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Soybean protein isolate gel particles as foaming and emulsifying agents

Kentaro Matsumiya[1,2,*] and Brent S. Murray[1]

[1] Food Colloids Group, School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK

[2] Laboratory of Quality Analysis and Assessment, Division of Agronomy and Horticultural Science, Graduate School of Agriculture, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan

*To whom correspondence should be addressed. Tel: +81-774-38-3748. Fax: +81-774- 38-3746. Email: matumiya@kais.kyoto-u.ac.jp

Mailing address: Laboratory of Quality Analysis and Assessment, Division of Agronomy and Horticultural Science, Graduate School of Agriculture, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan

Abstract

In order to enhance functional properties of commercial soybean protein isolate (SPI), SPI microgel particles as foaming and emulsifying agents were studied. Microparticulation of heat-set SPI macrogels containing no added and various added salts was systematically carried out using a high-speed blender, an ultrasonicator and a high-pressure jet homogenizer. Among the tested conditions, the smallest gel particles were achieved via the high-pressure jet homogenization process under conditions of no added salts. Conversion of ordinary high molecular weight commercial SPI proteins into the counterpart gel particles enhanced foam stabilizing properties of the suspensions and stability against creaming and freeze-thaw triggered instability of the emulsions, while the enhancement was not necessarily achieved for low-molecular-weight partially hydrolysed SPI. This can be attributed to the different steric repulsive effects of the gel particles.

Key words

Soybean protein isolate; gel particle; Pickering stabilization; emulsion;~~ suspension; ~~foam; stability

1. Introduction

Food products including oils exist as o/w or w/o emulsions where one phase is dispersed as small droplets in the other immiscible phase; for example, fruit cordials, milk beverages and salad dressings (Dickinson, 1992). Even though emulsions differ in their appearance, texture and so on, they are all thermodynamically unstable in principle, and therefore usually subjected to various kinds of periodical destabilization such as creaming, aggregation and coalescence of oil droplets in their shelf life before consumption (McClements, 2004a). In order to slow down the destabilization processes, surface-active food macromolecules like proteins and polysaccharides have been widely and extensively used for emulsion production in the food industry.

Surface-active macromolecules adsorbed at the oil-water interface during emulsification not only lower the interfacial tension and improve emulsification efficiency but also form relatively thick layers around the emulsion oil droplets (McClements, 2004b). These protective layers generate repulsive interactions, *i.e.*, steric and electrostatic forces between oil droplets that lead to improved stability against aggregation and coalescence on the shelf (Dalgleish, 2006). On the other hand, in the last decade, colloidal particle-stabilization known as Pickering stabilization has received increasing attention in the food science field because the even thicker particle layers at the oil-water interface produce even more effective barriers to droplet aggregation and coalescence (Dickinson, 2010).

Common mature examples of Pickering stabilization in foods include protein granules from egg yolk, casein micelles particularly in homogenized milk and ice creams (Dalgleish, 2003) and triglycerides crystals in margarines/spreads (Dickinson, 2010; Rousseau, 2013). Recently, growing numbers of colloidal particles suitable for making Pickering emulsions have been reported; *e.g.*, glyceryl stearyl citrate solid particles (Gupta & Rousseau, 2012), cellulose micro crystals (Wege, Kim, Paunov, Zhong & Velev, 2008), chitin nanoparticles (Tzoumaki, Moschakis, Kiosseoglou & Biliaderis, 2011), soy protein nanoparticles (Liu & Tang, 2013), zein protein particles (de Folter, van Ruijven & Velikov, 2012) and flavonoid particles (Yusoff & Murray, 2011). In addition to these reports regarding Pickering stabilization, insoluble egg or milk protein aggregate preparations are well-known to have positive influences on foam stability (Dickinson, 2010).

In this context, Destribats, Rouvet, Gehin-Delval, Schmitt & Binks (2014), in their latest work, have reported the utility of another novel class of protein-based particles, whey protein microgel particles. They employed a bottom-up approach in their research to produce the microgel particles by heating whey protein solutions sufficiently diluted to prevent the formation of brittle macrogels, within combination of microfiltration and spray-drying. They demonstrated that this new class of food-grade particles can successfully stabilize a food-grade oil-in-water emulsion for a long time with exceptional resistance to droplet coalescence that shows good promise for application in the food industry.

Soybean proteins, as well as whey proteins, are commercially and extensively used in food products due to their high nutritional value (Friedman, 1996). Since they are originally from plant resources, they need much less energy input for their production than those from animal resources, including milk and egg proteins, which is helpful for energy savings in overall food production (Pimentel & Pimentel, 2003). However, commercially available soybean proteins, denatured due to high-temperature pasteurization or drying processes, although practically easy to manufacture, do not necessarily have enough ability to stabilize oil-in-water emulsions compared to their native counterparts (McSweeney, 2008).

In the current study, in order to enhance the functional properties of soybean proteins, we describe the production of soybean protein gel particles under varied conditions to examine effects of the gel particles on the stability of foams and oil-in-water emulsions prepared with food-grade oils. Two kinds of soybean protein, ordinary commercial soybean protein isolate (SPI) and low-molecular-weight partially hydrolysed SPI (LMW-SPI) were employed to estimate the impact of steric repulsive effects of the gel particles. Because the commercial SPIs are not so soluble and disperse into water as suspensions, we employed a top-down approach, different from the latest work on whey proteins by Destribats *et al* .(2014), to produce the gel particles, whereby a SPI macrogel was first formed and then efficiently broken into microgel particles.

2. Materials and Methods

SPI and LMW-SPI were kindly donated by Fuji Oil Co. (Osaka, Japan). The protein content of the SPI and LMW-SPI was both > 90 wt%. LMW-SPI is partially hydrolysed by proteases with an average molecular weight of approximately 60,000 Daltons. Corn oil (Mazola, ACH Food Companies, Inc., UK) was purchased from a local supermarket in the UK. All other chemicals used were of Analytical grade. Ambient temperature was approximately 25 °C throughout all the experiments.

2.2. Sample preparation

2.2.1. Macrogel

SPI and LMW-SPI were mixed with deionized water and then the appropriate amount of salt solution was added to adjust the concentration of salts. The final concentration of SPI and LMW-SPI in the macrogels was 15 wt% and 20 wt%, respectively. The salt concentration in the final macrogels was set at 0 mM (no added salts), 60 mM for NaCl and 30 mM for $CaCl_2$ or $MgCl_2$. The concentrations of the salts were determined according to the gelation method reported by Kohyama, Sano & Doi (1995). The cationic salts were chosen based upon their use in the creation of *tofu* gels, which are proposed as comparable to the microparticulated SPI gels presented.

The SPI and LMW-SPI mixtures were stored in a water bath at 40°C for 30 min to allow enough hydration of the protein powders. The mixtures were respectively in paste and liquid forms and thereby required two kinds of equipment with different shearing speeds to ensure complete dispersion of the proteins. The mixtures were then dispersed by a hand blender with a puree masher attachment (HB711M, Kenwood) at Speed 1 (350 rpm) and a hand blender with one of twin steel beaters (HM320, Kenwood) at Speed 2 (1050 rpm) at ambient temperature to prepare SPI and

LMW-SPI suspensions, respectively. They were heated in a glass jar at 90 °C for 30 min in a water bath and then cooled down to room temperature in another water bath at ambient temperature. The suspensions were kept in a cold room at 4 °C overnight to fully set the macrogels.

