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502   Soybean protein isolate gel particles as foaming and emulsifying agents

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517 Abstract

518 In order to enhance functional properties of commercial soybean protein isolate (SPI), SPI microgel

519 particles as foaming and emulsifying agents were studied. Microparticulation of heat-set SPI

520 macrogels containing no added and various added salts was systematically carried out using a

521 high-speed blender, an ultrasonicator and a high-pressure jet homogenizer. Among the tested

522 conditions, the smallest gel particles were achieved via the high-pressure jet homogenization process

523 under conditions of no added salts. Conversion of ordinary high molecular weight commercial SPI

524 proteins into the counterpart gel particles enhanced foam stabilizing properties of the suspensions

525 and stability against creaming and freeze-thaw triggered instability of the emulsions, while the

526 enhancement was not necessarily achieved for low-molecular-weight partially hydrolysed SPI. This

527 can be attributed to the different steric repulsive effects of the gel particles.

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529 Key words

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

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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

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558 reports regarding Pickering stabilization, insoluble egg or milk protein aggregate preparations are

559 well-known to have positive influences on foam stability (Dickinson, 2010).

560   In this context, Destribats, Rouvet, Gehin-Delval, Schmitt & Binks (2014), in their latest work,

561 have reported the utility of another novel class of protein-based particles, whey protein microgel

562 particles. They employed a bottom-up approach in their research to produce the microgel particles by

563 heating whey protein solutions sufficiently diluted to prevent the formation of brittle macrogels,

564 within combination of microfiltration and spray-drying. They demonstrated that this new class of

565 food-grade particles can successfully stabilize a food-grade oil-in-water emulsion for a long time

566 with exceptional resistance to droplet coalescence that shows good promise for application in the

567 food industry.

568   Soybean proteins, as well as whey proteins, are commercially and extensively used in food

569 products due to their high nutritional value (Friedman, 1996). Since they are originally from plant

570 resources, they need much less energy input for their production than those from animal resources,

571 including milk and egg proteins, which is helpful for energy savings in overall food production

572 (Pimentel & Pimentel, 2003). However, commercially available soybean proteins, denatured due to

573 high-temperature pasteurization or drying processes, although practically easy to manufacture, do not

574 necessarily have enough ability to stabilize oil-in-water emulsions compared to their native

575 counterparts (McSweeney, 2008).

576   In the current study, in order to enhance the functional properties of soybean proteins, we

577 describe the production of soybean protein gel particles under varied conditions to examine effects of

578 the gel particles on the stability of foams and oil-in-water emulsions prepared with food-grade oils.

579 Two kinds of soybean protein, ordinary commercial soybean protein isolate (SPI) and

580 low-molecular-weight partially hydrolysed SPI (LMW-SPI) were employed to estimate the impact of

581 steric repulsive effects of the gel particles. Because the commercial SPIs are not so soluble and

582 disperse into water as suspensions, we employed a top-down approach, different from the latest work

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583   on whey proteins by Destribats et al .(2014), to produce the gel particles, whereby a SPI macrogel

584   was first formed and then efficiently broken into microgel particles.

585

586   2.   Materials and Methods

587   SPI and LMW-SPI were kindly donated by Fuji Oil Co. (Osaka, Japan). The protein content of the

588   SPI and LMW-SPI was both > 90 wt%. LMW-SPI is partially hydrolysed by proteases with an

589   average molecular weight of approximately 60,000 Daltons. Corn oil (Mazola, ACH Food

590   Companies, Inc., UK) was purchased from a local supermarket in the UK. All other chemicals used

591   were of Analytical grade. Ambient temperature was approximately 25 °C throughout all the

592   experiments.

593   2.2.   Sample preparation

594   2.2.1. Macrogel

595   SPI and LMW-SPI were mixed with deionized water and then the appropriate amount of salt solution

596   was added to adjust the concentration of salts. The final concentration of SPI and LMW-SPI in the

597   macrogels was 15 wt% and 20 wt%, respectively. The salt concentration in the final macrogels was

598   set at 0 mM (no added salts), 60 mM for NaCl and 30 mM for $CaCl_2$ or $MgCl_2$. The concentrations

599   of the salts were determined according to the gelation method reported by Kohyama, Sano & Doi

600   (1995). The cationic salts were chosen based upon their use in the creation of tofu gels, which are

601   proposed as comparable to the microparticulated SPI gels presented.

602   The SPI and LMW-SPI mixtures were stored in a water bath at 40°C for 30 min to allow enough

603   hydration of the protein powders. The mixtures were respectively in paste and liquid forms and

604   thereby required two kinds of equipment with different shearing speeds to ensure complete

605   dispersion of the proteins. The mixtures were then dispersed by a hand blender with a puree masher

606   attachment (HB711M, Kenwood) at Speed 1 (350 rpm) and a hand blender with one of twin steel

607   beaters (HM320, Kenwood) at Speed 2 (1050 rpm) at ambient temperature to prepare SPI and

608 LMW-SPI suspensions, respectively. They were heated in a glass jar at 90 °C for 30 min in a water

609 bath and then cooled down to room temperature in another water bath at ambient temperature. The

610 suspensions were kept in a cold room at 4 °C overnight to fully set the macrogels.

611 2.2.2. Gel-particle suspensions

612 The heat-set macrogels were placed in a water bath at 25 °C for 30 min before the following steps

613 and texture measurements described in section 2.3.1. The macrogels were coarsely mixed via a

614 spatula with deionized water and 1 wt% salts solution to adjust the concentration of salts. The final

615 concentration of SPI and LMW-SPI in gel-particle suspensions was 6.25 wt% and the final

616 concentration of salts was 0 mM (no added salts), 30 mM for NaCl and 15 mM for $CaCl_2$ and $MgCl_2$.

617 It should be noted that final SPI concentration status is based on the SPI weight, not based on the SPI

618 gel weight including water.

619   The above coarse mixtures were homogenized in a bottle-type juice blender (Blend Active VBL

620 096, Breville, UK), in order to limit bubble generation from the mixtures, for 2 min at ambient

621 temperature to obtain coarse gel-particle suspensions. This referred to later as the high-speed

622 blending stage and the resulting dispersions were subjected to short-term storage tests, ζ-potential

623 measurements and microscopic observations. SPI suspensions not subjected to the gelation process

624 were also studied as control samples.

