Electronic Supplementary Material (ESI) for Food & Function.

Materials and methods

Materials

Sodium caseinate (SC) was obtained from Armor Protéines Saint-Brice-en-Cogles, France) and contained 91% protein, 4% minerals, 0.7% fat, and 0.2% carbohydrates. ALG (Algogel®) was received from Cargill R&D Vilvoorde, Belgium. Sodium azide, sodium chloride, glycerol and HCl were obtained from Sigma Aldrich (Saint-Louis, USA). NaOH was bought from VWR Chemicals (USA) and refined sunflower oil was obtained from a local supermarket. Milli-Q water was used throughout the study. All materials were used without further purification or modification, and all samples were formulated and reported on a weight-by-weight basis (g/g).

Preparation of samples

SC and ALG powders were weighed into separate beakers at concentrations of 10% and 1%, respectively, in 100 mL Milli-Q water containing 0.02% sodium azide. These dispersions were then stirred continuously at room temperature (22-25 °C) till complete dissolution was obtained. The solutions were then stored overnight at 5 °C to ensure complete hydration of the biopolymers.

In the following day, the SC and ALG stock dispersions were mixed together to achieve various SC:ALG ratios (concentration of ALG was kept constant). Colloidal dispersions of SC:ALG at ratios of 4:1, 6:1, 8:1, and 10:1 were prepared by diluting and mixing the stock solutions. During the adjustment of pH, the protein-polysaccharide mixtures were initially sonicated with a Sonifier 250 (Branson Ultrasonics, US) set at output 9 and duty cycle 90 (%) followed by magnetic stirring. The final pH was 6.9-7.0 for all mixtures.

HIPE samples were prepared by homogenizing sunflower oil (concentration of 80%, ϕ_{oil} = 0.82) and appropriately diluted aqueous phase (20%) containing SC and ALG mixtures. All emulsions were homogenized for 30 s at 11000 rpm using an UltraTurrax IKA® T25 Basic (IKA-Werke GmbH & Co. KG, Germany) equipped with a dispersing rotor S25KV-25G. Glycerol (0.1%) was added after the first 30 s emulsification, followed by 90 s homogenization at 13500 rpm. The resulting self-standing emulsion gels were then stored at 5 °C prior to analysis on the following day.

HIPE samples were cast onto a surface of board paper covered with aluminium foil (10 x 10 cm^2) using a laboratory casting machine (Elcometer 4340, Manchester, UK) with the HIPE thickness around 1.875 mm. Drying of the HIPE sample was carried out by oven drying at 35 ^oC for 24 h. Afterwards, the dried oleofilm samples were manually peeled off using a flat spatula and stored in a dessicator at room temperature prior to analyses.

Emulsion characterization

The volume-weighted average droplet size (D[4,3]) of the emulsions was determined using a MasterSizer 3000 (Malvern Instruments Ltd, Malvern, Worcestershire, UK) equipped with a wet sample dispersion unit (Malvern Hydro MV, UK). Before measurement, all samples were diluted 20 times with the same pH as the solution. In the sample port, the samples were dispersed in mQ water at 1500 rpm until an obscuration of 2-6% was obtained. The background and sample integration times were 20 and 10 seconds, respectively. The optical properties were defined as refractive index 1.47 (sunflower oil), 1.330 (dispersant water) and absorption index 0.01, with the normal instrument calculation sensitivity and general purpose spherical particle shape selected. Results were calculated with the Mastersizer Version 5.54 software (Malvern, UK) to obtain the particle size distribution and volume-weighted average droplet size (D4,3). All measurements were done in triplicate.

Optical and cryo-scanning electron microscopy was utilized to study the microstructure of the samples. Optical microscopy was done on a Leica DM2500 microscope (Leica Microsystems, Belgium). For cryoSEM, samples were placed in the slots of a stub, plunge-frozen in slush nitrogen and transferred into the cryo-preparation chamber (PP3010 T Cryo-SEM Preparation System, Quorum Technologies, UK) where they were freeze-fractured and subsequently sputter-coated with Pt and examined in a JEOL JSM.

Oleofilm characterization

Thickness: The thickness of the oleofilms was measured using a micrometer. Five random locations around each oleofilm sample were used for average thickness determination.

Oleofilm samples were subjected to color measurement using a colorimeter. The color of the films was expressed as L*-value (lightness), a*-value (redness/greenness) and b*-value (yellowness/blueness). The total difference of color (ΔE*) and whiteness (WI) were calculated as follows

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

$$WI = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2}$$

Where ΔL*, Δa*, and Δb*are the differences between the color parameter of the samples and the color parameters of the white standards (L*=92.84, a*=-1.23, b*=0.47).

Oleofilms were measured at the wavelength of 600 nm using a UV-vis spectrophotometer. A greater transparency value was calculated using the following equation :

$$Transparency\ value = -\frac{\log T_{600}}{x}$$

Where T_{600} is the fractional transmittance at 600 nm and x is the oleofilm thickness (mm). The greater transparency value represents the lower transparency of film.

The contact angle (θ_w) of a sessile drop of water (10 µL) on oleofilm samples was measured using a drop shape analysis system DSA 10 Mk2 (Kruss GmbH, Germany). Contact angles were determined from approximating the contour of the imaged droplets with a circle fitting. The length of the measurement was 30 s with an increment of 0.5 s. The contact angle was the average value of the upper- and bottom-surface of the oleofilms.

Rheological measurements of HIPE and Oleofilm samples

Dynamic rheological measurements were carried out on an advanced rheometer AR 2000ex (TA Instruments, USA) equipped with a Peltier system for temperature control. A parallel plate geometry of 40 mm diameter was used and the geometry gap was set at a fixed gap (1500 µm) compromising with the average thickness of oleofilms. Preliminary oscillatory tests were performed to determine the fixed strain and frequency used in the frequency and amplitude sweep tests. A range of experiments including an amplitude sweep (stress = 0.1-1000 Pa for HIPE and 0.1-3000 Pa for oleofilms, frequency = 1 Hz), and frequency sweep (0.1-10 Hz, oscillatory stress = 2 Pa for HIPE and 50 Pa for oleofilms) were carried out at 20 °C. For temperature ramp evaluations, the selected oleofilm (1.0:0.1%) was subjected to time sweeps

at constant oscillatory stress (50 Pa) and fixed frequency (1 Hz). Creep recovery tests were carried out by subjecting the samples to a fixed stress below the yield stress (200 Pa) for 5 minutes followed by 10 minutes of recovery by decreasing the stress to zero. Stress relaxation tests were carried out by subjecting the samples to a fixed strain (100) for 10 minutes.

Data analysis

All experiments were carried out in triplicate using freshly prepared samples. All data analyses were done using MS-Excel 2010 and Origin 8.0. An analysis of variance (ANOVA) of the data was performed, and a least significant difference (LSD) with a confidence interval of 95% was used to compare the means.