2.2.2. Gel-particle suspensions

The heat-set macrogels were placed in a water bath at 25 °C for 30 min before the following steps and texture measurements described in section 2.3.1. The macrogels were coarsely mixed via a spatula with deionized water and 1 wt% salts solution to adjust the concentration of salts. The final concentration of SPI and LMW-SPI in gel-particle suspensions was 6.25 wt% and the final concentration of salts was 0 mM (no added salts), 30 mM for NaCl and 15 mM for $CaCl_2$ and $MgCl_2$. It should be noted that final SPI concentration status is based on the SPI weight, not based on the SPI gel weight including water.

The above coarse mixtures were homogenized in a bottle-type juice blender (Blend Active VBL 096, Breville, UK), in order to limit bubble generation from the mixtures, for 2 min at ambient temperature to obtain coarse gel-particle suspensions. This referred to later as the high-speed blending stage and the resulting dispersions were subjected to short-term storage tests, ζ-potential measurements and microscopic observations. SPI suspensions not subjected to the gelation process were also studied as control samples.

The coarse gel-particle suspensions were prepared for final homogenization using a high-speed blender (Ultraturrax T25, IKA, Germany) at ambient temperature at 24,000 rpm for 5 min, an ultrasonic homogenizer (VC 130, Sonics & Materials Inc., USA) at an amplitude of 100 for 5 min within a water bath at ambient temperature for prevention of overheating or alternatively by passing the coarse gel particle suspension through a high-pressure homogenizer (Jet homogenizer with a 60:40 chamber, University of Leeds, UK) at 240 bar to make fine gel-particle suspensions (Burgaud, Dickinson & Nelson, 2007). These fine suspensions were subjected to particle size analysis and foaming tests. The suspensions after the high-pressure homogenization were diluted with deionized water to make suspensions containing 1-5 wt% SPI for the foam stability tests.

2.2.3. Emulsions stabilized by gel particles

The fine gel particle suspensions produced via the jet homogenizer containing 6.25 wt% SPI or LMW-SPI were diluted with appropriate amount of deionized water and then mixed with corn oil at a weight ratio of 8:2 aqueous: oil. The mixtures were preliminarily homogenized by a hand blender (Rosso hand Blender, Russell Hobbs, UK) for 2 min at ambient temperature to make coarse emulsions. These emulsions were then subjected to jet homogenization under the same conditions as described above.

2.3. Sample characterization

2.3.1. Texture measurements

Texture analysis was conducted on SPI and LMW-SPI macrogels at ambient temperature using a TA.XT2 texture analyser (Stable Micro Systems, UK) equipped with a 1/4 inch spherical stainless steel probe. The analysis conditions were set as follows: Test mode = compression (penetration), Pre-test speed = 5.00 mm/sec, Test speed = 1.00 mm/sec, Post-test speed = 5.00 mm/sec, Penetration distance = 20.00 mm, Trigger force = 5.0 g. The fracture force (N) and the proportionality constant of initial linear parts of force (N) vs distance (mm) curves were reported to characterise the hardness and elasticity of the macrogels.

2.3.2. Storage stability tests

Non-gelled SPI and LMW-SPI suspensions and gelled particle suspensions were stored at 25 °C for 24 h and visually observed to examine for creaming or sedimentation.

2.3.3. ζ-potential measurement

Non-gelled SPI and LMW-SPI suspensions and gel particle suspensions were 1250x diluted with aqueous phase containing the same salt concentration. ζ-potential of the gel particles was measured by a laser-Doppler ζ-potential analyser (Zetasizer, Malvern Instruments, UK) using a refractive index of 1.450-0.001i.

2.3.4. Light microscopy

The microstructures of SPI and LMW-SPI suspensions and gel-particle suspensions were observed with a conventional light microscope (Optishot, Nikon, Japan) and recorded by a digital camera linked to imaging software (Leica Microsystems, USA).

2.3.5. Particle size analysis

The size distribution of gel particles and emulsion oil droplets was measured by a laser-diffraction particle size analyser (Mastersizer 3000, Malvern Instruments, UK). Sample emulsions were appropriately diluted with deionized water to avoid multiple scattering. A refractive index of 1.45 was used to calculate the particle size distribution based on the Mie theory. The particle size of the emulsions was reported as the surface-weighted mean diameter, $d_{3,2}$

2.3.6. Foam stability tests

The fine gel-particle suspensions were poured into a whip cream dispenser (ICO Brand 0.5L stainless steel whip cream dispenser, ICO Trading UK Ltd., UK) and then foamed with N_2O gas that can be directly charged by a dedicated charger. The foam was poured into a measuring cylinder and the time-dependent behaviour was recorded by a video camera at ambient temperature. The foam half-life, *i.e.*, the time when half volume of the liquid had drained into the bottom of measuring cylinder was taken as a measure of foam stability.

2.3.7. Emulsion stability tests

The SPI and LMW-SPI gel-particle emulsions were stored in an incubator at 25°C for 4 weeks. Creaming stability was evaluated by measuring the creaming index described before: Creaming index = (Height of serum layer/ Height of total emulsion) (Demetriades, Coupland & McClements, 1997). Evidence for aggregation and coalescence of emulsion oil droplets was obtained via the Mastersizer with and without prior ultrasonic treatment for 30 sec. Samples for the long-term stability tests had 0.05% (w/v) sodium azide added as an antimicrobial agent. Freeze-thaw stability of the emulsions was examined after 1 week storage in a freezer at -18°C. The frozen emulsions were thawed in a water bath at 40°C for 30 min and then visually observed. Destabilized free oil was determined as described earlier (Palanuwech, J., Potineni, R., Roberts, R. F., & Coupland, 2003).

2.4. Statistics

All experiments were conducted in triplicate with freshly prepared samples of suspensions or emulsions. Statistical analyses were performed using Microsoft Excel ver. 2010 for Windows.

3. Results and Discussion

3.1. Characterisation of SPI macrogels and gel particles

It is well-known that the rheological properties of SPI gels depend on pH and salt type and concentration (Bryant & Julian McClements,1998). Fig. 1a indicates the surface hardness of various heat-set SPI macrogels, taken as the peak of the force vs distance curves. Clear breakage was not clearly observed for almost all the samples except for the SPI macrogels with no added salts. Fig. 1b shows elasticity of the SPI and LMW-SPI macrogels as measured by the slope of initial linear part of the force versus distance curves. Even though a higher concentration of LMW-SPI (20 wt%) was used compared to SPI (15 wt%), the SPI macrogel had a higher elasticity than the LMW-SPI macrogel. This is probably because the LMW-SPI consisted of partially hydrolysed proteins that were less able to form well-organized molecules networks, with lower water holding capacity.