625   The coarse gel-particle suspensions were prepared for final homogenization using a high-speed

626 blender (Ultraturrax T25, IKA, Germany) at ambient temperature at 24,000 rpm for 5 min, an

627 ultrasonic homogenizer (VC 130, Sonics & Materials Inc., USA) at an amplitude of 100 for 5 min

628 within a water bath at ambient temperature for prevention of overheating or alternatively by passing

629 the coarse gel particle suspension through a high-pressure homogenizer (Jet homogenizer with a

630 60:40 chamber, University of Leeds, UK) at 240 bar to make fine gel-particle suspensions (Burgaud,

631 Dickinson & Nelson, 2007). These fine suspensions were subjected to particle size analysis and

632 foaming tests. The suspensions after the high-pressure homogenization were diluted with deionized

30

633   water to make suspensions containing 1-5 wt% SPI for the foam stability tests.

634   2.2.3. Emulsions stabilized by gel particles

635   The fine gel particle suspensions produced via the jet homogenizer containing 6.25 wt% SPI or

636   LMW-SPI were diluted with appropriate amount of deionized water and then mixed with corn oil at a

637   weight ratio of 8:2 aqueous: oil. The mixtures were preliminarily homogenized by a hand blender

638   (Rosso hand Blender, Russell Hobbs, UK) for 2 min at ambient temperature to make coarse

639   emulsions. These emulsions were then subjected to jet homogenization under the same conditions as

640   described above.

641   2.3. Sample characterization

642   2.3.1. Texture measurements

643   Texture analysis was conducted on SPI and LMW-SPI macrogels at ambient temperature using a

644   TA.XT2 texture analyser (Stable Micro Systems, UK) equipped with a 1/4 inch spherical stainless

645   steel probe. The analysis conditions were set as follows: Test mode = compression (penetration),

646   Pre-test speed = 5.00 mm/sec, Test speed = 1.00 mm/sec, Post-test speed = 5.00 mm/sec, Penetration

647   distance = 20.00 mm, Trigger force = 5.0 g. The fracture force (N) and the proportionality constant of

648   initial linear parts of force (N) vs distance (mm) curves were reported to characterise the hardness

649   and elasticity of the macrogels.

650   2.3.2. Storage stability tests

651   Non-gelled SPI and LMW-SPI suspensions and gelled particle suspensions were stored at 25 °C for

652   24 h and visually observed to examine for creaming or sedimentation.

653   2.3.3. ζ-potential measurement

654   Non-gelled SPI and LMW-SPI suspensions and gel particle suspensions were 1250x diluted with

655   aqueous phase containing the same salt concentration. ζ-potential of the gel particles was measured

656   by a laser-Doppler ζ-potential analyser (Zetasizer, Malvern Instruments, UK) using a refractive index

657   of 1.450-0.001i.

658 2.3.4. Light microscopy

659 The microstructures of SPI and LMW-SPI suspensions and gel-particle suspensions were observed

660 with a conventional light microscope (Optishot, Nikon, Japan) and recorded by a digital camera

661 linked to imaging software (Leica Microsystems, USA).

662 2.3.5. Particle size analysis

663 The size distribution of gel particles and emulsion oil droplets was measured by a laser-diffraction

664 particle size analyser (Mastersizer 3000, Malvern Instruments, UK). Sample emulsions were

665 appropriately diluted with deionized water to avoid multiple scattering. A refractive index of 1.45

666 was used to calculate the particle size distribution based on the Mie theory. The particle size of the

667 emulsions was reported as the surface-weighted mean diameter, $d_{3,2}$

668 2.3.6. Foam stability tests

669 The fine gel-particle suspensions were poured into a whip cream dispenser (ICO Brand 0.5L stainless

670 steel whip cream dispenser, ICO Trading UK Ltd., UK) and then foamed with N_2O gas that can be

671 directly charged by a dedicated charger. The foam was poured into a measuring cylinder and the

672 time-dependent behaviour was recorded by a video camera at ambient temperature. The foam

673 half-life, i.e., the time when half volume of the liquid had drained into the bottom of measuring

674 cylinder was taken as a measure of foam stability.

675 2.3.7. Emulsion stability tests

676   The SPI and LMW-SPI gel-particle emulsions were stored in an incubator at 25°C for 4 weeks.

677 Creaming stability was evaluated by measuring the creaming index described before: Creaming

678 index = (Height of serum layer/ Height of total emulsion) (Demetriades, Coupland & McClements,

679 1997). Evidence for aggregation and coalescence of emulsion oil droplets was obtained via the

680 Mastersizer with and without prior ultrasonic treatment for 30 sec. Samples for the long-term

681 stability tests had 0.05% (w/v) sodium azide added as an antimicrobial agent. Freeze-thaw stability

682 of the emulsions was examined after 1 week storage in a freezer at -18°C. The frozen emulsions were

683   thawed in a water bath at 40°C for 30 min and then visually observed. Destabilized free oil was

684   determined as described earlier (Palanuwech, J., Potineni, R., Roberts, R. F., & Coupland, 2003).

685   2.4.   Statistics

686   All experiments were conducted in triplicate with freshly prepared samples of suspensions or

687   emulsions. Statistical analyses were performed using Microsoft Excel ver. 2010 for Windows.

688

689   3.   Results and Discussion

690   3.1.   Characterisation of SPI macrogels and gel particles

691   It is well-known that the rheological properties of SPI gels depend on pH and salt type and

692   concentration (Bryant & Julian McClements,1998). Fig. 1a indicates the surface hardness of various

693   heat-set SPI macrogels, taken as the peak of the force vs distance curves. Clear breakage was not

694   clearly observed for almost all the samples except for the SPI macrogels with no added salts. Fig. 1b

695   shows elasticity of the SPI and LMW-SPI macrogels as measured by the slope of initial linear part of

696   the force versus distance curves. Even though a higher concentration of LMW-SPI (20 wt%) was

697   used compared to SPI (15 wt%), the SPI macrogel had a higher elasticity than the LMW-SPI

698   macrogel. This is probably because the LMW-SPI consisted of partially hydrolysed proteins that

699   were less able to form well-organized molecules networks, with lower water holding capacity.

700   Both the macrogels were significantly strengthened by addition of the salts in a similar way. The

701   strengthening effects of the divalent cations, Ca^{2+} and Mg^{2+} were much higher than those of the

702   monovalent cation, Na^+ due to the higher ability of divalent ions to act as bridging agents between

703   neighbouring molecules (Mine, Murakami, Azuma, Yoshihara, Fukunaga, Saeki & Sawano, 2005).

704   On the other hand, for both the SPIs, there were no noteworthy differences between macrogels made

705   with Ca^{2+} and Mg^{2+}, while Mine et al. reported that the breaking stress required for tofu varied with

706   the types of the divalent cations (Desfougeres, Lechevalier, Pezennec, Artzner & Nau, 2008).

