the absorbed silk fibroins. There was no significant change in ζ-potential within the range of silk fibroin mass fraction (0.2–3.0 %), with the average being (–30.7±1.4) mV. Since the emulsion droplets are coated with a charged biopolymer, electrostatic repulsion is expected to play an important role to prevent aggregation (*13,14*).

The mean particle diameter (d_{32}) of emulsion decreased as the mass fraction of silk fibroin increased from 0.2 to 1 %, its minimum being (0.47±0.05) μm containing 1 % (by mass) of silk fibroin (Fig. 1). The large aggregates were observed under the microscope (d_{32}>10 μm), and rapid creaming (CI_{CL}>40 %) was observed in

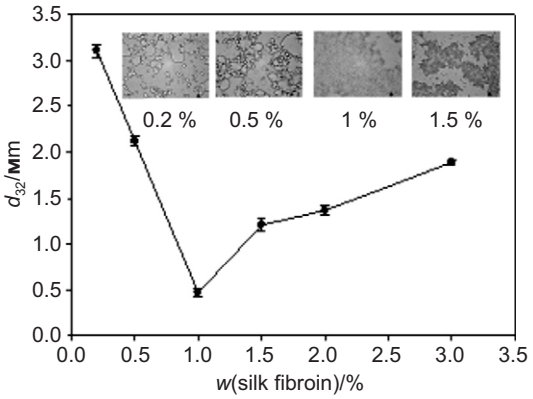

Fig. 1. Influence of silk fibroin mass fraction on the mean particle diameter (d_{32}) of corn oil-in-water emulsions. Inserted is the morphology of relevant emulsions under microscope 400×; more staining means worse dispersion

the emulsions containing 0.2 and 0.5 % (by mass) of silk fibroin, whereas no aggregation or creaming were observed at 1 % (by mass) silk fibroin. These results can be attributed to the following reasons: (*i*) the total droplet surface area that can be stabilized by the increased mass fraction of silk fibroin; (*ii*) the rate at which the droplet surfaces were covered with increased mass fraction of silk fibroin; (*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, therefore leading to the formation of smaller droplet sizes.

However, the mean particle diameter (d_{32}) of the emulsion increased again when the mass fraction of silk fibroin was over 1.5 %. Apart from this, the foam was observed after homogenizing. Therefore, the foam capacity of the silk fibroin was measured (Fig. 2). There was no foam capacity when the silk fibroin mass fraction was very low (*i.e.* 0.2 or 0.5 %), after which it increased with the increase of silk fibroin mass fraction. The highest foam capacity was 136 % at the silk fibroin mass fraction of 3 %. This observation suggests that higher silk fibroin mass fraction enhances foaming capacity, which can be attributed to the above conclusion that the average particle diameter increased with the higher silk fibroin mass fraction. In addition, oil destabilization mea-

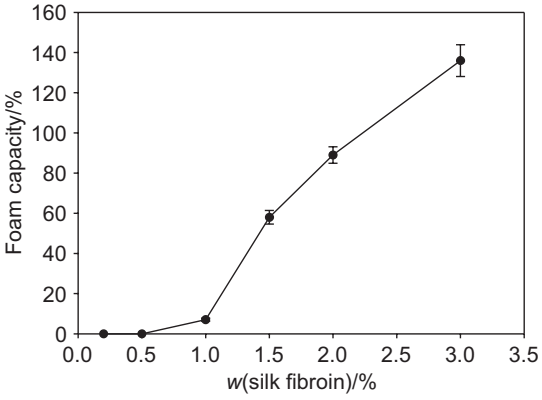

Fig. 2. Influence of silk fibroin mass fraction on the foam capacity of corn oil-in-water emulsions

surements (Fig. 3) confirmed that there was appreciable 'oiling-off' in the emulsion with higher silk fibroin mass fraction. At high silk fibroin mass fraction (≥1.5 %), there was more than 15 % of destabilized oil in the emulsions. All these results indicate that O/W emulsions can be prepared using silk fibroin concentration. However, in order to avoid the formation of foam during homogenizing, 1 % (by mass) of silk fibroin was used to prepare the emulsion (*i.e.* silk fibroin-to-oil mass ratio of 1:10) in all subsequent experiments, because this mass fraction enabled the production of emulsions containing relatively small droplets that were stable to creaming.