Both the macrogels were significantly strengthened by addition of the salts in a similar way. The strengthening effects of the divalent cations, Ca^{2+} and Mg^{2+} were much higher than those of the monovalent cation, Na^{+} due to the higher ability of divalent ions to act as bridging agents between neighbouring molecules (Mine, Murakami, Azuma, Yoshihara, Fukunaga, Saeki & Sawano, 2005). On the other hand, for both the SPIs, there were no noteworthy differences between macrogels made with Ca^{2+} and Mg^{2+}, while Mine *et al*. reported that the breaking stress required for tofu varied with the types of the divalent cations (Desfougeres, Lechevalier, Pezennec, Artzner & Nau, 2008).

Fig. 2 describes the gravitational behaviour of the gel particle suspensions made with Ca^{2+} and

Mg^{2+} under static conditions stored at 25 °C for 24 h. For both the SPI and LMW-SPI, control suspensions without gelation and gel particle suspensions including no added salts, no gravitational phase separation was observed, suggesting that the gelation processes did not significantly change the dispersion state or structural features of the original SPI particles. However, the divalent cations induced precipitation of the SPI gel particles and creaming of the LMW-SPI gel particles. Both the SPI macrogels tended to be more elastic when the divalent cations were added (Fig. 1b) but, once they were broken into microgel particles, they behaved in opposite directions against gravity, i.e., downward and upward (Fig. 2). These phenomena can be attributed to different structural features of the suspended particles or varied apparent specific gravity, presumably related to different degrees of air incorporation during creation of the macrogels based on the separate preparation methods and/or disparate affinity of the SPI particles to air. One possibility is that the precipitating SPI gel particles prepared with the divalent cations could be utilized as a density adjuster of emulsion oil droplets if they adsorb at the oil-water interface.

Fig. 3 shows microstructural images of SPI gel particles obtained by light microscopy. The SPI gel particles with no added salts seemed to be dispersed in the aqueous phase as well as the original SPI particles without the gelation, whereas the SPI gel particles with Ca^{2+} and Mg^{2+} formed relatively large aggregates after homogenization. These results correspond to those obtained by the short-term storage test described above, which suggests that the different gravitational behaviour depending on the type of added salt can be explained by the different structural features of the aggregates, such as different degrees of compactness or fractal geometry, probably caused by the different screening effects. Similar results were obtained for the LMW-SPI gel particles.

To confirm the screening effects of the added salts, the ζ-potential of the SPI gel particles was measured (Fig. 4). The ζ-potential of the SPI and LMW-SPI microgel particles were both significantly decreased by the presence of added salts, particularly for the divalent ions, in almost the same way, in line with greater electrostatic screening effects of Ca^{2+} and Mg^{2+}. However, neither the microscopic observations nor the ζ-potential measurements can fully explain how the different salts affected the internal microstructure, density and water holding capacity of the individual microgel particles.

For the practical use of the gel particles as surface-active agents, the adsorption efficiency at the oil-water or air-water interfaces should be considered. In particular, emulsion formation efficiency via particles predominantly depends on the size of dispersed surface-active particles due to their relatively slow adsorption kinetics compared to the rate of interface formation during homogenization (McClements, 2004a). This was why the coarse gel particles were further homogenized to reduce their particle size. Fig. 5 shows the particle size distribution of the fine gel particle suspensions prepared by the various homogenization conditions and various salt concentrations. The size distribution of the SPI and LMW-SPI coarse gel particles homogenized by the blender expressed as bold lines reasonably corresponds to the microscopic observations under all added salt conditions (Fig. 3). The results indicate that the large particles observed via the microscope in the SPI suspensions were strongly coagulated aggregates that were not be easily dissociated even by the high dilution used for particle size analysis. On the other hand, according to statistical correlation analysis performed, representative values from the distributions of SPI and LMW-SPI coarse gel particles, that is $d_{3,2}$, $d_{4,3}$ and d_{50} (Table 1) did not clearly correlate with either surface hardness or elasticity of the SPI and LMW-SPI macrogels obtained from texture tests (Fig. 1), suggesting that the size of SPI gel particles does not necessarily depend on the texture properties of the macrogels but does relate to the original structure of macrogel networks, etc.

The particle size of SPI coarse and fine gel particle suspensions was larger than that of LMW-SPI gel particle suspensions under all salt conditions, particularly larger under conditions of no added salt or NaCl added conditions (Fig. 5 and Table 1). The particle size of LMW-SPI gel particle suspensions containing added $CaCl_2$ and $MgCl_2$ was larger than that of the suspensions containing no added salts and added NaCl, while it was rather smaller than that of all SPI gel particle suspensions. For all suspensions, the size of fine gel particles generally decreased in the order Blender < Blender + High speed blender < Blender + Ultrasonicator < Blender + Jet homogenizer. Thus, the smallest gel particles were obtained via a combination of homogenization processes: the juicer blender followed by the jet homogenizer under conditions of no added salts and added NaCl.

3.2. Foam and emulsion-stabilizing properties of gel particles

3.2.1. Foam systems

Foams were prepared via the whip cream dispenser using various concentrations of the non-gelled SPI and fine gel particle suspensions without any added salts. Fig.6 shows the stability of foams foamed with N_2O gas expressed as the half-life. The stability with the SPI non-gelled control suspensions and with the SPI gel particle suspensions both increased with increasing protein concentration, while foam stability with the LMW-SPI suspensions and gel particles did not increase so significantly with protein concentration. The stability of foams with the SPI gel particles was significantly higher than that with the counterpart SPI suspensions, whereas the stability with the LMW-SPI gel particles and suspensions were similar to each other. These results indicate that for ordinary high molecular weight commercial SPI, conversion for gel particles could be a useful tool for improving their foam stabilizing properties. This improvement is presumably based on stronger Pickering stabilization effects produced by the particles. Desfougeres, Lechevalier, Pezennec, Artzner & Nau (2008) argued that foam stability depends on conditions that favour protein aggregation rather than the presence of aggregates themselves. In our case, there is a possibility that the SPI gel particles tended to aggregate via hydrophobic attractive interactions or they enhanced the viscoelastic properties of the air-water interface by their increased water holding capacity.

3.2.2. Emulsion systems

Fig. 7 describes the creaming index of emulsions containing 20 wt% of oil stabilized by SPI and LMW-SPI non-gelled proteins, i.e., suspensions (SPI Sus and LMW-SPI Sus) and fine gel particles (SPI Gel and LMW-SPI Gel) without any added salts. For SPI Sus and Gel, the emulsions containing

3 wt% protein or more were almost stable against creaming for 4 weeks, while those containing 1 or 2 wt% were not, indicating that 3 wt% of proteins is at least required to prepare stable SPI emulsions. Creaming stability of the SPI Gel-based emulsions was higher than that of the SPI Sus-based ones, albeit the difference was not so significant. Creaming stability of LMW-SPI Sus and Gel-based emulsions were much lower than that of SPI ones. For LMW-SPI Sus and Gel, creaming was only prevented completely in the emulsion containing 5 wt% of LMW-SPI non-gelled proteins. Apart from the emulsions containing 5 wt% of LMW-SPI, the stability of LMW-SPI Gel emulsions was generally higher than that of the Sus emulsions. These results show that conversion to gel particles may enhance the creaming stability of the emulsions, probably due to enhanced steric repulsion between gel particles at the oil droplet surfaces and/or due to a possible increased viscoelasticity of the emulsions in the presence of interactive microgel particles (Dickinson, 2015).