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

708   Mg^{2+} under static conditions stored at 25 °C for 24 h. For both the SPI and LMW-SPI, control

709   suspensions without gelation and gel particle suspensions including no added salts, no gravitational

710   phase separation was observed, suggesting that the gelation processes did not significantly change

711   the dispersion state or structural features of the original SPI particles. However, the divalent cations

712   induced precipitation of the SPI gel particles and creaming of the LMW-SPI gel particles. Both the

713   SPI macrogels tended to be more elastic when the divalent cations were added (Fig. 1b) but, once

714   they were broken into microgel particles, they behaved in opposite directions against gravity, i.e.,

715   downward and upward (Fig. 2). These phenomena can be attributed to different structural features of

716   the suspended particles or varied apparent specific gravity, presumably related to different degrees of

717   air incorporation during creation of the macrogels based on the separate preparation methods and/or

718   disparate affinity of the SPI particles to air. One possibility is that the precipitating SPI gel particles

719   prepared with the divalent cations could be utilized as a density adjuster of emulsion oil droplets if

720   they adsorb at the oil-water interface.

721   Fig. 3 shows microstructural images of SPI gel particles obtained by light microscopy. The SPI

722   gel particles with no added salts seemed to be dispersed in the aqueous phase as well as the original

723   SPI particles without the gelation, whereas the SPI gel particles with Ca^{2+} and Mg^{2+} formed

724   relatively large aggregates after homogenization. These results correspond to those obtained by the

725   short-term storage test described above, which suggests that the different gravitational behaviour

726   depending on the type of added salt can be explained by the different structural features of the

727   aggregates, such as different degrees of compactness or fractal geometry, probably caused by the

728   different screening effects. Similar results were obtained for the LMW-SPI gel particles.

729   To confirm the screening effects of the added salts, the ζ-potential of the SPI gel particles was

730   measured (Fig. 4). The ζ-potential of the SPI and LMW-SPI microgel particles were both

731   significantly decreased by the presence of added salts, particularly for the divalent ions, in almost the

732   same way, in line with greater electrostatic screening effects of Ca^{2+} and Mg^{2+}. However, neither the

733   microscopic observations nor the ζ-potential measurements can fully explain how the different salts

734   affected the internal microstructure, density and water holding capacity of the individual microgel

735   particles.

736   For the practical use of the gel particles as surface-active agents, the adsorption efficiency at the

737   oil-water or air-water interfaces should be considered. In particular, emulsion formation efficiency

738   via particles predominantly depends on the size of dispersed surface-active particles due to their

739   relatively slow adsorption kinetics compared to the rate of interface formation during

740   homogenization (McClements, 2004a). This was why the coarse gel particles were further

741   homogenized to reduce their particle size. Fig. 5 shows the particle size distribution of the fine gel

742   particle suspensions prepared by the various homogenization conditions and various salt

743   concentrations. The size distribution of the SPI and LMW-SPI coarse gel particles homogenized by

744   the blender expressed as bold lines reasonably corresponds to the microscopic observations under all

745   added salt conditions (Fig. 3). The results indicate that the large particles observed via the

746   microscope in the SPI suspensions were strongly coagulated aggregates that were not be easily

747   dissociated even by the high dilution used for particle size analysis. On the other hand, according to

748   statistical correlation analysis performed, representative values from the distributions of SPI and

749   LMW-SPI coarse gel particles, that is $d_{3,2}$, $d_{4,3}$ and d_{50} (Table 1) did not clearly correlate with either

750   surface hardness or elasticity of the SPI and LMW-SPI macrogels obtained from texture tests (Fig. 1),

751   suggesting that the size of SPI gel particles does not necessarily depend on the texture properties of

752   the macrogels but does relate to the original structure of macrogel networks, etc.

753   The particle size of SPI coarse and fine gel particle suspensions was larger than that of

754   LMW-SPI gel particle suspensions under all salt conditions, particularly larger under conditions of

755   no added salt or NaCl added conditions (Fig. 5 and Table 1). The particle size of LMW-SPI gel

756   particle suspensions containing added $CaCl_2$ and $MgCl_2$ was larger than that of the suspensions

757   containing no added salts and added NaCl, while it was rather smaller than that of all SPI gel particle

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758 suspensions. For all suspensions, the size of fine gel particles generally decreased in the order

759 Blender < Blender + High speed blender < Blender + Ultrasonicator < Blender + Jet homogenizer.

760 Thus, the smallest gel particles were obtained via a combination of homogenization processes: the

761 juicer blender followed by the jet homogenizer under conditions of no added salts and added NaCl.

762 3.2. Foam and emulsion-stabilizing properties of gel particles

763 3.2.1. Foam systems

764 Foams were prepared via the whip cream dispenser using various concentrations of the non-gelled

765 SPI and fine gel particle suspensions without any added salts. Fig.6 shows the stability of foams

766 foamed with N_2O gas expressed as the half-life. The stability with the SPI non-gelled control

767 suspensions and with the SPI gel particle suspensions both increased with increasing protein

768 concentration, while foam stability with the LMW-SPI suspensions and gel particles did not increase

769 so significantly with protein concentration. The stability of foams with the SPI gel particles was

770 significantly higher than that with the counterpart SPI suspensions, whereas the stability with the

771 LMW-SPI gel particles and suspensions were similar to each other. These results indicate that for

772 ordinary high molecular weight commercial SPI, conversion for gel particles could be a useful tool

773 for improving their foam stabilizing properties. This improvement is presumably based on stronger

774 Pickering stabilization effects produced by the particles. Desfougeres, Lechevalier, Pezennec,

775 Artzner & Nau (2008) argued that foam stability depends on conditions that favour protein

776 aggregation rather than the presence of aggregates themselves. In our case, there is a possibility that

777 the SPI gel particles tended to aggregate via hydrophobic attractive interactions or they enhanced the

778 viscoelastic properties of the air-water interface by their increased water holding capacity.

779 3.2.2. Emulsion systems

780 Fig. 7 describes the creaming index of emulsions containing 20 wt% of oil stabilized by SPI and

781 LMW-SPI non-gelled proteins, i.e., suspensions (SPI Sus and LMW-SPI Sus) and fine gel particles

782 (SPI Gel and LMW-SPI Gel) without any added salts. For SPI Sus and Gel, the emulsions containing

783   3 wt% protein or more were almost stable against creaming for 4 weeks, while those containing 1 or

784   2 wt% were not, indicating that 3 wt% of proteins is at least required to prepare stable SPI emulsions.