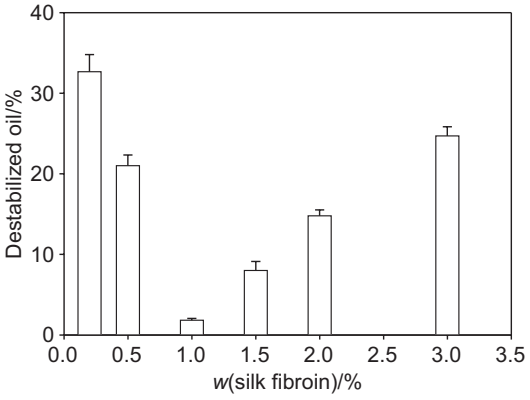

Fig. 3. Influence of silk fibroin mass fraction on the amount of destabilized oil in 10 % (by mass) corn oil-in-water emulsions

Surface activity of silk fibroin

The ability of protein to form and stabilize emulsions is dependent on their ability to adsorb to interfaces and on the amount of protein required to saturate the interface (*15*). The foam capacity is also dependent on it. In an attempt to explain the emulsifying ability and foam capacity of silk fibroin, the surface activity of silk fibroin was measured.

The dependence of surface tension (γ) of silk fibroin mass fraction in 10 mM phosphate buffer solution was tested (Fig. 4). As the silk fibroin mass fraction increased, the surface tension decreased from 71.5 mN/m in the absence of silk fibroin to a constant value of 58.8 mN/m when the interface became saturated with silk fibroin of 1.5 %, then sharply dropped to 54.9 mN/m with the continued increase in silk fibroin mass fraction to 3 %. The surface activity of silk fibroin was relatively low when concentrations were lower. This can be attributed to its relatively high hydrophobic properties.

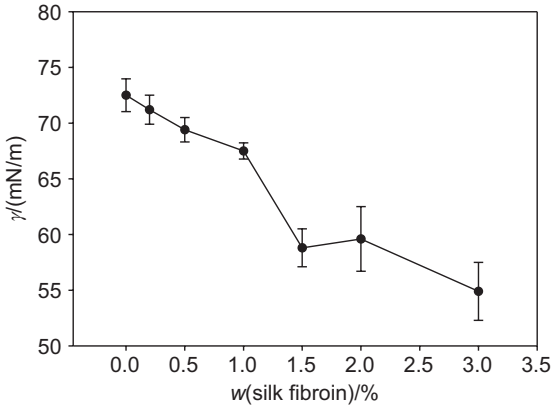

Fig. 4. Influence of silk fibroin mass fraction on the surface tension of silk fibroin solutions at 30 °C

Generation of foam involves the development of a protein film surrounding a gas bubble and the packing of gas bubbles into an overall structure. The spontaneous adsorption of proteins from the solution to the air/aqueous interface is of central importance for their foaming performance. This phenomenon is thermodynamically favourable due to the simultaneous dehydration of the hydrophobic interface and hydrophobic portions of the protein. Hydrophobic patches on the surface of a protein initially drive this process, and surface hydrophobicity has been correlated with improved foaming properties (*16*). Once contacts are made with the interface, flexibility within the molecules can expose previously buried hydrophobic portions into the interface, potentially leading to interfacial denaturation of the molecules and subsequent reduction in surface tension (*17*). This has been correlated with improved foamability. In order to avoid its foamability during emulsion processing, silk fibroin mass fraction of 1 % in the emulsion system was selected.

Influence of pH on the emulsion stability

The influence of storage pH (2–8) on the droplet charge (ζ-potential), mean particle diameter (d_{32}), and creaming stability of 10 % (by mass) of corn oil-in-water emulsions stabilized with silk fibroin was measured (Figs. 5–7).