To estimate aggregation and coalescence of emulsion oil droplets, the particle size distributions of the emulsions were monitored for 4 weeks. Similar results were obtained for SPI Sus and SPI Gel-based emulsions (Figs. 8a and b). The initial mean particle diameter of the SPI Sus and Gel emulsions decreased with an increase of protein content up to 3 wt% but plateaued out above it. Significant particle size changes were not clearly observed for both the SPI emulsions with and without prior ultrasonic treatment, demonstrating that aggregation and coalescence of the SPI Sus and Gel stabilized oil droplets did not readily occur under the test conditions.

The initial mean particle diameter of the LMW-SPI Sus and Gel-based emulsions changed depending on the protein concentration and it was slightly different for each set of emulsions (Figs. 8c and d), whereas the non-gelled proteins and gel particle sizes of LMW-SPI were almost the same (Table 1). Although the detailed mechanism is unclear, we can point out a possibility that the LMW-SPI Gel particles were more difficult to adsorb at the newly created oil-water interface during emulsification than non-gelled proteins due to the structural changes induced by the gel conversion. Whilst aggregation and coalescence of oil droplets were not observed for SPI Sus and Gel emulsions, slight aggregation and coalescence were observed for LMW-SPI Sus and Gel ones particularly containing 3-5 wt% proteins according to the particle size changes after the ultrasonic treatment and the 4-week storage (Figs. 8c and d). This is maybe because the thickness of interfacial layer consisting of LMW-SPI Sus and Gel was insufficient to prevent oil droplets from coalescing. The particle size of LMW-SPI Sus and Gel emulsions was reduced with more amount of proteins in all cases. The reason why the higher the protein content apparently caused more extensive aggregation and coalescence is difficult to explain but possibly that relatively smaller free LMW-SPI Sus and Gel promoted depletion flocculation of the oil droplets via the attractive forces.

Fig. 9 shows the freeze-thaw treated SPI and LMW-SPI stabilized emulsions. For SPI Sus, LMW-SPI Sus and LMW-SPI Gel, the emulsions clearly separated into a cream layer at the top and serum layer including solid-like precipitates at the bottom, whereas the SPI Gel based emulsions containing 3 wt% proteins or more did not separate. It is well-known that freeze-thaw treatment produces a much stronger and more cohesive soybean curd (*tofu*) gel (Kalichevsky-Dong, Ablett, Lillford & Knorr, 2000). The same phenomena might have occurred in the SPI gel particles at the oil droplet surfaces, leading to more resistance of the SPI stabilized emulsions to phase separation. In the previous study by Palazolo, G. G., Sobral, P. A., & Wagner, J. R. (2011), thermally-denatured soybean isolates-based emulsions were shown to be more resistant against freeze-thaw treatments than native ones. In our case, emulsions stabilized by the thermally-denatured commercial-grade SPI became much more resistant via the heat-set gelation step that can introduce hydrated layers enhancing repulsive forces to the protein molecules on the oil droplet surfaces.

The destabilized free oil from the emulsions containing 1 wt% of SPI-Sus and Gel and LMW-SPI Sus and Gel was 1.2 ± 0.9, 2.5 ± 1.2, 1.6 ± 1.3, 7.5 ± 2.6 (wt%), respectively, whilst for all emulsions containing 2-5 wt% protein zero free oil was detected, within experimental error. One-cycle freeze-thaw treatment might not be enough for elucidating clearer differences in oil phase separation amongst the samples.

4. Conclusions

We have demonstrated that SPI microgel particles imparted increased stability to foams and emulsion oil droplets, probably via their enhanced steric repulsive forces in such colloidal systems. Formation of microgel particles could be useful tool for enhancing functional properties of other polysaccharides or proteins that exhibit difficulties in their normal modes of solubilisation.

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(Note)