785   Creaming stability of the SPI Gel-based emulsions was higher than that of the SPI Sus-based ones,

786   albeit the difference was not so significant. Creaming stability of LMW-SPI Sus and Gel-based

787   emulsions were much lower than that of SPI ones. For LMW-SPI Sus and Gel, creaming was only

788   prevented completely in the emulsion containing 5 wt% of LMW-SPI non-gelled proteins. Apart

789   from the emulsions containing 5 wt% of LMW-SPI, the stability of LMW-SPI Gel emulsions was

790   generally higher than that of the Sus emulsions. These results show that conversion to gel particles

791   may enhance the creaming stability of the emulsions, probably due to enhanced steric repulsion

792   between gel particles at the oil droplet surfaces and/or due to a possible increased viscoelasticity of

793   the emulsions in the presence of interactive microgel particles (Dickinson, 2015).

794   To estimate aggregation and coalescence of emulsion oil droplets, the particle size distributions

795   of the emulsions were monitored for 4 weeks. Similar results were obtained for SPI Sus and SPI

796   Gel-based emulsions (Figs. 8a and b). The initial mean particle diameter of the SPI Sus and Gel

797   emulsions decreased with an increase of protein content up to 3 wt% but plateaued out above it.

798   Significant particle size changes were not clearly observed for both the SPI emulsions with and

799   without prior ultrasonic treatment, demonstrating that aggregation and coalescence of the SPI Sus

800   and Gel stabilized oil droplets did not readily occur under the test conditions.

801   The initial mean particle diameter of the LMW-SPI Sus and Gel-based emulsions changed

802   depending on the protein concentration and it was slightly different for each set of emulsions (Figs.

803   8c and d), whereas the non-gelled proteins and gel particle sizes of LMW-SPI were almost the same

804   (Table 1). Although the detailed mechanism is unclear, we can point out a possibility that the

805   LMW-SPI Gel particles were more difficult to adsorb at the newly created oil-water interface during

806   emulsification than non-gelled proteins due to the structural changes induced by the gel conversion.

807   Whilst aggregation and coalescence of oil droplets were not observed for SPI Sus and Gel emulsions,

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808 slight aggregation and coalescence were observed for LMW-SPI Sus and Gel ones particularly

809 containing 3-5 wt% proteins according to the particle size changes after the ultrasonic treatment and

810 the 4-week storage (Figs. 8c and d). This is maybe because the thickness of interfacial layer

811 consisting of LMW-SPI Sus and Gel was insufficient to prevent oil droplets from coalescing. The

812 particle size of LMW-SPI Sus and Gel emulsions was reduced with more amount of proteins in all

813 cases. The reason why the higher the protein content apparently caused more extensive aggregation

814 and coalescence is difficult to explain but possibly that relatively smaller free LMW-SPI Sus and Gel

815 promoted depletion flocculation of the oil droplets via the attractive forces.

816   Fig. 9 shows the freeze-thaw treated SPI and LMW-SPI stabilized emulsions. For SPI Sus,

817 LMW-SPI Sus and LMW-SPI Gel, the emulsions clearly separated into a cream layer at the top and

818 serum layer including solid-like precipitates at the bottom, whereas the SPI Gel based emulsions

819 containing 3 wt% proteins or more did not separate. It is well-known that freeze-thaw treatment

820 produces a much stronger and more cohesive soybean curd (tofu) gel (Kalichevsky-Dong, Ablett,

821 Lillford & Knorr, 2000). The same phenomena might have occurred in the SPI gel particles at the oil

822 droplet surfaces, leading to more resistance of the SPI stabilized emulsions to phase separation. In

823 the previous study by Palazolo, G. G., Sobral, P. A., & Wagner, J. R. (2011), thermally-denatured

824 soybean isolates-based emulsions were shown to be more resistant against freeze-thaw treatments

825 than native ones. In our case, emulsions stabilized by the thermally-denatured commercial-grade SPI

826 became much more resistant via the heat-set gelation step that can introduce hydrated layers

827 enhancing repulsive forces to the protein molecules on the oil droplet surfaces.

828   The destabilized free oil from the emulsions containing 1 wt% of SPI-Sus and Gel and LMW-SPI

829 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

830 containing 2-5 wt% protein zero free oil was detected, within experimental error. One-cycle

831 freeze-thaw treatment might not be enough for elucidating clearer differences in oil phase separation

832 amongst the samples.

833

834   4.   Conclusions

835   We have demonstrated that SPI microgel particles imparted increased stability to foams and emulsion

836   oil droplets, probably via their enhanced steric repulsive forces in such colloidal systems. Formation

837   of microgel particles could be useful tool for enhancing functional properties of other

838   polysaccharides or proteins that exhibit difficulties in their normal modes of solubilisation.

839

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913

914   (Note)

915   All the figures and tables are magnified for peer-reviews. Their actual sizes on the published paper

916   will be adjusted and reduced by the publisher if the manuscript is accepted.

917   ---

918   Fig. 1

(a)

(b)

919

920

921   Fig. 2

SPI   LMW-SPI

| 0 h | 3 h | 24 h | | 0 h | 3 h | 24 h |

20 mm

Control   Control

| 0 h | 3 h | 24 h | | 0 h | 3 h | 24 h | | 0 h | 3 h | 24 h | | 0 h | 3 h | 24 h |

No Salts   NaCl   No Salts   NaCl

| 0 h | 3 h | 24 h | | 0 h | 3 h | 24 h | | 0 h | 3 h | 24 h | | 0 h | 3 h | 24 h |