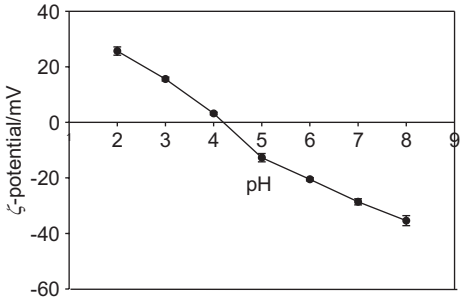

Fig. 5. Influence of pH on ζ-potential of corn oil-in-water emulsions stabilized with silk fibroin (1 %, by mass)

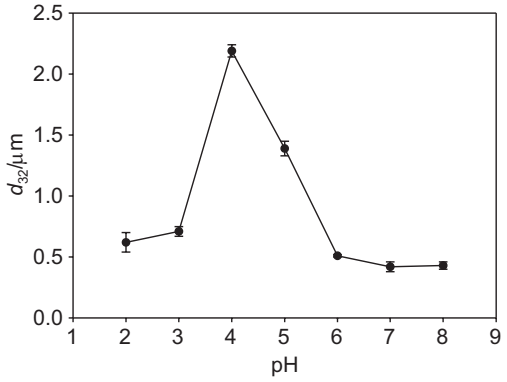

Fig. 6. Influence of pH on the mean particle size of corn oil-in-water emulsions stabilized with silk fibroin (1 %, by mass)

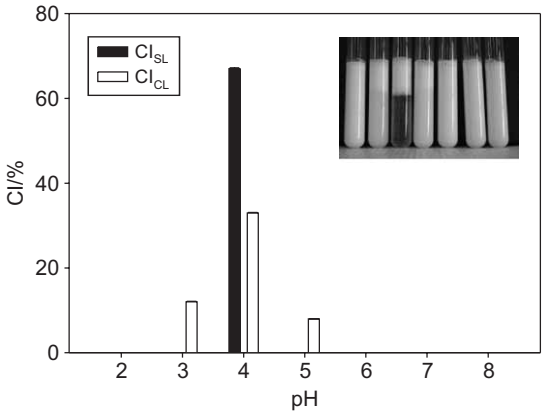

Fig. 7. Influence of pH on the cream stability of corn oil-in--water emulsions stabilized with silk fibroin (1 %, by mass). Inserted is the cream stability of emulsions at various pH, separation signifies unstability

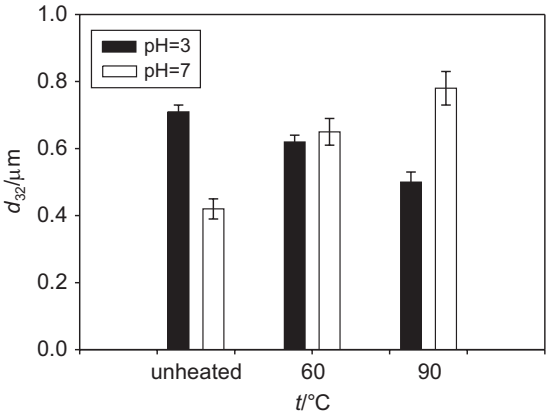

Fig. 8. Influence of thermal processing on the mean particle size of corn oil-in-water emulsions stabilized with silk fibroin (1 %, by mass)

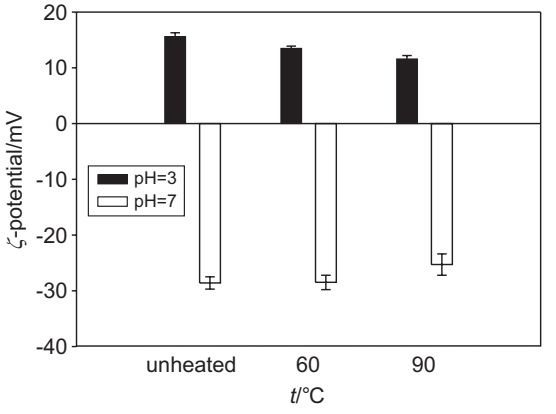

Fig. 9. Influence of thermal processing on ζ-potential of corn oil-in-water emulsions stabilized with silk fibroin (1 %, by mass)