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Fig. 1

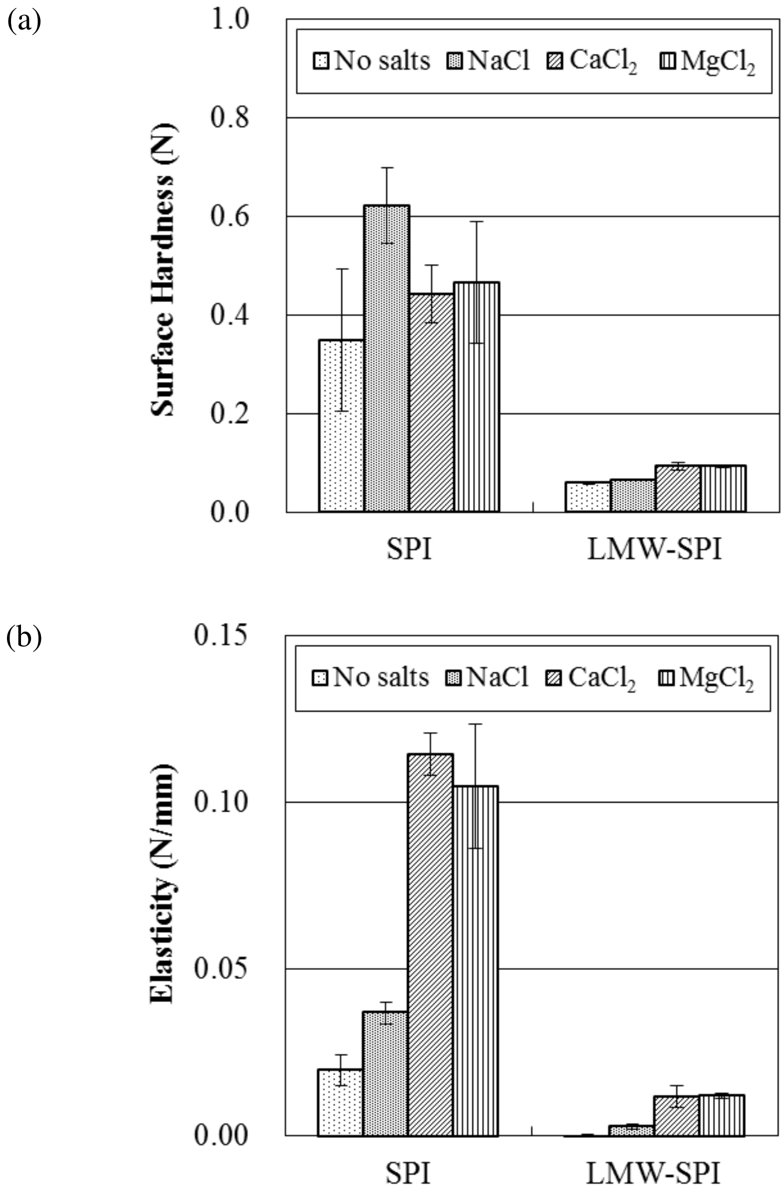


Fig. 2

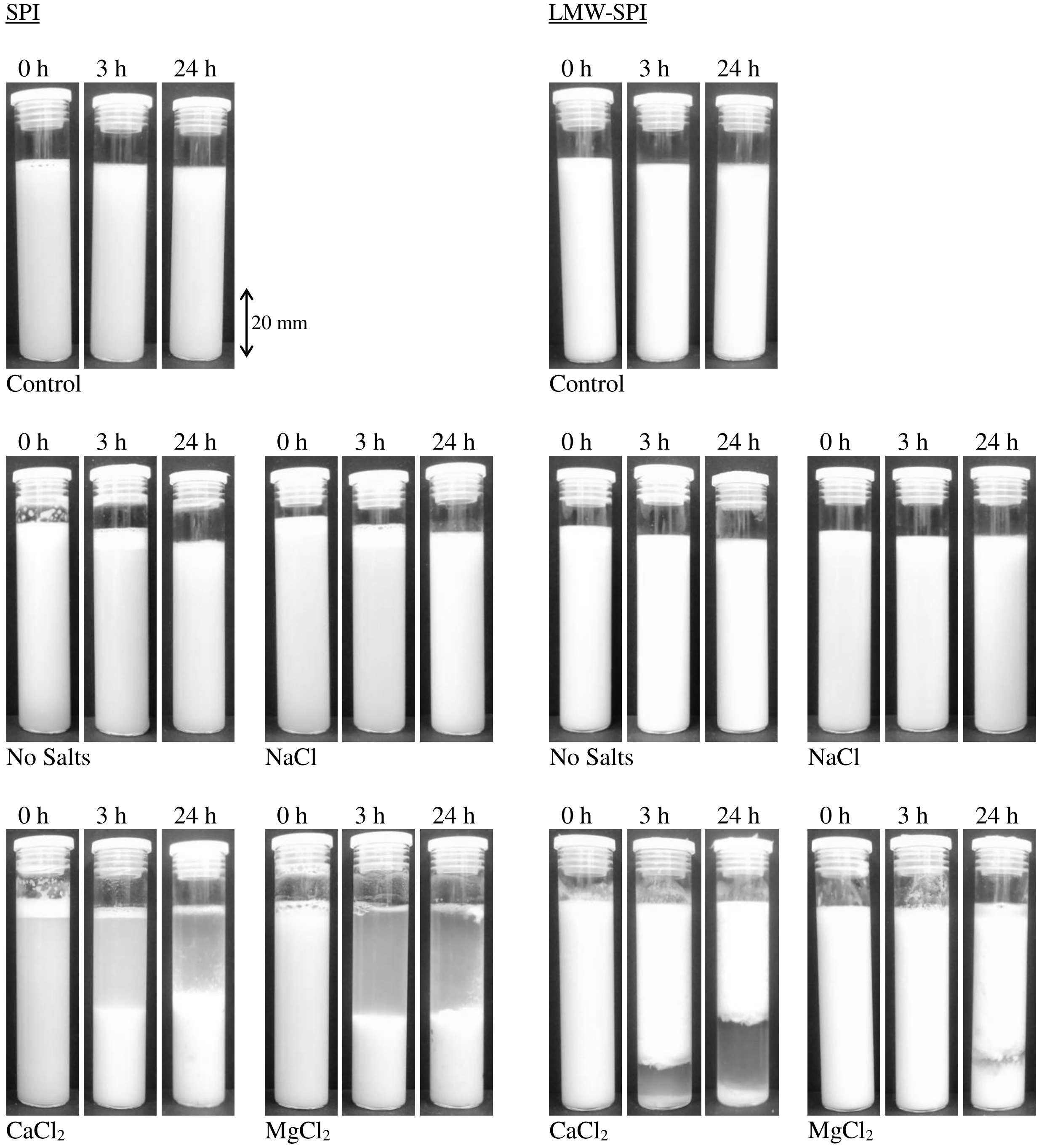
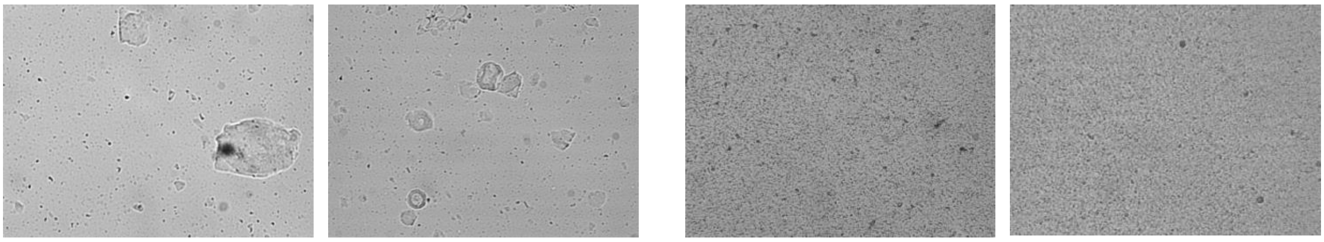


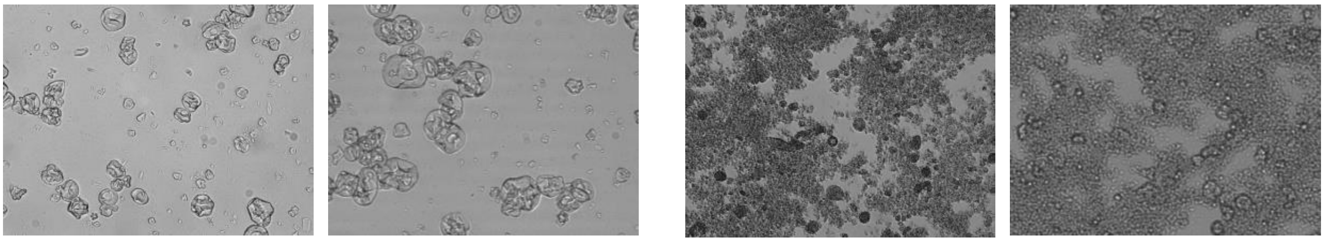
Fig. 3

SPI LMW-SPI

(Non-gelled) Control (Non-gelled) Control



No added salts NaCl No added salts NaCl



$CaCl_2$ $MgCl_2$ $CaCl_2$ $MgCl_2$

Fig. 4

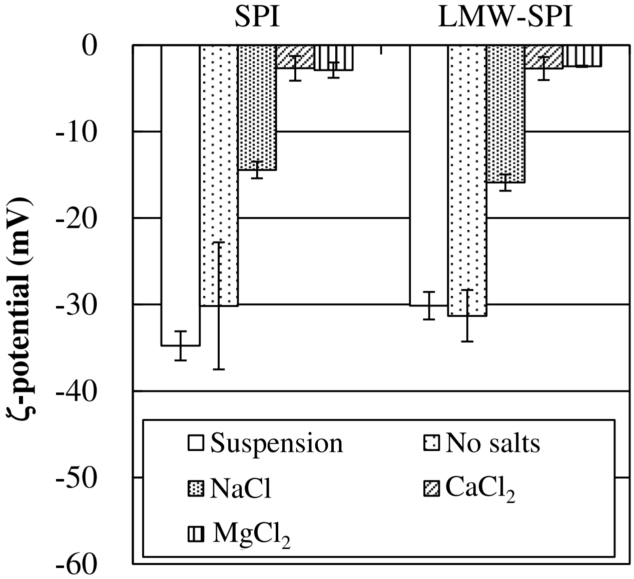

SPI
LMW-SPI
0
-10
-20
-30
-40
-50
-60
ζ-potential (mV)
Suspension
No salts
NaCl
CaCl2
MgCl2