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

922

923

44

924   Fig. 3

SPI
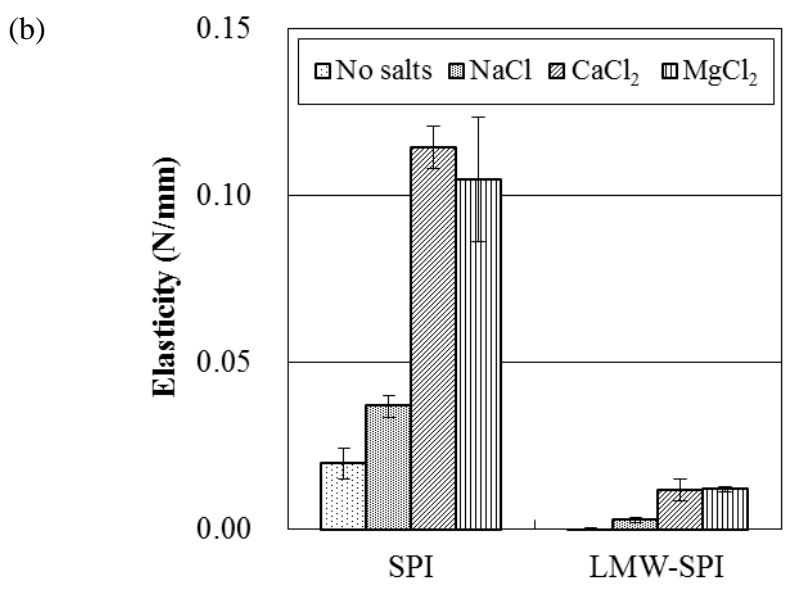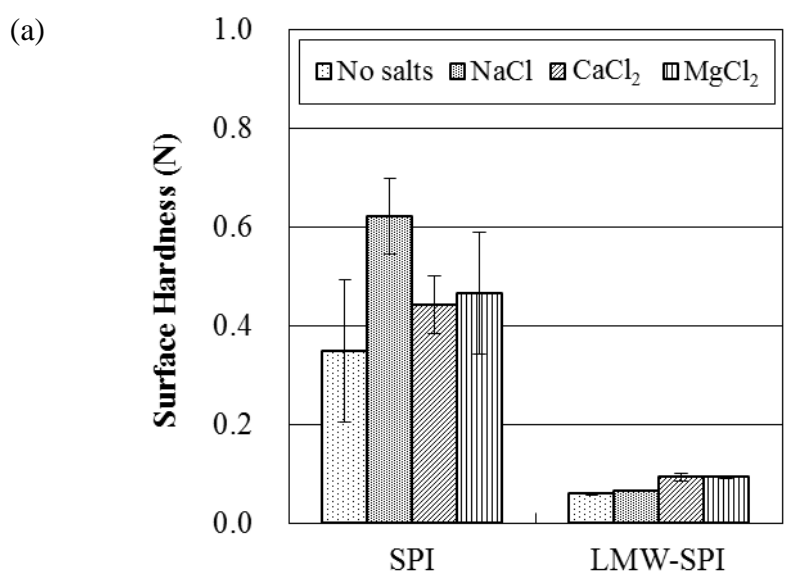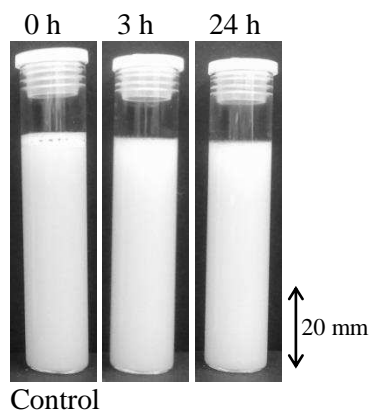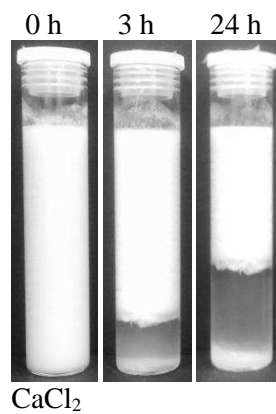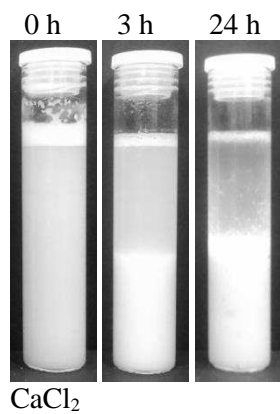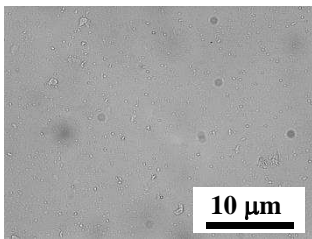
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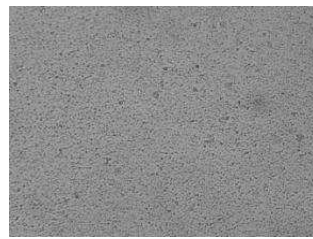
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(Non-gelled) Control
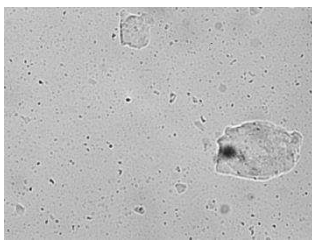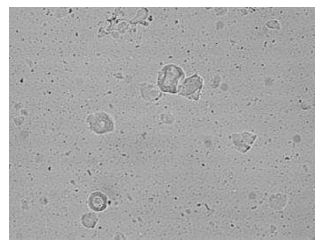
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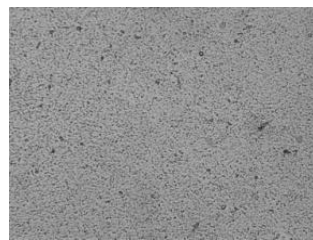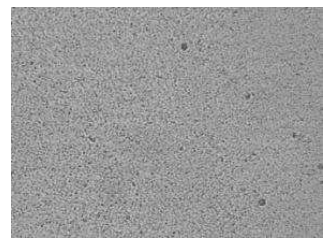
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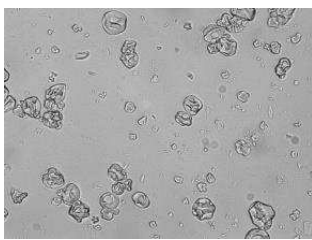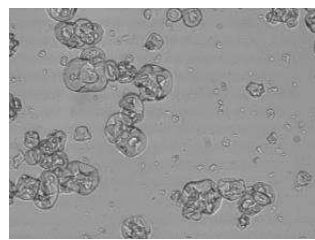
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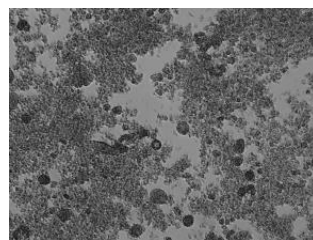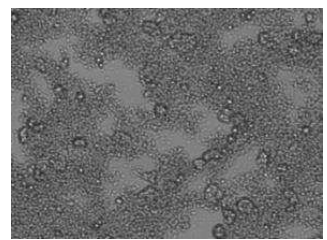
$CaCl_2$ $MgCl_2$