The ζ-potential was relatively high (25.7 mV) at pH=2.0, but it became less positive with increasing pH until it reached a value of zero at around pH=4.0, and then became increasingly negative as the pH increased further, until it reached a value of –35.4 mV at pH=8.0. The droplets in protein-stabilized emulsions have zero net charge around the isoelectric point (IEP) of the adsorbed proteins. Our ζ-potential *vs*. pH measurements suggested that the pI values of the silk fibroin were around pH=4.0. This value is quite similar to the pI value reported by the other researchers (*18*).

The mean particle diameter (d_{32}) remained relatively small (<1 μm) from pH=2.0 to 8.0 except at pH=4.0, indicating that little droplet aggregation occurred during storage. The creaming stability of the emulsions was relatively good at all pH except at intermediate pH=4.0. These results suggest that silk fibroin-stabilized emulsions remain relatively stable to droplet aggregation.

Influence of thermal processing on emulsion stability

The purpose of these experiments was to examine the influence of thermal processing on the stability of emulsion droplets coated with silk fibroin. The influence of holding temperature on the particle size and droplet charge of 10 % (by mass) of corn oil-in-water emulsions stabilized by 1 % (by mass) of silk fibroin at pH=3.0 and 7.0 was measured. Emulsions were held at 60 and 90 °C for 20 min, then cooled down to room temperature, and stored for 1 day prior to analysis (Figs. 8 and 9).

Results showed that the changes of ζ-potential and mean particle diameter (d_{32}) were relatively small even in high temperature treatment, which implied that the emulsion coated with silk fibroin was quite stable during thermal processing. At pH=7.0, the ζ-potential of emulsions decreased compared to unheated emulsion: –28.5 mV at 60 °C and –25.3 mV at 90 °C. The d_{32} of the droplets at this pH increased from 0.65 μm at 60 °C to 0.78 μm at 90 °C ($p<0.05$). The increase of particle size can be attributed to the decrease of ζ-potential, which reduces the electrostatic repulsion between the droplets. The ζ-potential of droplets at pH=3.0 decreased (13.5 and 11.6 mV at 60 and 90 °C, respectively). However, the d_{32} also decreased from 0.62 μm at 60 °C to 0.50 μm at 90 °C. The reduction of ζ-potential should increase the particle size if the electrostatic repulsion is the main force in the system. In principle, silk fibroin is a protein which has two chains. It is possible that the absorbed silk fibroin undergoes some form of conformational change upon heating above a critical temperature that decreases the attraction between the droplets, for example, due to increased exposure of hydrophobic groups at pH=3.0, which decreases droplet flocculation.

Influence of salt on emulsion stability

The purpose of this experiment was to examine the influence of NaCl on the stability of silk fibroin emulsions. Emulsions were prepared containing different NaCl concentrations (0–250 mM), and their pH was adjusted to 7.0 and 3.0, respectively (Figs. 10 and 11).

Studies have shown that the ζ-potential of silk fibroin-stabilized droplets in the emulsions remained negative and positive at pH=7.0 and 3.0, respectively, at all NaCl concentrations. Nevertheless, the magnitude of ζ-potential decreased as the concentration of NaCl increased, which can be attributed to the electrostatic screening effects and ion binding effects. There was also a much smaller reduction in the magnitude of the ζ-potential