Fig. 5

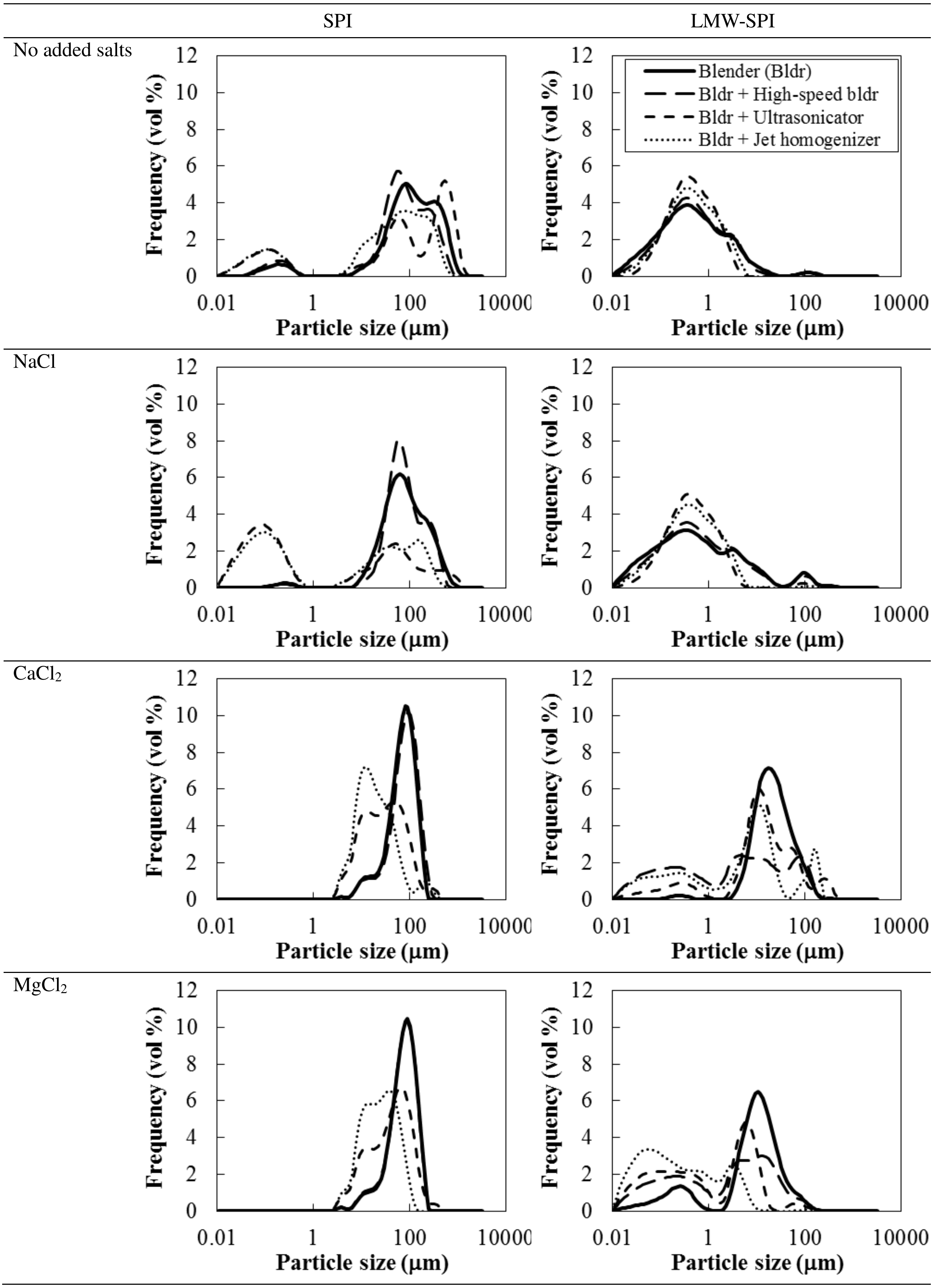

SPI
LMW-SPI
No added salts
NaCl
$CaCl_2$
$MgCl_2$
Blender (Bldr)
Bldr + High-speed bldr
Bldr + Ultrasonicator
Bldr + Jet homogenizer
Frequency (vol %)
Particle size (μm)
0
2
4
6
8
10
12
0.01
1
100
10000