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927   Fig. 4
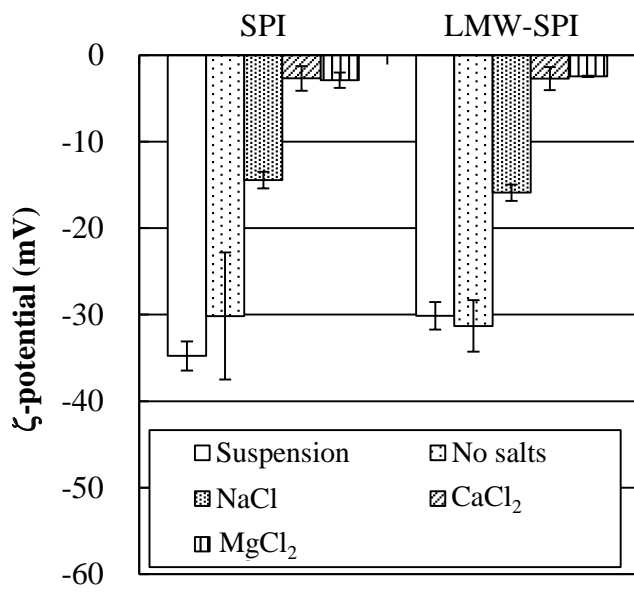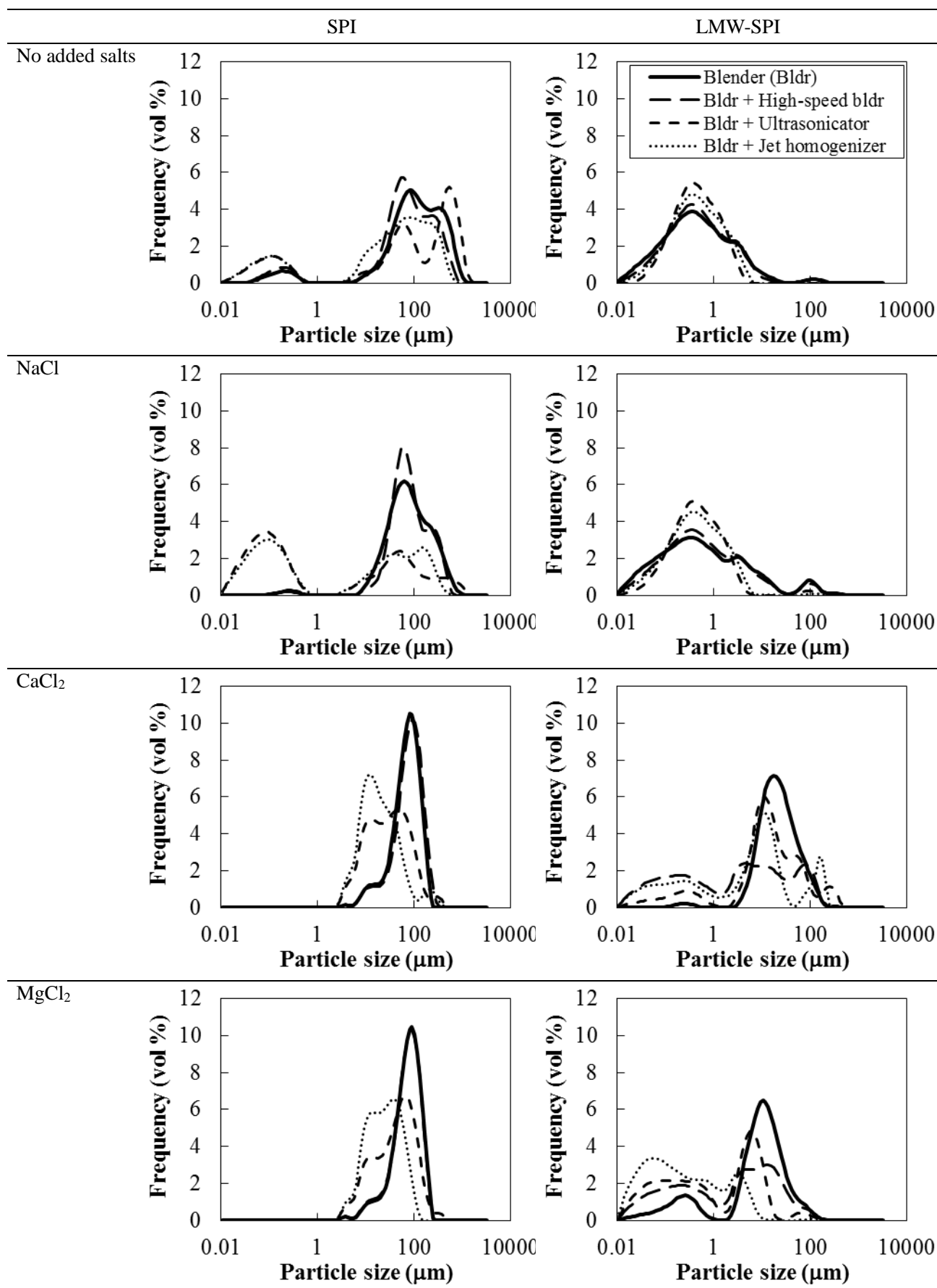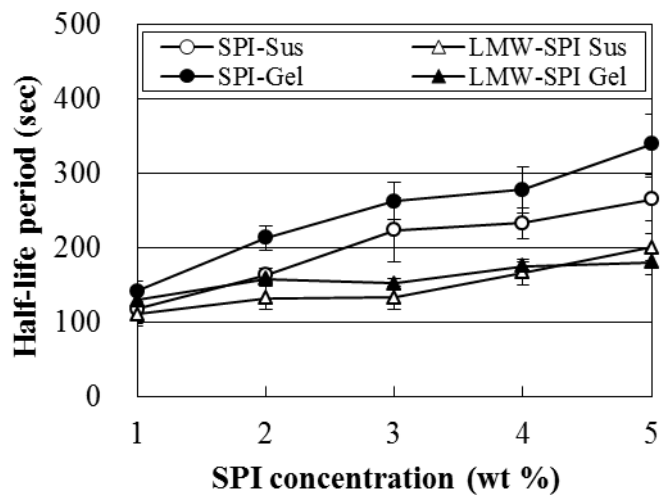
46