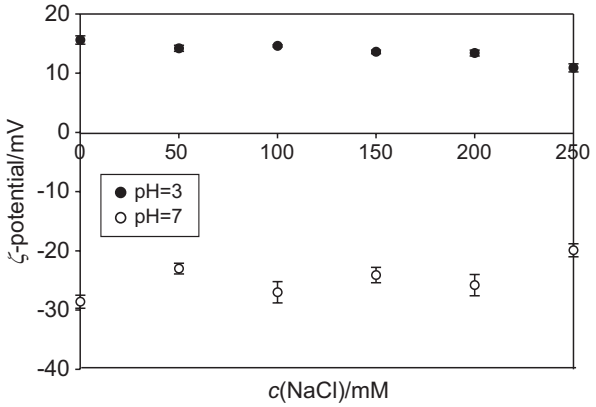

Fig. 10. Influence of NaCl on ζ-potential of corn oil-in-water emulsions stabilized with silk fibroin (1 %, by mass)

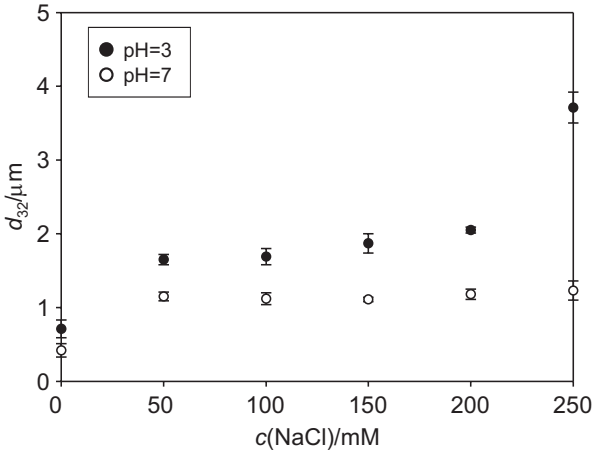

Fig. 11. Influence of NaCl on the mean particle size of corn oil--in-water emulsions stabilized with silk fibroin (1 %, by mass)

with increasing NaCl concentration at pH=7.0 than at pH=3.0. The most likely reason for this difference is that the monovalent Na^+ ions are counterions for the anionic droplets in the emulsion, whereas the monovalent Cl^- ions are counterions for the cationic droplets in the emulsion. Also, the Na^+ ions are more sensitive to binding to the droplets coated with silk fibroin at pH=3.0. This was also confirmed by the mean droplet diameter (d_{32}) measurements, which showed that the magnitude of d_{32} increase at pH=7.0 was smaller than that at pH=3.0 with increasing NaCl concentration, presumably because electrostatic screening and ion binding effects reduced more electrostatic repulsion at pH=3.0 than at pH=7.0 between the oil droplets. It was also found that the mean particle diameters (d_{32}) at pH=3.0 and 7.0 sharply increased even when 50 mM of sodium chloride were added. This would account for the fact that lower amount of NaCl was needed to promote droplet aggregation in the emulsion. The creaming stability (data not shown) of emulsion was relatively high (CI_{SL}>50 %) even at lower concentration of salt. All these results suggest that silk fibroin-stabilized emulsions were unstable against droplet aggregation even at lower ionic strength, when electrostatic interactions would be screened. Hence, it can be concluded that polymeric steric repulsion plays a less important role than electrostatic repulsion in preventing the droplets from aggregating.

Conclusions

This study has shown that silk fibroin, which can be isolated from silkworm cocoon, is very effective in stabilizing oil-in-water emulsion by absorbing to the surface of lipid droplets. It has also helped to identify optimum mass ratio of corn oil and silk fibroin (corn oil/silk fibroin=10:1) for the preparation of O/W emulsions. The electrical properties and aggregation stability of the silk fibroin-coated O/W emulsions were determined as a function of pH, ionic strength, and thermal processing because these environmental stresses are commonly encountered in the food industry. The silk fibroin-coated O/W emulsions proved to be stable to aggregation at pH values sufficiently far from their isoelectric point (pH=4.0) at relatively low salt concentrations (<50 mM), and they were stable to thermal processing (<90 °C). Silk fibroin isolated from the cocoon may prove to be a useful new source for homogenizing bulk lipids to form emulsions. In further study, we intend to improve the stability of droplets coated with silk fibroin under high ionic conditions. We also intend to examine the interfacial properties between oil and silk fibroin, which may contribute to better understanding of the emulsification capability of silk fibroin.

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