Fig. 6

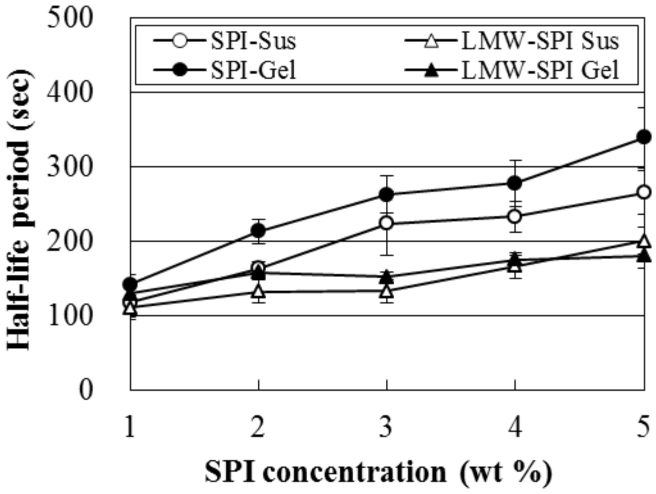
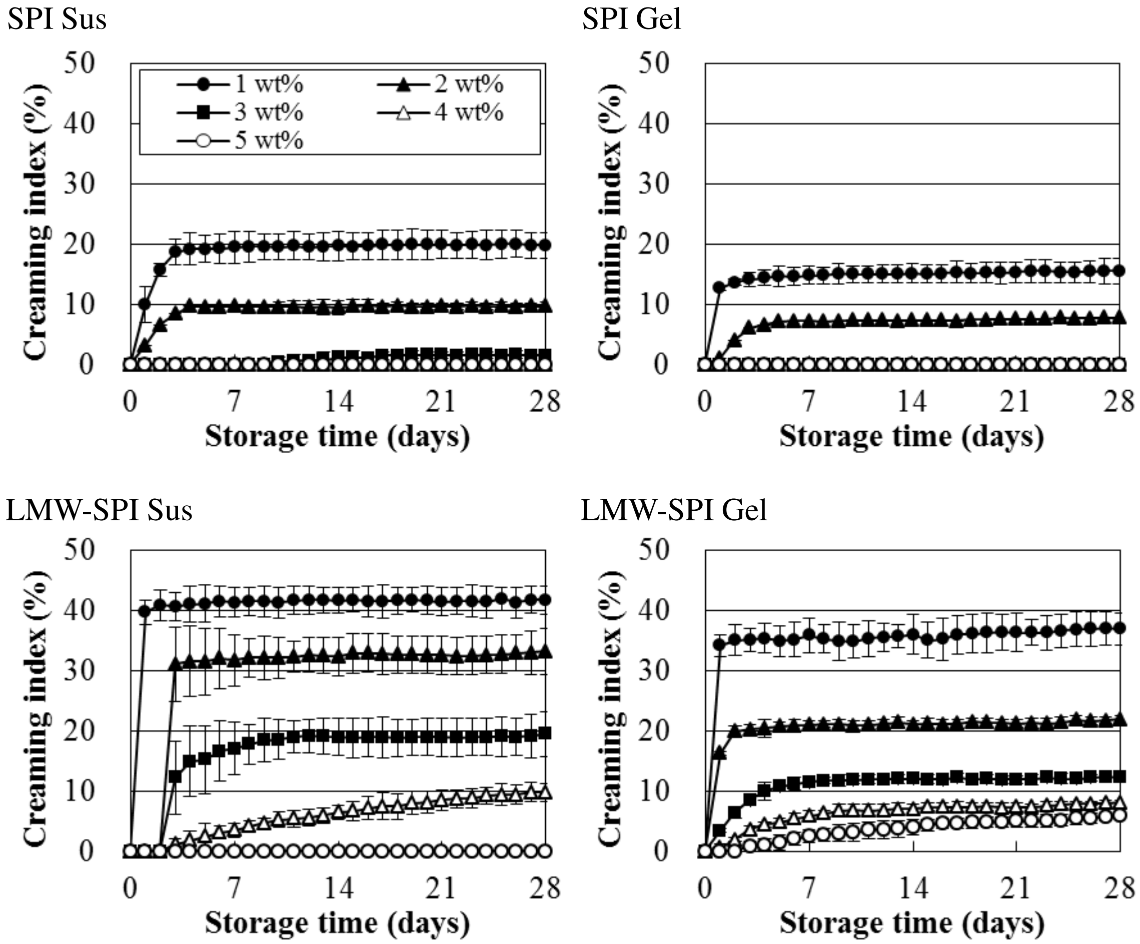

SPI-Sus
LMW-SPI Sus
SPI-Gel
LMW-SPI Gel
Half-life period (sec)
500
400
300
200
100
0
1
2
3
4
5
SPI concentration (wt %)