Fig. 5

47

931   Fig. 6

932

933

934   Fig. 7

SPI Sus

SPI Gel

LMW-SPI Sus

LMW-SPI Gel

935
936

49

937   Fig. 8

938

939   Fig. 9

SPI-Sus

| 1 wt% | 2 wt% | 3 wt% | 4 wt% | 5 wt% |

U   U   U   U   U

SPI-Gel

| 1 wt% | 2 wt% | 3 wt% | 4 wt% | 5 wt% |

U   U   S   S   S
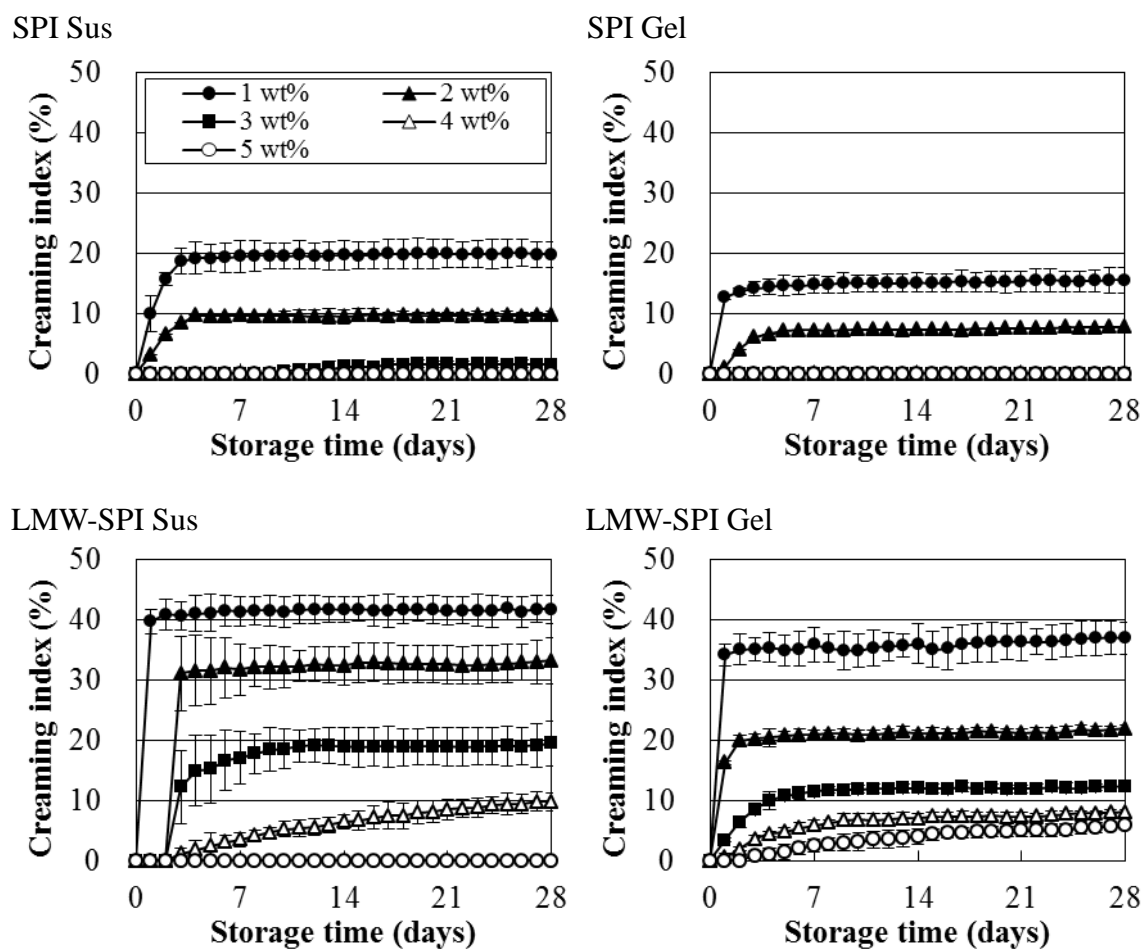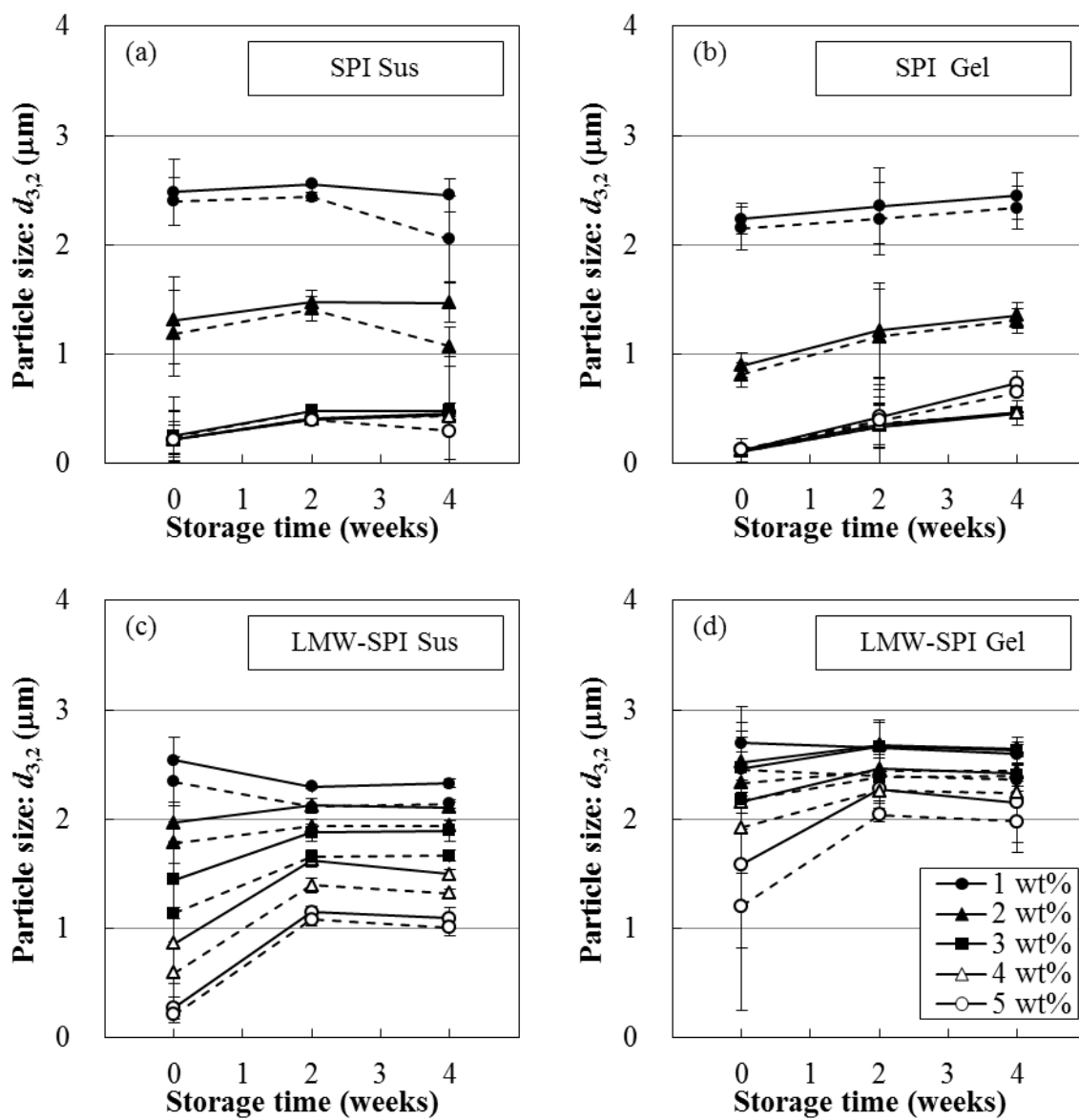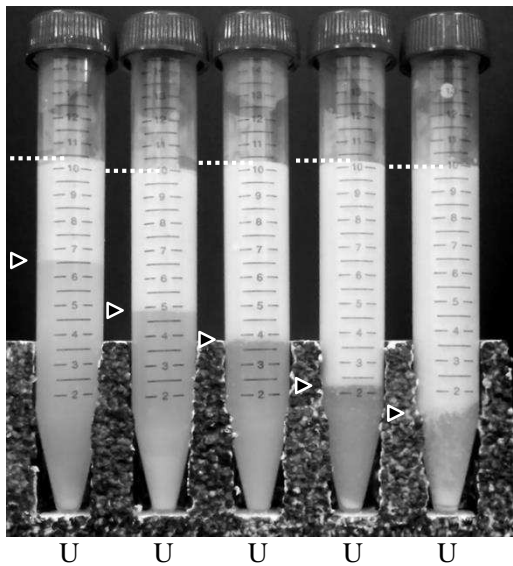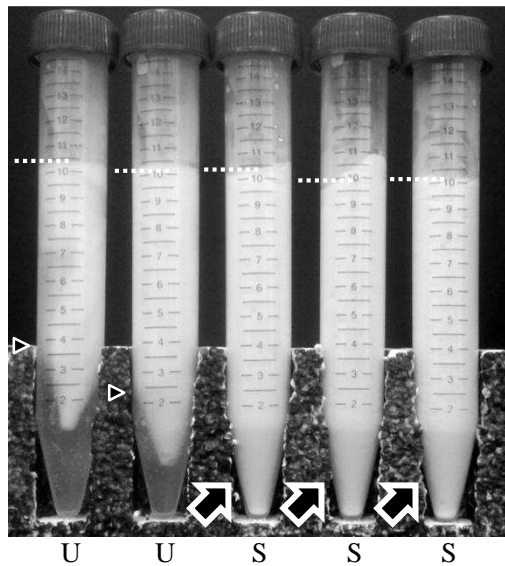
LMW-SPI Sus

| 1 wt% | 2 wt% | 3 wt% | 4 wt% | 5 wt% |

U   U   U   U   U
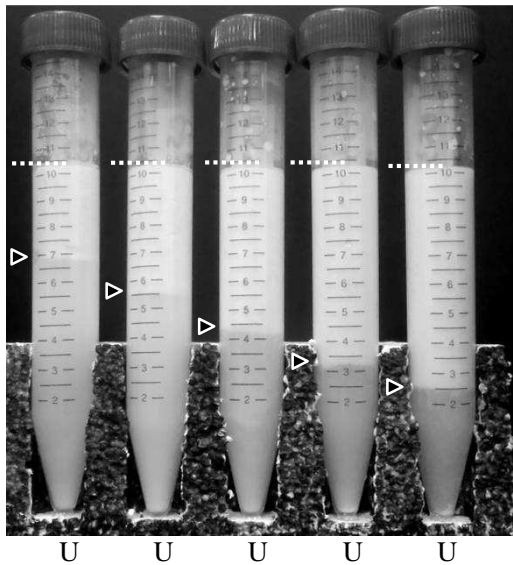
LMW-SPI Gel

| 1 wt% | 2 wt% | 3 wt% | 4 wt% | 5 wt% |

U   U   U   U   U

940

941
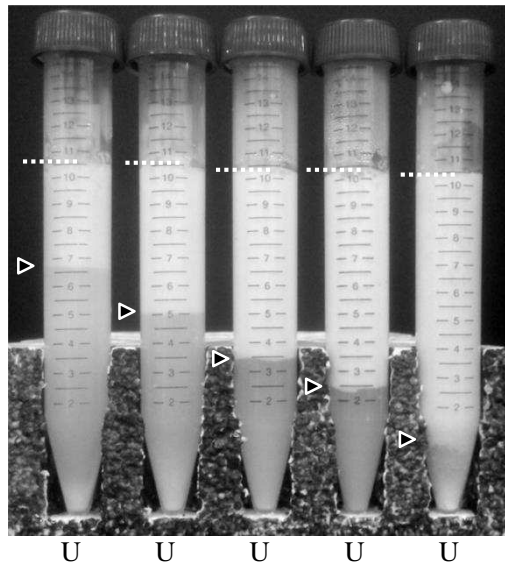
51

942   Figure captions

943

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

945   particle suspensions.

946

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

948   Texture analysis was conducted at 25 °C using a 1/4 inch spherical stainless steel probe

949   under penetration mode. The fracture force (N) and the proportionality constant of

950   initial linear parts of force (N) vs distance (mm) curves were reported as the surface

951   hardness and elasticity of the macrogels.

952

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

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

955

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

957   The microstructures of SPI and LMW-SPI coarse gel particle suspensions were

958   observed with a conventional light microscope at 25 °C.

959

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

961   SPI and LMW-SPI coarse gel particle suspensions were 1250x diluted with aqueous

962   phase containing the same salt concentration. ζ-potential was measured at 25 °C.

963

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

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

966

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

968   The gel particle suspensions were foamed with N_2O gas using a whip cream dispenser

969   at 25 °C.

970

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

972   SPI and LMW-SPI gel particle emulsions were stored at 25°C for 4 weeks. Creaming

973   stability was expressed as creaming index (Demetriades et al., 1997).

974

975   Fig. 8. Aggregation and coalescence stability of SPI and LMW-SPI gel

976   particle-stabilized emulsions.

977   Surface area-weighted mean particle size was obtained with (solid lines) and without

978   (dotted lines) prior ultrasonic treatment for 30 sec.

979

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

981   Freeze-thaw stability of the emulsions was examined after 1 week storage in a freezer at

982   -18°C followed by thawing in a water bath at 40°C for 30 min. Appearance of the

983   thawed emulsions were recorded with a digital camera. White dotted line and triangle

984   show the top of emulsions and the interface of cream and serum layers, respectively.

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

986