Fig. 7



SPI Sus

SPI Gel

LMW-SPI Sus

LMW-SPI Gel

Fig. 8

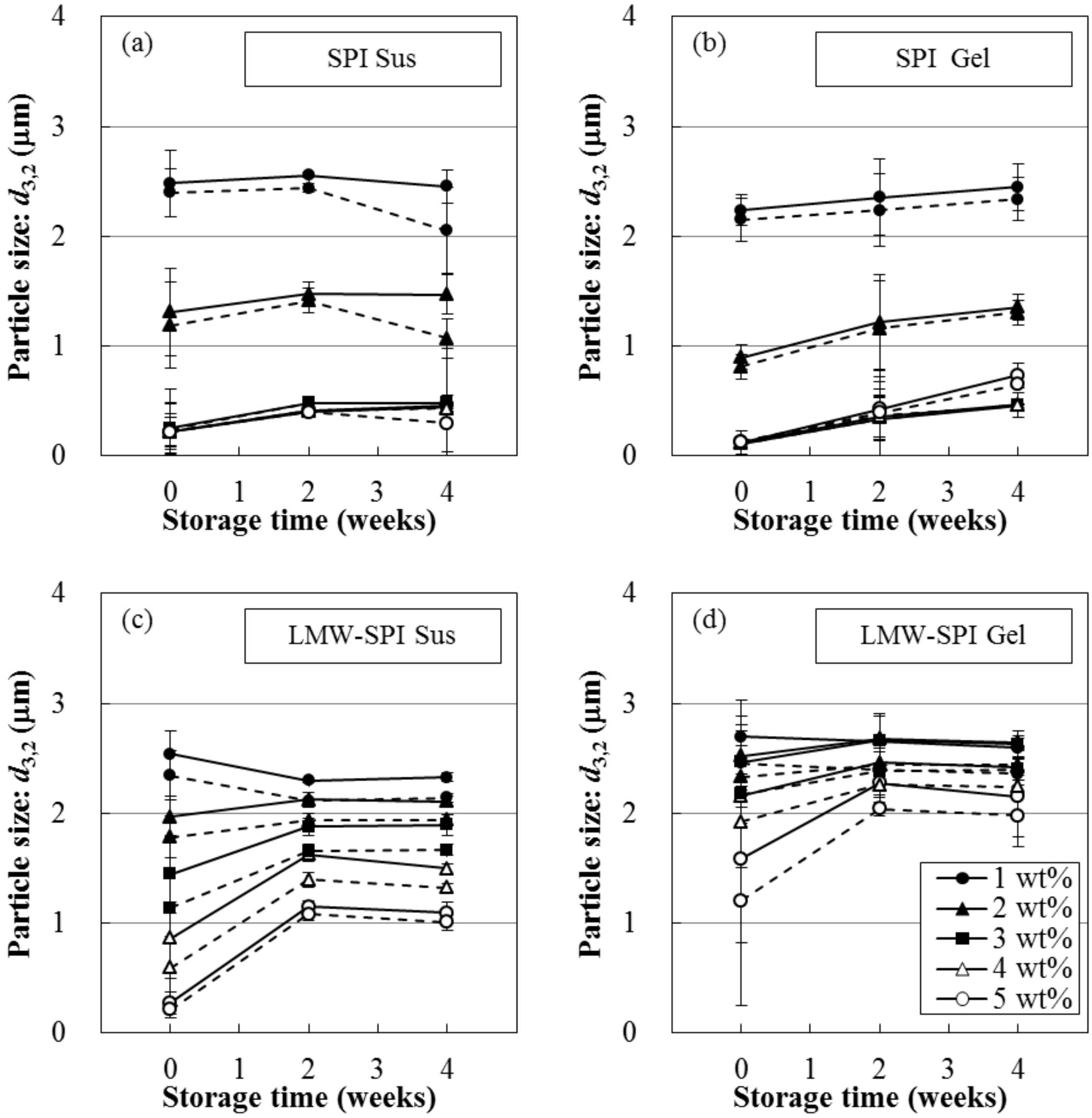
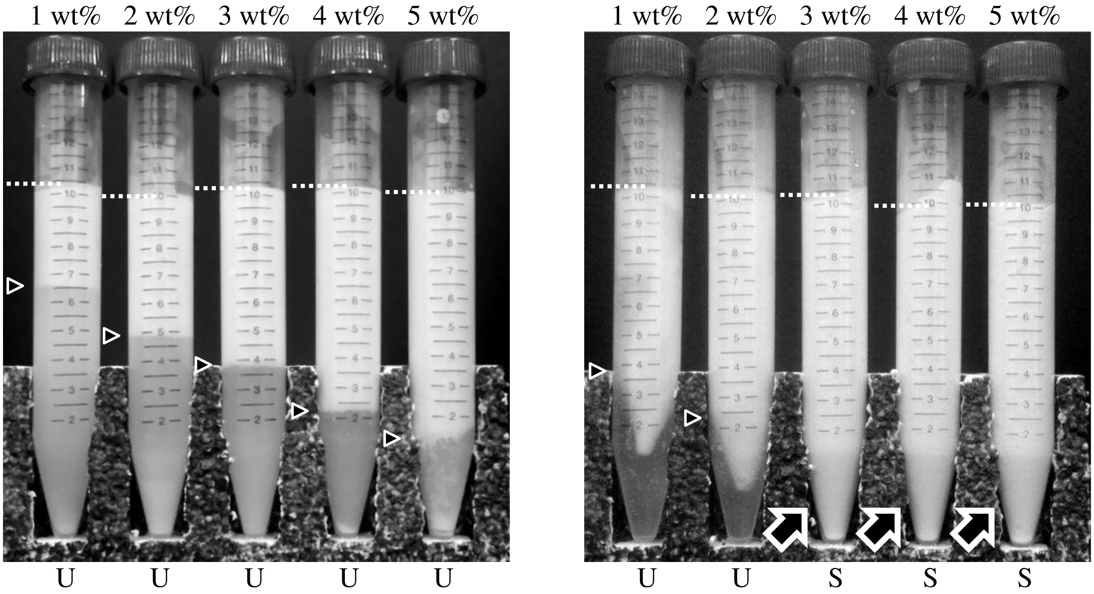
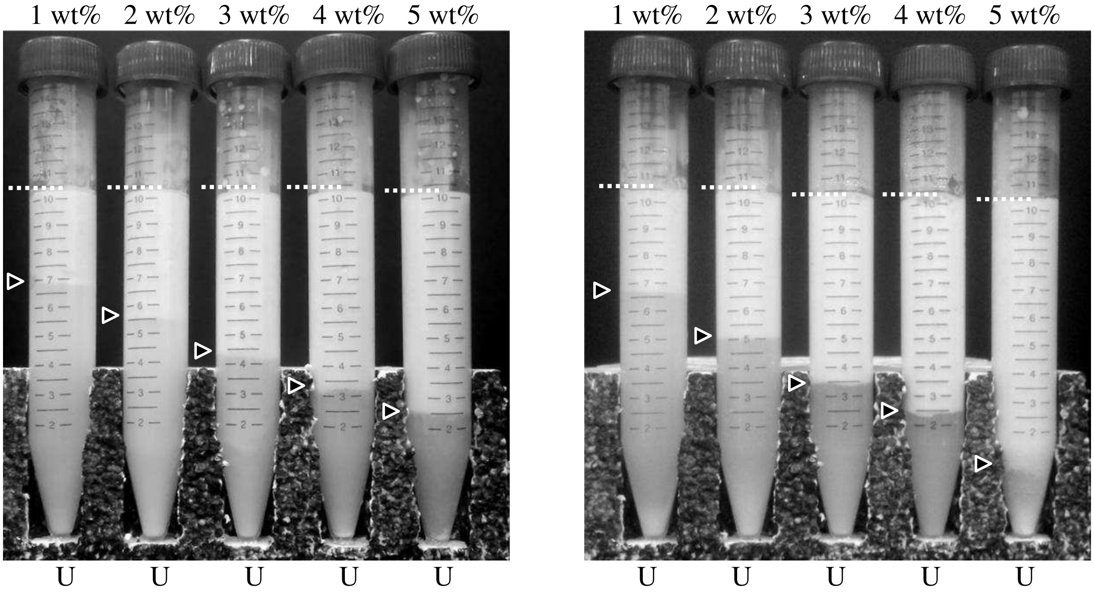

(a)
SPI Sus
(b)
SPI Gel
(c)
LMW-SPI Sus
(d)
LMW-SPI Gel
Particle size: $d_{3,2}$ (µm)
Storage time (weeks)
0
1
2
3
4
1 wt%
2 wt%
3 wt%
4 wt%
5 wt%

Fig. 9


SPI-Sus

SPI-Gel


LMW-SPI Sus

LMW-SPI Gel

Figure captions

Table 1. Representative values of particle size distribution of SPI and LMW-SPI gel particle suspensions.

Fig. 1. Texture properties of SPI and LMW-SPI macrogels.

Texture analysis was conducted at 25 °C using a 1/4 inch spherical stainless steel probe under penetration mode. The fracture force (N) and the proportionality constant of initial linear parts of force (N) vs distance (mm) curves were reported as the surface hardness and elasticity of the macrogels.

Fig. 2. Gravitational behaviour of SPI and LMW-SPI gel particle suspensions.

SPI and LMW-SPI coarse gel particle suspensions were stored at 25 °C for 24 h.

Fig. 3. Micrograph of SPI and LMW-SPI gel particle suspensions.

The microstructures of SPI and LMW-SPI coarse gel particle suspensions were observed with a conventional light microscope at 25 °C.

Fig. 4. ζ-potential of SPI and LMW-SPI gel particles.

SPI and LMW-SPI coarse gel particle suspensions were 1250x diluted with aqueous phase containing the same salt concentration. ζ-potential was measured at 25 °C.

Fig. 5. Particle size distribution of SPI and LMW-SPI gel particle suspensions.

A refractive index of 1.45 was used to calculate the particle size distribution.

Fig. 6. Stability of foams made with SPI and LMW-SPI gel particle suspensions

The gel particle suspensions were foamed with N_2O gas using a whip cream dispenser at 25 °C.

Fig. 7. Creaming stability of SPI and LMW-SPI gel particle-stabilized emulsions.

SPI and LMW-SPI gel particle emulsions were stored at 25°C for 4 weeks. Creaming stability was expressed as creaming index (Demetriades *et al.*, 1997).

Fig. 8. Aggregation and coalescence stability of SPI and LMW-SPI gel particle-stabilized emulsions.

Surface area-weighted mean particle size was obtained with (solid lines) and without (dotted lines) prior ultrasonic treatment for 30 sec.

Fig. 9. Freeze-thaw stability of SPI and LMW-SPI gel particle-stabilized emulsions.

Freeze-thaw stability of the emulsions was examined after 1 week storage in a freezer at -18°C followed by thawing in a water bath at 40°C for 30 min. Appearance of the thawed emulsions were recorded with a digital camera. White dotted line and triangle show the top of emulsions and the interface of cream and serum layers, respectively.

U: Unstable against serum separation, S: Stable against serum separation